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Peripheral Chemoreceptors: Function and Plasticity of the Carotid Body

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Abstract

The discovery of the *sensory* nature of the carotid body dates back to the beginning of the 20th century. Following these seminal discoveries, research into carotid body mechanisms moved forward progressively through the 20th century, with many descriptions of the ultrastructure of the organ and stimulus-response measurements at the level of the whole organ. The later part of 20th century witnessed the first descriptions of the cellular responses and electrophysiology of isolated and cultured type I and type II cells, and there now exist a number of testable hypotheses of chemotransduction. The goal of this article is to provide a comprehensive review of current concepts on sensory transduction and transmission of the hypoxic stimulus at the carotid body with an emphasis on integrating cellular mechanisms with the whole organ responses and highlighting the gaps or discrepancies in our knowledge. It is increasingly evident that in addition to hypoxia, the carotid body responds to a wide variety of blood-borne stimuli, including reduced glucose and immune-related cytokines and we therefore also consider the evidence for a polymodal function of the carotid body and its implications. It is clear that the sensory function of the carotid body exhibits considerable plasticity in response to the chronic perturbations in environmental O_2 that is associated with many physiological and pathological conditions. The mechanisms and consequences of carotid body plasticity in health and disease are discussed in the final sections of this article.

Introduction

The scientific history of peripheral chemoreceptors, especially that of the carotid body is often taken as beginning from the mid-18th century following the first recorded description, by Albrecht von Haller in 1762 of the “ganglion exiguum,” but could be argued to have begun earlier with the public defence of a dissertation describing the “ganglion minutuum” by his student, Taube in 1743 (819). In this, is given a description of the anatomical dissection of a ganglion-like mass of tissue located in the region where the common carotid artery bifurcates to form the internal and external carotid arteries. The presumption then, and held for almost the following 150 years, was that the tissue must have a glandular function sub-served by a motor innervation (186, 376, 432, 492). Our present understanding of the *sensory* nature of the carotid body can therefore be acknowledged to start only from the beginning of the 20th century with the influential publication of a paper by Kohn in 1900 (454) who classified the carotid body as a chromaffin tissue but, importantly, did not

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consider the amassed, detailed microscopical evidence sufficient to believe the organ to be a gland. Consequently, the first direct evidence of a sensory, rather than a motor function for the carotid body arose principally from a correct, anatomical and histological determination of the afferent nature of its innervation by the sinus branch of the glossopharyngeal (IXth cranial) nerve and was the work of Fernando de Castro, from the Cajal Institute in Madrid (Fig. 1), who also made the astute observation that its gross anatomy and location made it most likely to "… *detect qualitative variations in the blood, a function which by reflex action will have an influence on the functional activity of other organs*" (189). Shortly after de Castro's seminal work, a functional role for the carotid body in cardiorespiratory homeostasis was established in Ghent, Belgium, by Jean-Francois Heymans and his son, Corneille Heymans (377), through the deductions made from a series of cross perfusion experiments performed with vascularly isolated, carotid bifurcations in the dog. This work subsequently led to the Nobel Prize in 1938 (375) being awarded to Corneille (Jean-Francois having deceased by this time) "for the discovery of the role played by the sinus and aortic mechanisms in the regulation of respiration." Why de Castro may have been overlooked for this prestigious award is detailed in an interesting historical account (191). Following these seminal discoveries, research into carotid body mechanisms moved forward progressively through the 20th century, with many descriptions of ultrastructure of the carotid body and stimulus-response measurements at the level of the whole organ, which were elegantly reviewed by Fidone and Gonzalez in the Handbook Chapter published in 1986 (269). The next major advance in our understanding occurred in the latter part of the 20th century, with the initial isolation of enzymically isolated, individual carotid body cells (702, 705) leading to the first descriptions of the cellular responses and electrophysiology of isolated and cultured type I and type II cells (229, 540, 541, 703, 704, 850), from which the modern history of the carotid body could be said to begin.

In this article, our goal is to provide a comprehensive review of sensory transduction of the hypoxic stimulus at the carotid body with an emphasis on integrating cellular mechanisms to whole organ responses and highlighting the gaps in our knowledge. As the carotid body, in addition to blood gas composition, may also respond to a wide variety of blood-borne physicochemical stimuli, including temperature, glucose, and immune-related cytokines, we further discuss the evidence for a polymodal nature of carotid body sensory function. Recent studies provide rather compelling evidence that the sensory function of the carotid body exhibits considerable plasticity in response to chronic perturbations in environmental O_2. Consequently, we discuss the mechanisms and consequences of carotid body plasticity in health and disease in the final section of this article.

Anatomical Location of Carotid Bodies

The carotid bodies are located bilaterally in the neck, at the rostral end of the left and right common carotid arteries where these bifurcate into pairs of internal and external carotid arteries. In the cat, dog, rat, and mouse the carotid bodies are most often positioned as single, distinct capsules, isolated from the major vessel from which they derive their blood supply (16) and are usually located close to the ventral surface of the superior cervical ganglion. In contrast, in the rabbit and guinea pig, the carotid bodies are more often found encasing the arteries that provide the cerebral blood supply in these species (158). Carotid body wet weight increases with the size of the animal and ranges from around 60 μg in the rat to 600 μg in the cat (561) and up to around 13 mg in the adult human (361). The long axis length of the carotid body is variable, but is generally around 750 μm in rat (372), 1.5 mm in the cat (860), and between 2 and 3 mm in man (361).

Chemoreceptor tissue of similar histology to the carotid body is also found within the mediastinum and abdomen. In the thorax, such tissue has been identified on the basis of its

topographical distributions along the aorta, pulmonary trunk, and subclavian arteries, its blood supply and its innervation (165). Of these various tissues, only, so-called, group 3 and 4 bodies receive a systemic arterial blood supply in the adult and it is these that are commonly called “aortic” bodies. Innervation is largely afferent with fibers passing in the aortic nerve branch of the vagus, but, like the carotid body, there is also an efferent innervation from sympathetic fibers and possibly also from parasympathetic fibers (614, 615). These, aortic body chemoreceptors have been credited with a natural, sensory function in cats (495), but only a sparse number of such cells have been identified in the aortic arch region of rats (236) and may be entirely absent, either morphologically or functionally, from rabbit and mouse (139) where their normal function remains not fully determined.

The most caudal location of peripheral chemoreceptor tissue is intraabdominally, associated with the origin of the coeliac artery as it branches from the abdominal aorta and runs with branches of the abdominal vagal nerves in the rat and mouse (39, 393). This tissue, in the rat, is responsive to arterial hypoxia and to locally applied sodium cyanide (39, 393) but its functional contribution to cardiorespiratory reflexes is minimal in the presence of the other peripheral chemoreceptor tissues.

In its extra cervical locations, chemoreceptor tissue is more diffusely arranged in small groupings of cells and so less likely to exist as a single, distinct unit. Burleson and Milsom (121, 582) suggest that peripheral chemoreceptor tissue became consolidated into carotid bodies by the evolutionary transition from aquatic and bimodal breathing in fish to obligate air breathing in mammals as well as with the consequent loss, in mammals and birds, of intracardiac shunts, thus shifting the functional emphasis away from a need for multiple chemosensitive sites responding to varying internal or external fluctuations in stimuli, toward the single, primary site seen in mammals. Consequently, the embryological derivation of the carotid body cells arises as a condensation of neural crest cells, subsequent to their migration within the mesenchyme of the third branchial arch in mammals, a structure homologous to the first gill arch of fish. The mechanism that prevents migration once the cells have reached their target site is unknown.

Cell Types of the Carotid Body

The ultrastructure of the carotid body received particular attention during the period of the 1950s to the 1980s and many studies aimed to ascribe a structure-function relation to the organ, based on light- and electron-microscope observations. Given the relatively limited ability to ascribe function using fixed tissue, these studies were remarkably insightful and still form the basis of much of our modern understanding of the carotid body.

The carotid body is a conglomeration of type I and type II cells. Regarding the nomenclature, type I cells were also commonly, and interchangeably, referred to as “glomus” (523), “chief” [attributed to Sato, 1932 in Adams (16)], “epithelioid” (190), or simply “chemoreceptor” (754) cells but the designation of the organ’s predominant cell as type I (194) appeared to be favored and most commonly accepted over time, presumably due to its lack of connotation to any specific anatomy or function. Carotid bodies are endowed with a rich vascular supply and an afferent and efferent innervation that lies within a protective surrounding capsule of connective tissue of varying, species-dependent, density that also infiltrates it, thus providing a matrix for blood vessels and nerves which, in most species other than perhaps the cat (523), gives the organ its characteristic lobular, or “glomerula,” structure when viewed microscopically. The close association of chemoreceptor tissue with tissues such as sympathetic neurons and cranial nerves IX (glossopharyngeal) and X (vagus) may be explained by the migratory, neural crest origin of type I and type II cells (37, 674). This was first identified definitively in birds, by le Douarin

and colleagues (515, 677) who utilized a quail-chick marking methodology and confirmed, less categorically, in mammals, by an electron microscopy study of rat carotid body development (455, 463) and in a Cre/Lox genetic recombination study of neural crest fate mapping in mice (417). Fibroblasts, autonomic ganglion cells and mast cells (362) are also found within the connective tissue of the organ (16, 335) but are not believed to have any direct, functional role in chemosensing.

The number of type I cells per carotid body appears related to the size of the organ with the rat carotid body containing up to 12,000 type I cells and the cat up to 60,000 such cells (561, 860). Type I cells are the most abundant cell type within the carotid body with typically between a minimum of 3 and up to a maximum of 8 (although more typically, 4–5) such cells identified for each type II cell in most species (194, 195, 335, 561, 860), although the ratio may be higher in primates (335). This ratio underlies the general arrangement of these cells in clusters or glomeruli consisting typically of 3 to 5 type I cells, spaced apart from each other by approximately 15 to 20 nm and enveloped almost entirely by the thin, cytoplasmic processes of a single type II cell (previously termed the “sustentacular or sheath” cell (754) or “pericyte” (193, 194, 523).

Taken together with its capillary blood supply and its innervation, the glomerulus is considered as the functional unit of the organ (775). However, whilst generally agreed to be of importance, the physiological consequence of numerous, separate functional units of the carotid body is poorly studied or understood and much of our present comprehension of chemoreceptor behavior is consequently based on a tacit assumption that the various cellular components of the carotid body act individually, with their sum action combining to produce afferent action potentials of appropriate frequency.

The type I cell

That type I cells are of neural origin is strongly suggested by the presence of numerous immunological neural and glial cell markers and growth factors, including enolase (461), tyrosine hydroxylase (TH), protein gene product 9.5 (PGP9.5), βIII-tubulin, glial fibrillary acidic protein (GFAP) (425, 426), as well as basic fibroblast growth factor (bFGF), glial cell line-derived neurotrophic factor (GDNF), and brain-derived neurotrophic factor (BDNF) (406). However, unlike most neural crest-derived cells, type I cells do not depend upon nerve growth factor or corticosteroids for their long-term survival in culture (278) although bFGF has been shown to act as a necessary mitogen in perinatal carotid body type I cells, increasing DNA synthesis and cell survival (629). As regards to their similarity to chromaffin cells of adrenal medulla, it is worth noting that sympathectomy of the carotid body does not alter the microscopic structure of type I cells or their innervation (523) and therefore they do not conform to classical descriptions of chromaffin cells of the adult adrenal medulla, but may rather more resemble denervated, fetal sheep (166), or isolated neonatal rat adrenal medullary cells that secrete catecholamines in response to hypoxia (828). Additionally, nerve growth factor fails to induce neurite outgrowth or expression of neuronal markers in neonatal type I cells, whilst it does in chromaffin tissue derived from the adrenal medulla (407), demonstrating functional differences between these two related cell types.

A further differentiation of the embryological derivation of type I cells into neuroendocrine rather than neural derivatives of the sympathoadrenal neural crest was confirmed (430), by the selective expression on these cells of carbohydrate epitopes binding peanut agglutinin and this has been subsequently exploited as a means of identifying type I cells in live culture through the successful use of fluoresceinated peanut agglutinin markers in patch clamp and calcium imaging studies on isolated carotid body cells (442). However, the more intriguing question remains open of whether the carotid body might possess a neuroendocrine function,

in addition to its more recognized one of chemosensing, and is supported by its rich blood supply and the known presence within it of numerous neuropeptides, including substance P, neurokinin A, atrial natriuretic peptide (ANP), endothelin, and galanin (474).

Type I cells are generally oval or polygonal shaped, in microscopic section, with a diameter that ranges, in the rat, between 8 and 15 μm (372, 565) and often have cytoplasmic processes emanating outward for up to three times the cell diameter (565). These processes may increase in length with age, at least in the rat (917). The cat type I cell is somewhat more regular in shape and has fewer processes (372). Type I cells are spaced approximately 30 nm apart from each other in a glomerulus, but also exhibit tight junctions at intervals around their surface. The cell shape tends to become more spherical, and generally devoid of processes, when type I cells are initially isolated *in vitro*, giving it a characteristic birefringent appearance under phase contrast. If cell culture is maintained for 48 h or more, cytoplasmic processes are recovered with cells attaining a more bipolar and irregular shape (278, 784, 850). Type I cells have a large [commonly at least half the diameter of the cell (754)], spherical nucleus, with a single prominent nucleolus, which is surrounded by an outer shell of cytoplasm containing numerous, unremarkable distributions of cell organelles including a rich array of mitochondria, lysosomes, lipid droplets, glycogen particles, and a widely distributed endoplasmic reticulum with free ribosomes (72, 85, 561). The characteristic feature of the type I cell is, however, the presence of numerous, Golgi apparatus-associated, vesicles with either small, clear or larger, electron-dense cores located particularly (371, 566, 858), but not solely, adjacent to sensory nerve terminals. These represent two recognized classes of secretory vesicles (188, 433), that differ on the basis of their appearance, biochemical content (826), the kinetics of their fusion with the plasma membrane (620), and their origin and functions (38, 188, 411, 557, 855).

The clear core vesicles of type I cells (449, 452, 858, 860) are generally fewer in number than the dense core vesicles and have been much less remarked upon in morphological studies of the carotid body. By analogy to other catecholaminergic tissues, clear core vesicles may form a heterogeneous group of different ontogeny with some arising via terminal membrane endocytosis, whilst others may represent smooth endoplasmic reticulum or small “dense-cored” vesicles devoid of catecholamine (831). Their function is not fully established, but they are presumed to be the storage site for certain, non-catecholamine neurotransmitters and modulators, including acetylcholine (ACh) (626). They are usually small, with a diameter of approximately 40 nm, and are located throughout the type I cell cytoplasm, though with a higher probability at the plasma membrane apposed to adjacent type II cells or to sensory nerve terminals, though not necessarily at membrane specializations associated with synapses (858). Aggregations of clear core vesicles are also localized within the fine, cytoplasmic processes of type I cells (566).

Dense-core vesicles differ from clear synaptic vesicles in a number of ways other than their obvious morphological appearance. The biochemical composition of the vesicle, the mode of stimulation required for synaptic release, the type of calcium channels involved in the exocytotic process, and the time course of recovery after stimulation are all distinguishing features (956). In the carotid body, the dense-core vesicles appear to vary in size, not just across species but also within a species and even within a single type I cell, but are often within the range of 50 to 150 nm in diameter in most nonprimate mammals (448, 452, 522, 601, 859) but can be significantly larger in the macaque monkey (335). A variation in density has also been noted, with the highest density of 8 vesicles per um^2, ascribed to the monkey (335). The vesicles contain various neurochemicals; primarily catecholamines (523) and chromogranin neuropeptides (336) but, presumably also, adenine nucleotides and Ca^{2+} (914) giving the type I cell the appearance of adrenal medullary chromaffin cells, albeit that the dense core vesicles in the type I cell may, in most cases, only be up to half as large as

their counterparts in the adrenal medulla (176, 319). The small size of the granules and the relative small mass of carotid body tissue, makes quantification of vesicular content difficult, but the use of insulated, carbon electrodes and amperometry with either enzymically dispersed, single, rabbit type I cells (849), or conglomerations of cells in a thin slice rat carotid body preparation (670) has revealed that type I cells from both species secrete catecholamine (presumed to be dopamine), with a single quantal charge of 44 to 46 pC. Assuming two electro oxidation reactions per catecholamine molecule and the presence of no other transiently released, oxdizable species, the charge measured can be used to calculate that approximately 1.4×10^5 molecules of catecholamine are stored per vesicle. With no evidence to suppose that either adenosine triphosphate (ATP) or chromogranin-like peptides can undergo redox reactions at the holding potential (+ 750 mV) of the amperometric carbon electrode, this estimate can be taken as reliable and demonstrates that the type I cell dense core vesicle contains approximately 20 times less catecholamine per vesicle than the much larger, 250 to 300 nm diameter, vesicles of chromaffin cells of the adrenal medulla (909), and around five times greater quantal content than the smaller, 60 to 70 nm diameter, vesicles found in sympathetic ganglion cell varicosities (952). Further supporting the storage of catecholamine within the dense-core vesicles is the immunohistocytochemical and immunofluorecent localization of TH within these vesicles (429, 628).

Various studies have attempted to distinguish between type I cells on the basis of dense-core vesicle size and density and the results are equivocal. Some of this may be due to artefacts arising from either the various fixative processes utilized to visualize the cells and their constituents or from the degree of disruption to cell homeostasis during the surgical process of organ removal and isolation. Thus, some authors have stated they could find no differences between the various type I cells on the basis of vesicular diameter (859), whilst others have described two distinct populations (192) and can showa bimodal distribution in the dense-core vesicle diameter (366, 566). If vesicular density and shape are also included as distinguishing characteristics, then up to four populations may exist in the cat and monkey (335, 601).

McDonald and Mitchell (565, 566) ascribed the term type A to those type I cells that had, on average, 30% larger and 150% more numerous dense core vesicles than another subset that they termed, type B, with both subsets existing, in the rat, in approximately equal numbers. In addition, they reported that, in the rat, type B cells had a greater extent of cytoplasmic processes that were thinner, but greater in number, length, and branching than those of type A cells and also that type A cells received an afferent innervation whilst type B cells received little or no innervation. Type A and type B cells also made pre- and postsynaptic contact with each other, with large dense-core vesicles clustered around synaptic junctions of the presynaptic element and it was stated that the type B cell was the more likely of the two cell types to be presynaptic to another type I cell (565).

The existence of structural differences between type I cells has been supported by other morphological studies (448, 601, 802). Further, the specific distinction into type A and type B cells with possible different functions (565), was endorsed by a study in which α-bungarotoxin was used to distinguish ACh receptor binding sites on type I cells with the finding that type A cells showed little or no binding whilst type B cells showed plentiful binding distributed across the entire cell surface (142). These authors, as had McDonald and Mitchell previously (565, 566), suggested that the difference in cell type might demonstrate a difference in function with only type A cells acting as the presynaptic elements in a composite receptor system.

The difficulty of ensuring that, when isolated, type I cells retain their *in vivo* physiology is a real concern that cannot easily be identified and thus any potential for variation in type I cell function most often is not considered when the biophysical properties of these cells are being determined. However, differences do exist *in vitro* between isolated type I cells and type I cells retained in clusters, with considerable variation in response to changes in pH (666) or Po_2 (99) being observed. Whilst this may be due to a loss of type I-type I or even type I-type II interaction when cells are isolated from their natural clustering, there does still appear to be a possibility for functional heterogeneity *within* type I cells as, when studied systematically, an intracellular Ca^{2+} response to hypoxia was observed in only 20% of isolated type I cells (99) and a number of type I cells that showed a robust, secretory response to high extracellular K^+ solutions, did not respond to hypoxia in a thin slice rat carotid body preparation (670). Whether these cells that did not respond to hypoxia might have been classed as the noninnervated, type B cells of McDonald and Mitchell's classification (565) could not have been ascertained by these functional studies. Finally, different sensitivities to hypoxia between afferent responses in a single animal have been commonly observed (870) and whilst it is usually taken that this may represent varying stimulus intensities at the site of origin of the action potential, it is not yet possible to exclude that some of this variation may also reside within the heterogeneity of type I cells. It is, however, perhaps equally or even more likely that the subtypes of type I cells described to date represent an artificial grouping of such cells, by researchers, into distinct groups whereas the reality may be simpler with a continuum of variation that reflects turnover of a single cell type (698).

Role of the type I cell—the primary transducer?—The isolated type I cell, in culture, can mount a vigorous, physiological response to hypoxia (see later sections) and consequently has been ascribed a primary transducer role with its particular morphology, histochemistry, and relations to other cells in the carotid body presumed to form the basis of its presynaptic, neurosecretory function. However, its ability to respond to hypoxia in isolation does not prove, definitively, that the type I cell is a primary transducer element of a composite receptor system, as it may simply act to modulate responses generated, for example, in adjacent, afferent nerve terminals that might themselves, therefore, be considered as the primary elements (73, 218, 565, 587).

In an attempt to answer the question more directly, a number of transplant and/or regeneration experiments were performed whereby, either the carotid body was reinnervated by afferent nerves other than the glossopharyngeal nerve (GPN) or where the carotid body or type I cells were selectively destroyed, so separating them from chemoafferent nerve endings of the sinus nerve. In animal studies, section of the sinus nerve afferent innervation of the carotid body abolished the sensory discharge from the proximal stump of sinus nerve, with a timescale related to the degeneration of nerve terminals in the carotid body (1, 373). Additionally, if the nerve section did not disrupt the afferent nerve cell bodies in the petrosal ganglion, the nerve regenerated, via axonal sprouts, and recovery of afferent discharges was observed that was shown to depend upon synapse formation with type I cells (943).

Reinnervation of the superior cervical ganglion with sinus nerve afferents also failed to establish chemosensitivity in the fibers (66). Similarly, if the sinus nerve was left intact but the carotid body, of rabbits, was destroyed by localized freezing *in situ* (864, 865), no afferent chemosensitivity or reflex ventilatory response to natural stimuli or pharmacological stimulation was detected even after allowing up to one year for regeneration of nerve afferent terminals into the scar tissue, despite, at that time, clear evidence of baroreceptor activity being restored. Destruction of the cat carotid body type I cells by 2 to 3 h of ischemia, induced by ligature of the carotid body arterial blood supply (597, 622), produced comparable findings to the earlier cryodestruction experiments, with

irreversible damage to type I cells being correlated to a lack of sensory function, even after reinnervation of sinus nerve afferents. It should be remembered, however, that destruction of the carotid body by such blunt instruments as cryocoagulation or ischemia is not type I cell selective and damage to type II cells as well as to the blood supply must also occur, thus potentially offering a different explanation for the subsequent loss of function [see (938)]. In addition, not all studies were so conclusive and, whilst a number of regeneration studies showed a return of chemosensitivity that might be ascribed to the nerve terminals, it was subsequently shown that the lack of total removal of carotid body tissue in these studies was a significant methodological concern (712).

Complementary studies utilized reinnervation of the carotid body with nerves that would not, normally, form synaptic contact with type I cells of the carotid body. Initial experiments were performed by suturing the cut, central end of either the vagus nerve or the superior laryngeal nerve to the cut distal end of the GPN and allowing sufficient time for regeneration of new synaptic contacts of these, normally incongruent, afferents with type I cells. In both cases, chemoafferent sensitivity was returned but such studies (189, 941) were criticized for not accounting for the presence of non-carotid body chemosensitive afferents in both the vagus and superior laryngeal nerves. Whilst certainly a possibility for the vagus nerve, with its aortic body and bronchoplumonary chemoafferents (616), the criticism may be less robust for the superior laryngeal nerve where the majority of sensory fibers are mechanosensitive and the few, chemosensitive afferents, whilst sensitive to hypercapnia, are not naturally sensitive to hypoxia. Nevertheless, the case for type I cells as primary chemotransducers had not been proven.

For simple, logistical reasons, initial experiments had utilized afferent nerves for suturing to the sinus nerve, that were located anatomically close to the carotid body and so, to obviate all such criticism, a subsequent experiment was performed that moved the carotid body from its location in the neck to enable grafting of a defined, nonchemosensitive afferent nerve supply. Thus, Eyzaguirre and colleagues (596) transplanted cat carotid bodies into the tenuissimus muscle in the thigh of the same animal and allowed them to be reinnervated by the muscle nerve. After three to six months, some 40% of the regenerating fibers were able to exhibit chemosensitivity (in addition to their natural mechanosensitivity) and electron microscopic examination showed that successful type I cell—axonal connections had been made and which were assumed to underlie the restoration of function. The question, however, has been raised (938) of how carotid body type I cells seemingly survived these graft experiments, yet were irreversibly and severely damaged at an ultrastructural level, by just 2 h of ischemia (597, 622) raising the possibility that damage to carotid body elements, other than, or as well as, type I cells, may be important for enabling the generation of afferent nerve action potentials. Notwithstanding this, much evidence points to the absolute requirement for a close juxtaposition of type I cells with afferent nerve endings and a presynaptic, transducer role for the type I cell appears the most likely possibility for this cell's function. Supporting this contention, isolated, petrosal neurons fail to exhibit any sensitivity to hypoxic hypoxia (29, 951) but can produce synaptic potentials and even action potentials when subjected to the same stimulus after they had been juxtaposed, via chemical synapses, to clusters of type I cells in an *in vitro*, coculture preparation (951).

Not all evidence is so supportive for a presynaptic role for type I cells and Donnelly and coworkers (218, 222) consider the generation of postsynaptic action potentials to occur, not via the presumed summation of synaptic depolarizing potentials in the nerve terminal consequent to neurotransmitter release from type I cells (352, 951), but instead via the slow modulation of channel noise associated with a persistent sodium current (735) located in the nerve terminal. These authors cite the ultrastructural findings of Gronblad (323) and Verna (862) who examined stimulus-induced, exocytotic fusion profiles in type I cells and,

independently, failed to observe such events occurring between the type I cell and nerve terminal. Such an assertion, if shown to be correct, would not reduce the significance of the type I cell in the transduction process but would, rather, relegate its role to that of a necessary, neuromodulator. In addition, the interpretation of data is further complicated by the detection of voltage-gated potassium channels and hyperpolarization-activated channels in carotid sinus nerve afferents (114) as some of these channels may themselves have intrinsic O_2 sensitivity. Resolution of the important issue of whether the chemoafferent nerve possesses any chemosensitivity *independent* of the carotid body that might affect the number and/or shape of action potentials may need to await future refinements in the technique of making electrophysiological recordings in the nerve terminal of an *in situ*, otherwise intact, carotid body which to date has only rarely been achieved (214, 352).

The type II cell

A single type II cell is intimately associated with a group of approximately 3 to 5 type I cells and is therefore found in relatively small numbers of less than 20% of the total cell count within a carotid body (194, 195). Type II cells and their processes do not completely encase the type I cells with which they are associated, thus permitting type I cells direct access, at certain points on their periphery, to other type I cells, to nerve endings, and to capillaries and so appearing to offer no additional diffusion barrier that might otherwise have had functional consequence. Whilst the proportion of type I and type II cells in rat and cat are similar, the type II cells of the cat appear to occupy twice the volume of the carotid body than they do in that rat (53). To date, no function has been ascribed to this difference.

Type II cell processes are also associated with nerve endings and fibers, at times completely enveloping nerve endings (196) many of which are of sympathetic, efferent origin (863). Again, no functional significance has been attributed to this. In histological appearance, type II cells lack the dense-core vesicles that are characteristic of type I cells and have slightly more flattened, kidney-shaped and chromatin-dense nuclei but are otherwise unremarkable in their appearance with no particular distinguishing features readily discernible under the microscope. They contain the expected distribution of organelles including mitochondria, moderately developed Golgi complexes, rough endoplasmic reticulum, and lysosomes (425). In cell culture, they are smaller than type I cells with a diameter, in the rabbit of between 5 to 7 μm (850) and are often spindle shaped in contrast to the ovoid shape of type I cells (923). It is worth noting that if the sinus nerve is cut, the type II cell, *in situ*, undergoes morphological change whilst the type I cell does not (1, 196, 373).

The major functional difference from type I cells, however, is that the type II cell appears more or less unexcitable (229, 671, 850, 923) containing, in the rabbit and rat, no discernible sodium or calcium currents and only a small, high threshold activated, slowly inactivating, outward K^+ current. Consequently perhaps, the type II cell does not respond electrophysiologically to hypoxia at stimulus levels that do alter ionic conductances in type I cells (541). Further, in contrast to type I cells of the rabbit, type II cells do not generate action potentials when depolarized (229).

Immunocytochemical studies have identified the presence, in the type II cell, of the calcium-binding, acidic protein, S-100 (461) and GFAP (628) as well as the intermediate filament, vimentin (425), all proteins associated with astrocytes, satellite cells of peripheral ganglia, or Schwann cells (163, 801). Taken together with the ultrastructural and electrophysiological evidence, it is not surprising that the type II cell has most often been ascribed a relatively undefined, supportive, glial-like role in the carotid body where it has been assumed to be a modified Schwann cell (380). However, although the proportion of type II to type I cells in the carotid body is relatively low, this proportion is significantly greater than the proportion of glial/Schwann cells to chromaffin cells in the adrenal medulla, which might support the

notion of a particular function for type II cells, either in the transduction process or in the provision of some other service not required, at least to the same extent, in the adrenal medulla. In this respect, it is worth noting that the mean intracellular Ca^{2+} concentration of isolated type I cells in culture is *ca*. 10 nM greater than that of type I cells maintained in clusters where type II cells are likely to remain in similar intimate contact with type I cells as seen *in vivo* and also that very high (> 200 nM), nonphysiological, intracellular concentrations of Ca^{2+} were only observable in isolated type I cells (416). Similar differences between clusters and isolated type I cells regarding their intracellular K^+ concentrations and pH have also been noted (666, 949), indicating perhaps a role for type II cells in maintaining ionic balance in clusters of type I cells.

The embryological origin of type II cells has been examined in mice where the generation of neural precursors of the peripheral and central nervous systems is under the control of transcription factors including *Mash1, Phox2a, and Phox2ba*, mammalian homologues of the *Drosophila achaete-scute (asc)* , complex. *Mash1* is essential for the development of sympathetic ganglia from uncommitted neural crest cells and *Mash1* null, mutant mice fail to survive post-natally as a consequence. However, such null mice, although able to develop a normal carotid body primordium, *in utero*, exhibited a failure to develop, specifically, type I but not type II cells within the carotid body, which subsequently exhibited a significant hypoplasia at birth (426). As such, this provides additional support for a glial-like role for type II cells (326) but, further, suggests a dual origin for the embryonic development of the carotid body with a requirement for neural cell lineages originating from the superior cervical ganglion for the development of type I cells and a different, neural crest-derived, ectomesenchymal cell lineage, from the wall of the third arch artery, being responsible for type II cell development (426). The possibility remains, however, that the absence of *Mash1* prevents differentiation of type II, mesenchymal cells into type I cells but this would then appear to require the presence of a functional sympathetic nervous system (426).

Role of the type II cell—It is now widely appreciated that glial cells are important functional elements of neuronal systems, expressing Ca^{2+} signaling responses to neurotransmitter stimulation (856) and it is therefore not surprising that the type II cell may turn out, with time, to have a larger role to play in the full expression of chemosensitivity and respiratory plasticity than has usually been presumed. Thus, whilst it has been confirmed that the type II cell is relatively unexcited by membrane depolarization (923), it was also shown, by these same authors, that $P2Y_2$ receptors were localized on the type II cell plasma membrane and it was suggested that the type II cell may therefore be a site of action for stimulus activated ATP release from type I cells, for example, during hypoxia. Activation of the G-protein-coupled $P2Y_2$ purinoceptor in identified type II cells, triggered Ca^{2+} release from internal stores that were shown to have an absolute requirement for replenishment from the extracellular site (923). By analogy to glial cells (856), it was suggested that Ca^{2+} signaling in type II cells might serve a paracrine role in the carotid body. This may be via the release from type II cells of neuroactive substances, including ATP, acting to stimulate afferent endings directly or by Ca^{2+}-dependent alterations in type II cell K^+ conductance leading to modification of K^+ buffering and hence neuronal excitability in the vicinity of type I cells and nerve endings (923). It is worth noting here that these same authors have described the presence of $P2Y_1$ receptors on type I cells (924). In many ways, this hypothesis has a great number of similarities to earlier ones in which the type II cell was also ascribed a modulatory role in chemotransduction (52, 420, 663), but via type I cell derived ACh rather than ATP. Further studies may be warranted using models in which the type I and type II cells remain in natural contact with each other to establish the true functional role of the type II cell in chemotransduction.

In addition to growth and dilation of the carotid body vasculature (367, 504, 505), type I cell hyperplasia has long been established as a major factor of the carotid body hypertrophy observable during chronic hypoxia (43, 568, 877), but the cellular and molecular basis of this process was unknown. Therefore, perhaps the most intriguing role suggested, to date, for type II cells is that of neural stem cells acting, via proliferation and differentiation, as progenitors of type I cells during periods of chronic hypoxia (671). This is particularly relevant given the limited mitogenic potential in type I cells, at least in the absence of nerve growth factors and hypoxia (629, 671). Thus, in the first demonstration of the potential for adult peripheral nervous system neural crest-derived tissue to exhibit pluripotent neurogenesis, Pardal et al. (671) showed that tyrosine hydroxylase positive (TH+) type I cells almost doubled in number within five to six days after application of chronic hypoxia to young adult and old mice and did so in association with an increase in replicating DNA. This proliferation was reversible upon return of animals to normoxia when approximately half of the carotid body now consisted of retained, newly formed cells. In an *in vitro* study of carotid body neurosphere formation, these authors were able to estimate that a single adult rat carotid body may contain some 200 to 300 stem cells with the potential, observed also in other neural crest-derived stem cells, to differentiate into multiple cell types; in this case type I cells and smooth muscle cells. The type II cell as the source of the progenitor cells was indicated by the observation of a, reversible, reduction in GFAP positive cells to < 10% of the normoxic population during *in vivo* exposure to chronic hypoxia, which was negatively correlated with the increase of TH+ cells. This was subsequently confirmed by a cell fate mapping study in transgenic mice (671) with each, normally quiescent, GFAP+ type II cell shown to be able to generate clonal, pluripotent, and chemoreceptive neurospheres of type I cells upon stimulation, via an intermediate GFAP−/nestin+ form. Thus, the carotid body exhibits a remarkable plasticity in the face of hypoxic insult and the type II cell appears central to this. Finally, it is worth noting that, in a study of human carotid body ultrastructure following pathological chronic hypoxia, a *proliferation* rather than a decrease in type II cells was observed that was associated with type I cell compression (364) and as such needs to be reconciled with the more recent animal studies in conjunction with the progression toward utilization of the carotid body in stem cell therapy (44, 244, 546, 583, 932, 933).

Innervation and Communications of the Carotid Body

Afferent innervation

The afferent neurons have their cell bodies in the petrosal ganglion, which is an anatomically distinct structure in the cat and rabbit, but is part of a larger complex with the jugular and nodose ganglia in the rat. The central terminations of these afferent fibers lie in the commissural or medial subnuclei of the nucleus tractus solitarius (68, 157, 185, 276, 392, 554, 668), a subcomponent of the dorsal respiratory group respiratory neurons in the brainstem and convey sensory information regarding cardiorespiratory homeostasis (421) in the form of graded action potential frequencies in fibers of the carotid sinus branch of the IXth cranial (glossopharyngeal) nerve.

In the rat, the carotid sinus nerve arises from the GPN at about 0.5 to 1.0 mm distal to the petrosal ganglion and is most often observable as a single bundle of axons of between 1.3 to 2.9 mm length and with an elliptical cross section having major and minor axis diameters of 77 and 46 μm, respectively (562). In the human, the carotid sinus nerve is approximately 2 9 mm in length, ranging between 15 and 50 mm (838). Both myelinated (A) and unmyelinated fibers (C) are found in the carotid sinus nerve (189, 190, 259) and the conduction velocities for these two types of fibers exhibit the expected distinction related to the degree of myelination, with A fibers of the cat having velocities between 4 and 53 m/s with a median value of 16 m/s, whilst the C fiber velocities varied only between 0.5 and 2.0 m/s (272).

Numerically, the C fibers are in the majority with around two to five times as many C fibers in the carotid sinus nerve as there are A fibers (190, 259). The A fiber population contains afferents of carotid body chemoreceptor and carotid sinus baroreceptor origin (16, 376) with approximately two-thirds of this population being chemoafferent (272). Given that the cat carotid sinus nerve has been estimated to contain some 600 to 700 A fibers (190, 259) this would predict that there should be around 400 to 450 chemoafferents in the nerve. Fidone and Sato (272) also measured a range of A fiber diameters in the carotid sinus nerve of 1 to 11 μm, with the majority being below 5 μm, and used data from Eyzaguirre and Uchizono (259) to calculate that A fiber baroreceptor afferents were most likely constrained to a smaller range of diameters of between 3 and 5 μm making the largest and smallest myelinated fibers in the carotid sinus nerve to most likely be chemoafferent in nature.

Around 54% of the C fiber population of the cat carotid sinus nerve is of autonomic, nonchemoreceptor and nonbaroreceptor origin with the remaining fibers being approximately two-thirds of baroreceptor origin (272), thus neatly reversing the ratio observed in A fibers and making just 15% of the C fibers of chemoafferent origin. The carotid sinus nerve has one blood vessel supplying it with a mean diameter of 12.4 μm and which arises from vessels supplying the GPN and ends at a venule on the rostral surface of the carotid body (562). Also at the rostral pole of the carotid body, the carotid sinus nerve divides into multiple bundles that mostly enter the organ to make contact with parenchymal cells. In some cases, the nerve fibers can appear to wholly encase a single type I cell (372). At the point of entry into the carotid body, the afferent fibers are now mostly nonmyelinated but covered in Schwann cell cytoplasm, which is gradually lost as the neurons enter the connective tissue of the organ in folds of type II cell cytoplasm. The small remaining fibers that do not enter the carotid body merge with sympathetic fibers from the superior cervical ganglion and can innervate the carotid sinus, joining there other baroreceptor afferents that had traversed through the carotid body.

The chemosensory nerve endings of the carotid body have a variable morphology in terms of their shape and size (561). Electron microscopy has revealed both bouton and calyx terminal structures that make variable amounts of synaptic contact of between 1 and 10 μm^2 with the type I cell surface (623) characterized by electron dense thickenings of less than 0.5 μm length on both sides of a synapse which, in the cat, is relatively constant at around 30 nm in width (371). According to McDonald and Mitchell (565) up to half of the type I cell population is not innervated, and so for the remaining type I cells to receive innervation, each nerve axon entering the carotid body must undergo extensive branching. This they do, with a single nerve fiber branching to make contact, or close relation, with 10 to 20 different type I cells (458, 565), or more (842). The contacts made by a single fiber can be of varying structure on different type I cells. A single type I cell may synapse with one to two different neurons (335, 561). The nerve terminals contain mitochondria that may be smaller and more electron dense than those found in type I cells (858) and differing amounts of clear core micro vesicles [e.g., ca. 34 vesicles/μm^2 of cytoplasm in the monkey; (335)] ranging between 40 and 70 nm in diameter. Some, very few, may possess also some dense-core microvesicles that are approximately half the diameter of those characteristically found in the type I cells (858), but all are otherwise unremarkable in appearance.

The very existence of clear vesicles in the nerve terminals (85, 195, 523) suggested that these nerve endings may be efferent and not afferent to the type I cell and thus challenged de Castro's original postulate of the sensory nature of these fibers. This led to a repeat of de Castro's intracranial carotid sinus nerve section experiments, which generated results apparently supporting an efferent innervation of type I cells (2, 73, 79, 85, 449). Additional support for this idea came from the finding that electrical stimulation of the carotid sinus nerve could depress basal and hypoxia-stimulated discharge frequency in chemoafferents

(613, 763, 764). This efferent nerve hypothesis required the afferent chemosensory fibers to be located elsewhere in the carotid body and the suggestion was made (73) that they may be the free nerve endings located adjacent to type II cells as described by de Kock and Dunn (195) with their cell bodies located in the brainstem. The type I cell would then become a modulator element rather than a transducer element. The existence of free nerve endings could not, however, be confirmed in a number of other studies (373, 458, 561, 565, 623) and de Castro's original assertion and findings, following nerve section, were soon replicated (373, 566, 623).

The afferent nature of the fibers terminating adjacent to type I cells was further confirmed by the demonstration of axoplasmic flow of radiolabelled amino acids in an anterograde direction from the petrosal ganglion to the carotid body, where some 60% to 90% of nerve terminals apposed to type I cells were shown to express the label just a few days after application (274, 789).

An "afferent" nerve synaptic morphology is also more likely to be observed at the nerve terminal: type I cell contacts of mice (452), guinea pigs (449, 456), and rabbits (857). The consequence of age should, however, also be considered as it appears that the carotid sinus nerve terminals within the carotid body may change their morphology between birth and adulthood acquiring, in the rabbit, a more afferent appearance, rich in mitochondria and relatively deplete of vesicles with maturity whilst appearing more "efferent-like" in the neonatal period (96). A similar maturation was reported in the rat, where the number of afferent-like endings but not efferent-like endings increased with developmental age up to 50 days postnatal (457). Whether these ultrastructural changes might underlie the postnatal maturation in hypoxia sensitivity of the carotid body (475) is not known.

The chemical content of the vesicles in the carotid sinus nerve terminals has been used to classify the endings into two distinct types. The first group have nerve endings rich in TH and can synthesise catecholamines, of which dopamine is the major final form, with those fewer endings containing noradrenaline attributable to a sympathetic nerve origin (33, 277). These catecholaminergic fibers make extensive synaptic, often calyciform, contact with type I cells. The second group contains neuropeptides, particularly substance P, tachykinin and calcitonin gene-related peptide (277, 481, 878), and also neuronal nitric oxide synthase (nNOS) (381, 720, 878, 882, 885) and choline acetyltransferase (878). Vasoactive intestinal polypeptide (VIP) and neuropeptide Y (NPY) have also been localized to nerve terminals in the carotid body but are attributed to a sympathetic neural rather than a chemoreceptor origin (396). The chemoreceptor-related peptides are colocalized within dense-core vesicles in the nerve terminals of the small (submicron), unmyelinated fibers of the carotid sinus nerve (483). These neurons, however, appear less likely to make direct synaptic contact with type I cells and terminate near type II cell processes suggesting, perhaps, a lesser or more "sluggish" involvement in transmission of chemosensitive information (483, 843) although unmyelinated fibers are known to convey such information (272). The two groups of afferents are also distinguished by the anatomical location of their cell bodies in the petrosal ganglion and their terminals in the NTS, with the TH+ neurons localized to the distal pole of the ganglion (277, 481) and the commissural nucleus of the NTS (554, 841).

Reciprocal synapses and gap junctions

Reciprocal synapses have also been observed with both pre and postsynaptic morphologies observed at multiple sites between a single afferent fiber and a single type I cell in the rat (373, 458, 459, 565) and in the cat (789). In addition, reciprocal synapses have been observed between type I cells (565). Based largely, but not solely on the anatomical observations, a number of authors have proposed functional hypotheses for the reciprocal synapse (586). Thus, McDonald and Mitchell (565, 566) suggested that, whilst the nerve

terminals adjacent to type I cells were almost certainly afferent, the nerve terminal itself was the transducing element of the carotid body, with the type IA (innervated) cell playing the role of an inter neuron acting, via reciprocal synapses between type IA and IB cells and between type IA and afferent nerves, in an inhibitory feedback loop to modulate chemosensitivity. These authors also made the interesting, although unrepeated, observation that the degree of feedback inhibition was significantly greater at levels of Po_2 above arterial normoxia and that the normal hyperbolic shape of the response curve to hypoxia could be made more linear by block of the feedback loop with phenoxybenzamine (586). Although they themselves state this may be an oversimplification, it is one of very few examples where the *shape* of the chemoreceptor response curve to hypoxia is considered.

Krammer (467) modified these ideas slightly to propose that the type IB cell be the chemosensitive element in the interacting anatomical arrangement proposed by McDonald and Mitchell (565) acting, when stimulated, to inhibit the type IA, inhibitory inter neuron and hence increase the afferent nerve discharge. Finally, Osborne and Butler (657) proposed, instead, that the reciprocal arrangement between type I cell and afferent nerve ending is one of a positive feedback loop with the sensory nerve being normally endogenously active but inhibited by O_2-dependent neurotransmission (presumed to be dopamine) from type I cells. In hypoxia, the release of inhibition from type I cells would then lead to increased afferent nerve discharge *and* a release of inhibitory (presumed ACh) transmitter from efferent nerve endings that would further suppress the type I cell. Although now disregarded, this hypothesis does have some correlate with the suggestion of Donnelly (218) that action potential generation in the sensory nerve is more likely an endogenous attribute of the nerve terminal, that can be modulated by the type I cell. It should be borne in mind that although reciprocal synapses appear only to make up less than 3% of all synapses in the rat and cat (561), thus making their functional role in chemtransduction somewhat less obvious, the idea that the type I cell may, *in vivo*, be acting in a neuromodulatory role cannot be entirely ruled out solely by studies performed on isolated cells *in vitro*.

An additional consideration for any hypothesis based upon the structure functions of the whole carotid body *in situ*, is the observation that the type I cell, in the rat, possesses connexons making up gap junctions that enable direct, bidirectional electrical communication between adjacent type I cells, as well as between type I cells and type II cells (62, 250). Additionally, some type I: nerve ending contacts appear also to be gap junctions (460) although the ultra-structural observation of the presence of such close (2 nm) appositions of membranes, between these cell types, has been considered a possible artifact of a modified freeze substitution method for preparation of ultrathin sections of the carotid body (460).

Gap junctional connections are also evidenced by the observation of dye transfer occurring through these routes between cells (including between type I cell and nerve ending) and by direct measure of electrical coupling between cells (150, 249, 415) which suggest that the passage of current is unidirectional from type I cell to nerve ending. Such physiological assessment of coupling often reveals a higher frequency of coupled cells than observed microscopically, where they may occur only between 3% of type I cells (561). Connexin 43 appears to be the main protein involved in these structures being observable both in cultured cells (4) and constitutively in the intact organ (146). In addition, connexin 32 has also been identified in cultured cells (628). Natural stimuli, including hypoxia and hypercapnia, as well as several putative neurotransmitters, uncoupled most type I cells tested (5, 6) but could increase the coupling coefficient between type I cell and nerve ending. The transcript and protein levels of connexin 43 in both type I cells and petrosal ganglion cells is measurably increased by two weeks of hypobaric hypoxia (146) and, as such, may be part of the adaptation to chronic hypoxia (see later sections). Such (un) coupling between parenchymal

cells of the carotid body seems to be aimed at facilitating the chemotransduction process by enabling electrotonic spread of currents in addition to chemical transmission and thus enabling both augmented chemosensitivity and cell synchronization to allow the carotid body to act as a functional syncytium.

The consequences of losing the various chemical and electrical communication pathways between type I cells during their isolation for *in vitro* experiments, may account, at least in part, for some of the differences observed between the various preparations studied. Such considerations are often overlooked in many hypotheses of chemoreception that are primarily focused on the transduction processes, yet the full expression of chemosensitivity depends, ultimately, on the generation of a postsynaptic action potential in the sensory nerve. It remains to be seen whether the complex interactions between the various cellular components of the carotid body and its specific innervation, might play a role in a form of elementary signal processing, perhaps via postsynaptic summation or action potential collision. To date, there have been very few studies directed at this question and chemoreceptor discharge is most often reported simply as a mean level of frequency, although time-related information does exist, as reported *in vivo* (480).

Efferent innervation

The efferent innervation arises primarily from the sympathetic fibers originating from the superior cervical ganglion constituting the ganglioglomerular nerve (308). Nerve endings with an efferent morphology make up only around 10% of the total number of synapses (458, 459, 561). A serial section reconstruction of the rat carotid body ultrastructure led Kondo (458, 459) to state that *all* nerve endings adjacent to type I cells were postsynaptic and that many of the apparent efferent endings were fibers which had also exhibited postsynaptic endings, for instance, on other type I cells. In addition, to the sympathetic innervation from the ganglioglomerular nerve, there are also a small number (<5% of total) of unmyelinated fibers of nonsympathetic origin (81, 458, 566, 615) that lie within the glossopharyngeal and carotid sinus nerves that are sensitive to variations in blood gas tensions (83) and whose stimulation can decrease chemoafferent discharge frequency (252, 614, 615). The soma of these efferents are not found in the central nervous system (CNS) (424) but lie instead within paraganglia of the GPN at its border with the carotid sinus nerve as well as more distally along the GPN (129, 130, 878, 882).

The efferent endings have bouton and calyx formations and so cannot be distinguished from afferent endings on these morphological terms but might be distinguished in terms of the size and number of their clear core vesicles (565) with up to five times more vesicles per μm^2 in the motor endings of the rat. Interestingly, it has been stated (371) that much of the controversy regarding the nature of the nerve terminals in the carotid body might not have been occurred if the animals studied by Biscoe and colleagues (79) had been rats instead of cats, as the former rarely show clear core or dense-core vesicles in their nerve terminals whilst such phenotypes are much more abundant in the cat (561).

Efferent innervation may best be considered as a modulating influence affecting carotid body chemosensitivity largely (256, 560, 732), but not solely (615, 634, 635, 764, 863) via a modulation of carotid body blood flow. The effect of sympathetic nerve stimulation upon carotid body afferent discharge is most often excitatory although it can be variable (256, 288, 555, 585, 635) presumably due to the activation and location of specific adrenoceptor subtypes within the carotid body (32). Interestingly, however, if the carotid body is stimulated naturally, *in vivo*, ganglioglomerular nerve activity is reflexly increased leading to an *inhibition* of chemoafferent discharge (549). The inhibitory effects arising from increased ganglioglomerular nerve activity (efferent inhibition) is likely mediated by NO generated by neuronal NO synthase [nNOS; (131) and see later section on transmitters].

Vascular Supply, Blood Flow and Metabolism

Vascular supply

The carotid body is an extremely vascular organ (192) and has a characteristically high blood flow for its volume. Whilst this is often considered a functional characteristic of a chemoreceptor, it has only been verified for the carotid body and therefore may not be a prerequisite for its transducing function. In most species, the arterial supply to the organ is derived from larger, nearby arteries, including branches of the internal and external carotid, the occiptal and the pharyngeal arteries, with the precise origin of the blood supply showing some interindividual variation as well as being species dependent (158, 159, 564, 775).

The artery supplying the carotid body has a flexible wall, with an unusually thin media of smooth muscle cells and so can be stretched without difficulty by normal blood pressure oscillations (192). The main arterial supply soon divides into second-order arteries and terminal arterioles that are richly innervated by postganglionic sympathetic fibers from the superior cervical ganglion (85, 566, 861) and by parasympathetic fibers originating from intraglomic ganglion cells (129, 130, 561). The larger blood vessels have a relatively greater parasympathetic innervation, whilst the smaller vessels have a predominantly sympathetic innervation. In addition to this autonomic innervation, an observation, based upon electron microscopic observation of mouse carotid body (238), suggests that the type I cells may themselves be involved in the regulation of the vascular tone in the carotid body via close, <0.1 mm, type I cell-myocyte contacts. These may enable a physiological interaction between arterioles and the type I cells in a manner analogous to the vascular neuromuscular junctions of sympathetic nerve terminals and which has anatomical correlate in the so called glomus cell-smooth muscle (g-s) connections found in the carotid labyrinth of amphibia (399, 488).

Blood is distributed to chemoreceptor tissues through an intricate network of vessels and each glomerulus is supplied by two main forms of interconnecting capillaries (192, 562–564). The first type have a large diameter of between 8 and 28 μm, possess a fenestrated endothelium and are described as “tortuous” in their pathway through the carotid body, where they may turn back on themselves and often anastomose with other similar capillaries. Importantly, they make close (within 3 μm) contact with type I cells. The second type of capillaries are fewer in number and more typically narrow at between 6 and 12 μm diameter. These possess no fenestrations, are enveloped by pericytes and are often extra-glomerular in location, making little direct contact with type I cells. Blood is expelled from the organ via a prominent venous plexus on the surface of the organ and from there into the internal or external jugular veins.

Blood flow

The total blood flow of the carotid body has been estimated using direct measures of the volume of the venous drainage, hydrogen clearance, and microsphere methods and an agreed value of between 1000 and 2000 ml min^{-1} 100 g^{-1} has been reported across a number of species (14, 60, 160, 187). Given the low wet weight of tissue, it is easily calculated that this is a significant flow of greater than 10 times that observed per unit mass in the human cerebral circulation. Even this value may be an underestimate due to the inaccuracy of measuring the organ’s wet weight by gravimetric means (160). Whatever the precise value for total blood flow, at normal blood gas tensions these determinations may greatly exaggerate the flow directed toward transducing elements of the organ as much of the total flow may pass through arteriovenous shunts under the, presumed, control of precapillary sphincteric smooth muscle (563, 564). The existence of such shunts is, however, contested and anastomosing vessels > 20 mm in diameter were rarely observed

when tissues were examined after being perfusion fixed and serially sectioned (186). This however, contradicts the observation that latex microspheres with diameters between 20 and 50 μm could pass through the carotid body without lodging in its capillaries (636) and resolution of this anomaly is awaited.

The carotid body of the cat increases in size by almost 4-fold between fetal, neonatal, and adult form, yet the relative masses of its vascular and extravascular compartments shows a matched growth, so that the proportion of small blood vessels (5–12 mm diameter) supplying the type I and type II cells, remains a constant ratio of the total vascular volume and at 5% to 7% of the entire organ volume (161, 162). This suggests that during development blood delivery to the transducing elements is maintained and consequently, any alterations in sensitivity to particular blood-borne stimuli most likely occurs as a consequence of alterations in the O_2-related affinity of cellular protein sensors and/or downstream transducing elements in type I cells. In addition, across species the proportion of small vessel volume to total organ volume remained remarkably constant at between 5% to 6%, although the total organ volume varied more than 4-fold from *ca*. 0.045 mm^3 in rat, to 0.12 mm^3 in the nonprimate monkey and up to 0.200 mm^3 in cat (162). Thus, the vascular anatomy of the carotid body appears to be relatively fixed, at least in terms of volume, across species and during development, which may suppose a functional role for this arrangement. This may be both in the acute chemotransduction process and/or in facilitating the proliferation and differentiation of progenitor type II cells during sustained hypoxia (671), with the arrangement acting in both cases to determine tissue levels of Po_2 and hence the levels of stimulus intensity and thus mitogenic and angiogenic factors including vascular endothelial growth factor (VEGF) (835), bFGF (629), and endothelin-1 (ET-1) (662). More recently, it has been demonstrated that a 40% reduction of rabbit carotid body blood flow, from a mean control value of 1203 ml min^{-1} 100 g^{-1}, can augment chemosensitivity via an approximately 2-fold decrease in NO signaling and a 2-fold increase in angiotensin II signaling (207).

O_2 consumption

Values of carotid body resting O_2 consumption have been variable, with reports *in vivo* and *in vitro* ranging from as low as 0.3 ml 100 g^{-1} min^{-1} to as high as 9 ml 100 g^{-1} min^{-1} (113, 187, 264, 517, 633, 796, 904). This variability may be attributed, in part, to technical differences between experimental approaches, but also to the estimation of carotid body weight, the coefficient chosen for O_2 solubility and the complications arising from potential plasma skimming and the possible presence of arteriovenous anastomoses. Given these considerations, a value of between 1.0 and 1.5 ml min^{-1} 100 g^{-1}, may be a best estimate for resting O_2 consumption both for *in vivo* and *in vitro* carotid preparations (642, 643, 903). This value for the *whole organ* is similar to many other tissues and thus the high, arterial-like, venous Po_2 levels arising from the carotid body appears to reflect a flow in relative excess to metabolic demand such that only < 3% of the delivered O_2 is consumed per minute (187).

However, the measurements of total organ O_2 consumption, described above, may be misleading regarding any functional specialization in the carotid body, as they do not reveal the metabolic rate of the sensory cells themselves. Duchen and Biscoe (227), however, demonstrated that the resting O_2 consumption of individual type I cells was actually significantly *higher* than that of individual cells from other tissues, for example, dorsal root ganglion or adrenal medulla. They calculated that the type I cell must be operating near its Vo_2 max by comparing the fluoro-metric determination of NAD(P)H/NAD(P) auto fluorescence ratios, during stimulation with either the electron transport chain inhibitor, cyanide (CN^-) or the oxidative phosphorylation uncoupling agent, carbonyl cyanide-*p*-

trifluoromethoxy-phenylhydrazone (FCCP) to decrease or increase O_2 consumption to its minimum or maximum, respectively, The reasons for this high metabolic rate are not known, but these authors speculated that it may be due to a particularly "leaky" mitochondrial inner membrane or by the presence of a mitochondrial cytochrome oxidase with a low affinity for O_2, both of which would induce a high respiratory rate to maintain the mitochondrial membrane potential, or by an unusually high ATP consumption at rest. As oxygen is needed to provide the energy necessary to sustain the chemosensory responses during hypoxia, at some point, with increasing hypoxia, failure to sustain aerobic metabolism must occur. The high blood flow may function therefore as a reserve to ensure adequate supply of O_2 to active cells during hypoxia with the high metabolic rate of type I cells functioning to ensure the ability to sense physiological falls in Po_2.

Central to these functional considerations is the *tissue* level of Po_2, which is much lower than the Po_2 of venous blood draining the organ (12–14, 903) and is, in part, determined by the tissue blood flow. This local blood flow has been estimated in the monkey, cat and rat, to be between 30 and 100 ml 100 g^{-1} min^{-1} (162) and accounts for approximately 3% of the total blood flow, an estimate that concords with the observation that electrical stimulation of the preganglionic sympathetic nerves to the cat carotid body could decrease total blood flow to less than 5 ml/min, or just 8% of the prestimulated value without change in either tissue blood flow or Po_2 (14). Much of the carotid blood flow, in normal conditions, must therefore bypass the chemoreceptor tissue, thus potentially obscuring the relation between total blood flow, O_2 consumption and chemoafferent nerve discharge.

Thus, whilst in some studies, hypoxia and hypercapnia increased total blood flow and chemoafferent discharge, this was reported to occur with a decrease in overall O_2 consumption, yet decreases in total blood flow induced either by hypotension or sympathetic nerve stimulation could also reduce O_2 consumption but here only sympathetic nerve stimulation was able to augment chemoafferent discharge (731, 732). The only plausible explanation was that there must be a degree of stimulus-dependent control of the relatively low, local tissue blood flow and hence local Po_2 (11, 379, 502), but the only chemostimulant shown to be capable of decreasing local flow and thus local Po_2 is hypoxia (7, 11).

However, whilst other studies have also reported unchanged, or even decreased, O_2 consumption during hypoxia (113, 607, 904), more recently, the electrode-based methodology used by these studies has been questioned, such that it is now generally accepted that O_2 consumption increases significantly during hypoxia as might be expected by the concomitant increase in carotid body activity. Thus, exposure of an *in vitro* carotid body preparation to an hypoxia stimulus resulted in either a 44% increase in glucose consumption (from a basal value of around 120 n mol g^{-1} min^{-1}) and oxidation, measured using the 2-deoxyglucose technique (642, 643) or an increase in the rate of O_2 disappearance using a noninvasive, phosphorescence quenching method (757). In the latter study, a triphasic O_2 disappearance curve during stop-flow hypoxia was noted; O_2 disappearance being relatively low and linear as tissue Po_2 was made to fall from normoxia toward 10 mmHg, before it almost doubled in rate as Po_2 fell further toward 3 mHg after which the O_2 disappearance progressively slowed as Po_2 fell toward 0 mmHg. Chemoafferent discharge frequency was closely related to these changes, rising little during the fall in Po_2 toward 10 mmHg after which it rose steeply to a peak before falling again as Po_2 fell below 3 mmHg. These results most likely reflect the central importance of O_2 metabolism for the expression of hypoxia chemosensitivity, and hint strongly at its role, not only for the initiation of the transduction mechanism but also for the provision of energy for the increased cellular activity.

Physiological Consequences of Carotid Body Resection

A ventilatory response to hypoxia is regained with time in a number of species when the carotid bodies are absent or nonfunctional, thus signifying a redundancy in the normal respiratory control system and demonstrating the relative importance of this reflex. In humans, (Fig. 2A) extracarotid body chemoreceptor tissues appears to play no role in the acute, respiratory response to hypoxia as this reflex is entirely abolished following bilateral resection of the carotid bodies (384–387) or by local anesthetic block of the glossopharyngeal nerves (327). With time, however, an, albeit reduced, reflex sensitivity to hypoxia has been shown to return in humans in whom healthy carotid bodies had been previously resected for the aim of relieving the symptoms of respiratory diseases, such as asthma and chronic obstructive pulmonary disease (COPD) (906). The time required for this partial restoration of function can be as little as 6 to 10 weeks after resection in some studies (383) but appears to be greater than eight years in other studies (387, 545, 814) although the time course in these latter studies is limited by the time at which measurements could be taken. Whilst this recovery of function may occur via a slow augmentation in the sensitivity of aortic chemoreceptors to blood gas stimuli, the possibility of sinus nerve afferent regeneration remains a significant alternative possibility, at least in the longer time course measurements. Respiratory disease itself may alter chemosensitivity (311) and in, otherwise healthy, patients for whom bilateral carotid body tumor resection was performed, normocapnic hypoxia ventilatory sensitivity remained absent for periods of between 1 and 21 years (833), which may suggest an absolute requirement, in humans, for a degree of stimulation by hypercapnia for the observation of peripheral hypoxia sensitivity in the absence of carotid bodies (387, 814).

In most other, nonhuman, mammals a return to at least partial, reflex hypoxic sensitivity occurs generally much quicker following bilateral carotid sinus nerve section with the immediate hypoventilation and associated arterial hypercapnia at rest becoming restored toward *ca.* half normal, within a few weeks in rats (552), cats (790), goats (665), and pigs (544) with the returning function ascribed to aortic chemoreceptors in the cats, goats, and pigs and to abdominal chemoreceptors in rats. Part of the restoration of hypoxic reflex was attributed to central reorganization of aortic body sensory afferents in cats (790). In ponies (87) the partial restoration of function, following bilateral sinus nerve section, began from just nine weeks but took up to two years to reach a steady value of *ca.* 30% to 40% of the presection sensitivity to hypoxia. In dogs, no functional recovery to hypoxia was found at three weeks following postsinus nerve section (749), which was attributed by these authors to a lack of aortic chemoreceptor plasticity in this species, but may, instead, reflect an incomplete reorganization of aortic or ventral plasticity within this relatively short time frame. It is worth noting that baroreceptor afferents, as well as chemoafferents, will be lost in any sinus nerve section, such that baroreflex sensitivity will be affected by such a procedure (834).

Sensing Hypoxia—The Stimulus

The functional role of the carotid body in homeostasis is almost entirely defined by its response to hypoxia. Whether this is or is not appropriate, it is unquestionable that this single stimulus has provided the main focus for study of chemotransduction mechanisms over the past 70 to 80 years since the early pioneering studies (872). In response to falls in arterial Po_2, the carotid body produces a characteristically nonlinear, graded chemoafferent discharge in the carotid sinus nerve (34, 80, 255, 389, 872). The response, *in vivo*, is rapid in onset, with a short latency of just 0.2 to 0.3 s and a time to peak of between 1.2 to 2.6 s (90, 711) although it may be fractionally slower in onset (0.3–0.8 s) and time to peak (1–5 s) in other studies, most likely as a consequence of differences in the methodology used for

applying the stimulus (90, 281). The time to peak can, however, be significantly slower in *in vitro* preparations, most likely due to diffusion limitations (255). The response is also usually non-adapting if the hypoxia is not too severe or sustained. If hypoxia is severe, a form of adaptation has been described where a lowered steady value of discharge occurs some minutes after a peak (255) and undershoots in discharge frequency upon the return to normoxia after prolonged hypoxia, have also been reported (281, 480). In most cases, discharge can be sustained for the duration of the hypoxia episode, provided the intensity is not too severe.

The stimulus-response curve has been described, over the physiological range and at a constant PCO_2, as a single exponential with an offset or a hyperbola (see Fig. 3), both *in vivo* and *in vitro* (253, 255, 388, 389, 693, 757). The basal discharge in the cat can be between 0.2 and 2.3 Hz (74) but may vary with fiber type: A-type fibers generally exhibit a higher discharge in normoxia than C fibers with the former having a mean between 2 and 5 Hz whilst the latter is much lower at between 0 and 2 Hz (272). The underlying mechanism and functional consequence of these two populations in the carotid body is not known but may reflect generalized differences between these two fiber types when subserving similar modalities in other sensory systems (398). In the rat, mean basal discharge of single fibers in normoxia is typically between 0.2 and 2 Hz both *in vivo* (870) and *in vitro* (70), but no studies to differentiate between fiber type has been attempted in this smaller species.

The basal (and peak) discharge frequencies in few or multifiber preparations depend entirely upon the number of fibers recorded and on fiber recruitment and consequently discharge frequencies are often normalized to a fixed peak value. Irrespective of species, or fiber type, single and few-fiber chemoafferent discharge rises gradually from basal levels in normoxia as Po_2 is reduced and then more rapidly, as sometimes observed by an inflection in the curve from *ca*. 70 mmHg to reach peak discharges of usually between 10 and 20 times basal at Pao_2 values between *ca*. 20 to 30 mmHg (255, 389, 617, 870). Although the peak discharge in cats (82, 493) may be somewhat higher than in other species, we cannot be certain whether this is due to a genuine species difference in O_2 sensitivity or to a different response of cats to anesthesia/paralytic agents (870). In addition, the *in vivo* absolute level of peak discharge may also be fiber type dependent (272) and different fibers appear to have different thresholds and peaks (80, 272, 388) with both the basal discharge and the inflection in the curves of different chemoreceptor fibers occurring over a Pao_2 range of 60 to 110 mmHg (80, 255, 389, 870). This may reflect variability in the affinity range of an oxygen sensor(s) or gradients in tissue Po_2 within the organ (Fig. 4).

Below Pao_2 levels of 20 to 30 mmHg, the *in vivo* increase in discharge frequency gradually reduces and may even fall in some instances (80), often concomitantly with the failure to maintain adequate systemic blood pressure. This failure to sustain discharge may be an indirect effect upon the hypoxic response due to “a fall in $PaCo_2$ due to poor perfusion of the lungs as the cardiac output fell” (388), or it may simply be an artifact of failing to trigger electronic counters accurately when action potential amplitude falls at high discharge frequencies. Such a reduction in discharge in the face of severe Po_2 is, however, also commonly observed *in vitro* where considerations of adequate blood flow are not relevant (255, 757) and when single fiber recordings of frequency are carefully quantified to account for reductions in action potential amplitude (P. Kumar, unpublished observations). Consequently, the full Pao_2 response of the carotid body can be fitted by a sigmoidal function, which is also positioned appropriately as an inverse function of the hemoglobin O_2-dissociation curve. Indeed, such a relation was implied by the linear relation between arterial O_2 content and discharge as described by von Euler et al. (872) and later replicated by Hornbein (388).

A chemoreceptor discharge frequency is maintained at high, nonphysiological levels of Pao_2 in excess of normoxia, albeit at lower frequency, and there appears to be no absolute threshold to the chemoreceptor response, with basal discharge rates of up to 1 Hz still observable at a Pao_2 > 600 mmHg (80). If, however, a threshold is defined as the Pao_2 at which discharge first rises, rather than when it first appears, then a value of around 190 mmHg can be taken (80) although this is variable and can be as high as 400 mmHg in some instances. Intriguingly, there is some evidence to show that when Po_2 is increased from normoxia to hyperoxia, a decrease in chemoreflex-mediated sympathetic tone can be observed in humans (772) demonstrating a functional role for the small, absolute discharge in normoxia. The discharge is aperiodic, the inter spike intervals being randomly distributed with respect to each other, but, *in vivo*, an underlying rhythm related to the respiratory frequency can be observed if discharge is summed over a number of respiratory cycles (480) that is due to natural fluctuations in blood gas tensions brought about by tidal breathing (931). Whilst a role for these oscillations in the matching of ventilation to whole body metabolic rate has been proposed (58, 926), it is generally not accepted that this is a tenable viewpoint (631).

Carotid body chemoreceptor responses to hypoxia, recorded *in vivo*, are most often correlated with arterial Po_2 levels whilst responses *in vitro*, are correlated with the superfusate or perfusate Po_2. However, nonendothelial cells are exposed to *tissue* Po_2, which is normally lower than arterial Po_2. Any discussion regarding the natural stimuli of carotid body chemosensors, must therefore take into account the specific tissue stimulus intensity to which the organ is responding *in vivo* and which is likely determined by its high total blood flow, small arterial-venous Po_2 difference and rich efferent innervation. Carotid body tissue Po_2 in healthy mammals is a variable that depends, as its source, upon the inspired level of O_2 that, naturally, depends principally on the altitude.

In addition, the O_2 capacity of blood, which is largely determined by the hemoglobin concentration, the specific vascular structure of the organ and its blood flow, local diffusion concentrations, and finally the rate of cellular O_2 consumption all contribute to the specific tissue stimulus intensity. In addition, the contribution of these variables may vary throughout a single organ giving rise to a marked heterogeneity of tissue O_2 that can vary, in normoxaemia, between arterial levels and anoxia (519). Thus, at least in the acute time frame of seconds that the carotid body responds to a change in arterial Po_2, the tissue Po_2 and hence the stimulus presented to the type I cell, is determined almost exclusively by a balance between local vasodilator and constrictor influences and the O_2 consumption. If systemic hypoxia is maintained for hours or days, either physiologically in residents or visitors to altitude or, pathologically, by the deleterious consequences of cardiopulmonary disease, the tissue Po_2 may be altered independently of Pao_2 by vascular rearrangement, alteration in autonomic outflow, and/or changes in O_2 transport capacity.

With regard to chemosensing mechanisms, it thus becomes critical to determine whether tissue Po_2 in the carotid body is held relatively high or relatively low, compared to other nonchemosensitive, tissues, during acute systemic hypoxia. If held relatively high, then the chemosensor needs to be particularly sensitive to moderate falls in tissue Po_2, but if the tissue Po_2 is held relatively low, then these sensors need not express such exquisite sensitivity but their cells should perhaps be able to demonstrate the ability to maintain an increased metabolism despite reduced O_2 delivery. Conflicting data regarding the tissue level of Po_2 in the carotid body has been reported. Thus, Acker and coworkers (12) obtained, using O_2 electrodes, remarkably low carotid body tissue Po_2 values of 7 to 20 mmHg at a Pao_2 of 100 mmHg, while the relatively sharper electrodes of Whalen and Nair (902, 905) revealed higher values of between 40 and 70 mmHg throughout the organ. An optical, noninvasive phosphorescence quenching technique, avoiding tissue damage,

obtained a microvascular Po_2 value of 23 mmHg with equilibrated saline perfusion *in vitro* (757), but with blood perfusion *in vivo*, at a Pao_2 of 103 mmHg, this value increased to a relatively normal to high, microvascular Po_2 of 53 mmHg (502). *In vivo*, microvascular Po_2 fell with Pao_2 from normoxia but the rate of fall of microvascular Po_2 was increased as Pao_2 fell below 50 mmHg such that the arterial-microvascular difference was more than halved between a Pao_2 of 100 mmHg and 20 to 30 mmHg (Fig. 5). Some of this must be due to the shape of the Hb-O_2 dissociation curve and consequently the relation between Pao_2 and discharge must partly reflect this relation, although the shape of this relation is relatively unchanged when blood-free perfusion media is used, suggesting an inherent a linearity in the response curve. When chemoafferent discharge was correlated with intravascular Po_2 both *in vitro* and *in vivo*, discharge rose significantly only below 15 to 20 mmHg, peaking at 3 mmHg *in vitro* whilst *in vivo*, commensurate with the higher resting values of microvascular Po_2, chemodischarge rose from a microvascular Po_2 of 50 mmHg sharply toward a peak at the lowest recorded values of 20 mmHg (502, 757). An estimate of a microvascular P_{50} from their data would be around 30 mmHg. Clearly, there are some discrepancies regarding tissue Po_2 values in the carotid body that need to be resolved, but the present consensus is that the carotid body has a normal to high tissue Po_2, which suggests that the organ does not possess any particular specialization(s) that sets its tissue Po_2 differently from other tissues of comparable metabolic rate. It could also be used to suggest that the particularly high sensitivity to arterial Po_2 of the carotid body may be dependent upon a sensing mechanism of low O_2 affinity.

In addition, the maintenance of tissue Po_2 at high to normal levels may be necessary to enable the > 40% increase in carotid body metabolism that can occur during hypoxia (296, 298) and which has been ascribed largely to an increased activity of the Na^+-K^+ ATPase subsequent to cellular depolarization (643). Anaerobic metabolism would ensue when tissue Po_2 falls below 10 mmHg, which may account for the failure to sustain steady-state chemoreceptor afferent discharge *in vivo*, during severe hypoxia. The carotid body, therefore, appears unable to operate normally in these extremely low levels of tissue O_2, with physiological and pathological responses that are not part of the natural chemotransduction process being initiated. Thus, the presence of O_2 appears central to the neural response to hypoxia, presumably to enable the continued generation of ATP from oxidative phosphorylation, as implied from the impaired responses to hypoxia observed in the presence of electron transport chain inhibitors and uncouplers (605).

Knowledge of the stimulus intensity at the site of transduction is therefore important when making comparisons between different experimental models or when establishing potential transduction mechanisms (477). Where possible, responses, *in vivo* or *in vitro*, should always be referred to estimations or actual measurements of *tissue* Po_2. For example, as the precise horizontal position of any Po_2 stimulus-response determination will depend first and foremost upon the point at which the Po_2 was measured, responses from single, isolated type I cells would not be expected to occur until the media Po_2 fell below *ca*. 40 mmHg. Similarly, stimulus-response curves measured *in vivo*, with arterial blood Po_2 taken as the stimulus, would be right shifted relative to *in vitro* responses referred against superfusate Po_2 (693, 870) and it can be calculated that, for a 200 μm diameter rat carotid body superfused *in vitro* a maximum difference of up to 80 mmHg would occur between surface measured Po_2 and tissue Po_2, with the precise value dependent upon the depth of the tissue from the surface, the metabolic rate of the tissue and the solubility of O_2 (694); a difference both predictably and significantly greater than the 10 to 20 mmHg difference measured between Pao_2 and microvascular Po_2 *in vivo* (502). An effect of temperature upon the position of the response curve also cannot be discounted.

The sensed variable—O_2 tension or content?

The O_2 content of blood in mammals is largely determined by the hemoglobin concentration and its saturation, with a much smaller contribution from dissolved O_2. The saturation of hemoglobin is related in a sigmoidal function to the Po_2, according to the O_2-dissociation curve whilst the amount of dissolved O_2 is linearly related to the Po_2 according to its solubility. The question of whether O_2 content or partial pressure is sensed is a key one for our appreciation of the chemotransduction process and has been examined by utilising various forms of hypoxia as stimuli. Hypoxic hypoxia is a powerful stimulus of the carotid body and is defined as a generalized, low arterial Po_2 that is associated with a predictable decrease in the arterial content of O_2. It can be caused by inspiring low tensions of Po_2 at altitude or experimentally through alterations in inspired gas mixtures, through inadequate alveolar ventilation in respiratory diseases including COPD and sleep apnoea, impaired lung diffusion or by increase in the pulmonary shunt fraction.

In addition to hypoxic hypoxia, stagnant, or ischemic hypoxia and histotoxic hypoxia are also powerful stimuli of the carotid body (230, 556). Thus, sustained and significant hemorrhage leading to hypotension and reduced blood flow to the carotid body as well as metabolic poisons, including cyanide, all increase chemoafferent discharge. Application of hyperoxia can reverse the chemoexcitation induced by hypotension (513) and this finding was used to argue that hypotension-induced chemodischarge may be due to the accumulation of an anaerobic metabolite. However, although hypotension causes a reduction of the total carotid body blood flow (187, 730), this does not alter tissue Po_2, presumably due to a powerful autoregulation of local blood flow during changes in perfusion pressure (10, 11, 14). Carotid chemoafferent activity is thus unaffected as mean arterial blood pressure (MABP) falls between *ca*. 160 and 70 mmHg. A reduction of MABP below 60 mmHg causes a small increase of the chemoafferent activity, a response that is not as large as that of aortic chemoreceptors to a similar stimulus (499, 513, 516). Thus, only if hypoxia is also present, can reductions in MABP above 60 mmHg induce a significant discharge in carotid body chemoafferents.

As carotid body ischemic hypoxia and histotoxic hypoxia and systemic hypoxic hypoxia all induce hyperventilation, it is most commonly accepted that the adequate stimulus at the carotid body is the fall in Po_2 rather than O_2 content, or even Hbo_2 saturation. This may simply be due to the naturally small a-v O_2 content difference across the carotid body which would ensure that the a-v Po_2 difference was correspondingly small and perhaps even less than 4 mmHg (187, 237) meaning that a substantial change in the blood O_2 capacity would be required to observe any effect on carotid body tissue Po_2. Thus, inmost but not all cases, (498), the carotid chemoreceptor afferent discharge frequency remains unchanged by decreasing O_2 content, induced either by hemodilution (74) or by carbon monoxide (CO) poisoning of blood hemoglobin (230). HbCO needs to be > 20% before an increase in discharge is noted but even up to 50% HbCO (498), the effect is small and an explanation for this lack of excitation by HbCO may lie with the relatively high blood flow of the carotid body and thus its intrinsic inability to sense O_2 delivery *per se*. Indeed, the addition of CO to blood may even, through elevating Pao_2, decrease chemoafferent discharge slightly and hence either have no effect upon (149, 167) or even reduce ventilation (632), although it has been argued that the lack of ventilatory response might reflect a CO-induced CNS depression (579).

In marked contrast, the intrathoracic, aortic chemoreceptors are believed to be sensors of arterial O_2 content, increasing their afferent discharge significantly *in vivo* in an exponential-like fashion during an experimental protocol of graded increases in blood HbCO, induced by inhalation of sublethal doses of CO (346, 498). Whilst the anemia-induced increased discharge from aortic chemoreceptors was not associated with a ventilatory chemoreflex

response, variations in cardiovascular parameters, most notably blood volume and red blood cell count as well as systemic vascular resistance, were noted (182).

The aortic chemoreceptors may also to be sensitive to small, less than a few mmHg, falls in arterial blood pressure in marked contrast to the carotid body which does not respond to hypotension until blood pressure is below *ca.* 60 mmHg, at which point the tissue may be hypoxic (74). These findings suggest that O_2 delivery in aortic bodies may be more limiting than in carotid bodies, making them particularly sensitive to O_2 transport capacity. Blood flow through aortic chemoreceptors has not been measured but the anatomical and anecdotal evidence seems to indicate that it is little likely to be much different from the excess to metabolic requirement observed in carotid body blood flow, with blood being supplied by adjacent large arteries to a lobular, highly vascular tissue and with the color of the venous blood draining the aortic bodies of cats being described as arterial by Howe in his 1957 Ph.D. thesis, as described by De Burgh Daly (186). However, hyperoxia reduced the aortic chemoreceptor discharge induced by HbCO (498), which might indicate that the stimulus for both aortic and carotid chemoreceptors is most likely a reduced tissue Po_2. The possibility remains, however, that these two organs represent evolutionary different systems with their anatomical positions determining their precise cardiovascular or ventilatory function (582).

The question of species difference might be important also, as the mammalian recordings of aortic chemoafferent discharge in response to variations in O_2 capacity have all, to date, been performed on cats. In rats, which do not have any noticeable aortic tissue (236), the amplitude of the *ventilatory* response to Pao_2-independent, changes in arterial O_2 content induced by CO, was 60% of the reflex response to hypoxic hypoxia, which could be taken to suggest that rat carotid bodies may sense arterial O_2 content (304). How this may occur, in the absence of any noticeable limitation in O_2 delivery is not known and the possibility that the change in arterial blood O_2 content is translated to a (sensed) change in microvascular Po_2 is acknowledged. However, as rat carotid body experiments *in vitro* are most commonly employed in the absence of blood perfusion and with superfused, blood free solutions these models cannot resolve this issue. Perhaps further exploitation of the recording of rat carotid body chemoafferents *in vivo*, which has only been reported once (870), may offer greater insight to this key point. In the meantime, the assumption remains that carotid body chemoreceptors sense Po_2 and not O_2 content, whilst it appears that the aortic chemoreceptors *may* sense O_2 content.

Sensing Hypoxia—Transduction Processes in the Carotid Body

Since the late 1980s and the initial establishment of type I cell isolation procedures (229, 540, 541, 626, 679, 703), experiments in carotid body physiology have largely been aimed at an understanding of the cellular and molecular basis of hypoxia transduction in this cell type. The general hypothesis has subsequently arisen that transduction mechanisms in the type I cell can account for *in vivo* findings. In considering the full merit of any such hypothesis, at least for the detection of acute hypoxia, it should be able to account (i) for the particularly high sensitivity of the organ for changes in Po_2, (ii) its rapid, within seconds, speed of response, and (iii) for the modulating actions of other natural, systemic or pharmacological stimuli.

Much valuable information has been gained and a consensus appears in place regarding the main features of the transduction process. Thus, the cellular inclusions of the type I cell that have long suggested a neurosecretory function is now supported by strong evidence of an absolute requirement for Ca^{2+}-dependent neurotransmission between type I cells and afferent nerve endings. Neurotransmission is complex in the carotid body, with a large number of putative inhibitory and excitatory transmitter substances localized in and released

from type I cells and a corresponding extensive array of receptor subtypes on afferent nerve endings as well as on type I cells. The elevated type I cell intracellular Ca^{2+} that drives neurosecretion appears to be derived almost exclusively from extracellular sources, entering the cell through voltage-gated Ca^{2+} channels (VGCCs) subsequent to membrane depolarization, induced by the inhibition of a range of K^+ channels (the *Membrane Hypothesis*—see next section). Thus, the number of exocytotic profiles in microscopic sections of the carotid body is increased by the addition of a high, depolarising $[K^+]$ to the fixative solution and which is also Ca^{2+} dependent (323, 324). The entire process is initiated by an intracellular sensor mechanism that detects the stimulus and presumably initiates an intensity-dependent, membrane depolarization. Whilst a monotonic relation between any variable and falling Po_2 might indicate a transduction step, there is, however, no requirement for the sensitivity of any "downstream" element of the transduction pathway to correlate directly with the sensitivity of the O_2 sensor as the transfer functions between elements need not be linear. As almost all cells will respond in some way to falls in Po_2 if severe and/or sustained, perhaps the uniqueness of the type I cell may lie within the gain of these various transfer functions and/or perhaps their frequency responses rather than in any exquisite sensitivity of the sensor.

The nature of the O_2 sensor appears key to the understanding of carotid body chemotransduction. Whilst a number of hypotheses, each with its own particular appeal, have been generated and tested, none appear yet able to describe the chemoresponse in full and much controversy remains over details of virtually each step of the process. Some of the difficulty arises from a lack of detailed descriptions of O_2 affinity in many of the studies aimed at elucidating sensor mechanisms and the standardized generation of stimulus—response curves over a range of Po_2 would be of great assistance in the field. Consequently, therefore, there is little, if any, compelling evidence to suggest that a single O_2 sensor can be responsible for the entire stimulus-response characteristics of the carotid body. A compelling alternative to the concept of a single sensor is that of a range of sensors with varying affinities and thresholds that might better explain the entire response (434, 717, 887) or, indeed, provide an important failsafe redundancy for a vital homeostatic process.

The membrane hypothesis

O_2-sensitive K^+ channels—If the carotid body type I cell has an absolute requirement for voltage-gated, Ca^{2+} channel activation to initiate neurosecretion and transmission (see above), then it follows that type I cell membrane depolarization must be a key requirement in the hypoxia transduction process. However, until the late 1980s, there was relatively little data, and thus understanding, regarding the mechanism(s) by which type I cells might become depolarized by hypoxia. Some of this apparent disinterest may have arisen from the earlier, mistaken belief that type I cells were inexcitable as, although high extracellular $[K^+]$ had been shown to induce depolarization in type I cells (251, 318), this effect had been attributed to be secondary to changes in bath solution osmolarity with the suggestion that the type I cells were freely permeable to K^+ (62) and K^+ were not vital for maintenance of type I cell membrane potential (661). However, with the advent of patch clamp methodology and its application to isolated type I cells in culture, it became quickly clear that depolarization induced by closure of selective K^+ channels, located in the plasma membrane of type I cells, is a critical step in the hypoxia transduction process (Fig. 6) in rabbit (229, 370, 540, 850), rat (101, 679, 800), cat (151), and mouse (925).

The regulation of K^+ channels is complex, with significant functional variation depending upon their specific α- and/or β-subunit composition and their particular basal phosphorylation state. It is therefore perhaps inevitable that, although a number of K^+ channel subtypes have now been shown to be inhibited by hypoxia, there appears to be

significant between and within species as well as developmental age differences in the subtypes identified. These differences make difficult a definitive identity of the channel(s) responsible for initiating depolarization in type I cells (see (102, 543, 683) for recent reviews). There is, however, no known absolute requirement for a single class of K^+ channel to be involved in O_2 sensing, or for a single sensor to be associated with any single channel type, and thus a number of different K^+ channel types may play a role within a single cell to both induce and maintain the depolarization following natural stimulation. Nevertheless, these channels must have the property of being either directly, or indirectly, inactivated by hypoxia and should therefore possess a finite open conductance at the resting membrane potential, which, in the isolated rat type I cell, is usually found to be between −40 and −55 mV in many patch clamp recordings (106, 107, 921, 922), although a considerably lower value of −81 mV was estimated from more recent, single channel recordings (911) and from which intracellular [K^+] was calculated at 163 mM. In the rabbit, however, the intrinsically unstable membrane potentials of type I cells (850) make it hard to define an average value for a resting membrane potential, although values of between −67 to −33 mV (mean −48 mV) have been reported in this species (658).

O_2-sensitive channels should also show a high sensitivity to oxygen with their closure related to *in vivo* tissue levels of Po_2 in a manner that can be related to the stimulus-discharge response characteristics previously established for this organ. Thus, a new idea, termed the “membrane hypothesis” followed the first descriptions of a small conductance, fast inactivating (540), delayed rectifier (542) K^+ current in rabbit type I cells that was decreased, reversibly and selectively, by hypoxia, albeit initially shown with a low and complex Po_2 sensitivity (541). Regarding the link between single cell and whole organ hypoxia sensitivity, Montoro et al. (598) measured the effect of varying Po_2 between 160 mmHg and *ca*. 20 mmHg upon K^+ and Ca^{2+} currents as well as dopamine secretion, in isolated rabbit type I cells and showed, contrary to earlier findings, that the Ca^{2+} channel was also inactivated by hypoxia. They proposed a model whereby the inactivation of Ca^{2+} currents acted as a “brake” to neurosecretion until the hypoxia stimulus reached sufficient enough severity to enable the depolarization induced by concomitant, but independent, K^+-channel inhibition to disinhibit the Ca^{2+} channels, leading to a rapid and large increase in cytosolic [Ca^{2+}] that, from a threshold of *ca*. 180 nM, would initiate a burst of transmitter release into the synaptic cleft from a nonimmediately (i.e., greater than tens of milliseconds), nondocked pool of vesicles. They therefore suggested that a single type I cell could represent the physiological responses of the whole organ. However, Summers et al. (809) using HCO_3^- buffered solutions found that hypoxia activates L-type currents in glomus cells via a protein kinase C (PKC)-dependent mechanism. A modulatory role upon the channel properties could be played by subsequent feedback/forward mechanisms that would therefore act to alter O_2 sensitivity.

In the rabbit, the inhibitory effect of hypoxia upon Kv channels appeared to be membrane delimited, suggesting an intrinsic sensor mechanism that might be therefore be associated with either the a pore-forming subunits of the channel or with associated, auxiliary β-subunits. Consequently, these were termed Ko_2 channels to signify that hypoxia could induce depolarization directly via an effect on specific domains of the channel leading to a reduction in the channel open probability by up to 50% and without change in the single channel conductance of *ca*. 20 pS (302). It is now believed that $Kv_{4.1}$ (gene nomenclature: KCND1) and $Kv_{4.3}$ (KCND3) α-subunits, in the rabbit, most likely carry the Ko_2 current. Although $Kv_{3.4}$ (KCNC4) also contributes to the outward current, it is not an O_2-sensitive component as demonstrated by a significant attenuation of the hypoxia mediated inhibition of K^+ current and depolarization by anti-$Kv_{4.3}$ but not by anti-$Kv_{3.4}$ antibody (697, 765). Instead, it has been postulated that the action of $Kv_{3.4}$ in enabling fast membrane repolarization might act to increase the action potential frequency that is often a

characteristic of the response to hypoxia in rabbit type I cells (541). In the mouse, however, the Kv_3 subunit may play a more important role in mediating the K^+ current inhibited by hypoxia (697). In addition, a contribution to the resting membrane potential of rabbit type I cells by a voltage gated, human ether-a-go-go-related gene (HERG) channel has been described that, when blocked pharmacologically, can induce sufficient cell depolarization to raise intracellular [Ca^{2+}] and increase chemodischarge (658). The effect of hypoxia on this component of the total K^+ current is not yet known.

In the rat, a role for noninactivating, Ca^{2+}-dependent K^+ channels (principally the large conductance, maxiK (KC-NMx) subtype (679) and a heteromeric combination of tandem pore domain weak inward rectifying (TWIK)-related, acid sensitive K^+ channel (TASK-1) (KCNK3) and TASK-3 (KCNK9) subunits of the voltage-independent, acid sensitive tandem P domain K^+ channel family (101, 112) have been forwarded as candidate, O_2-dependent ion channels in type I cells. Although possessing K_V channels, these channels appear to have no intrinsic O_2 sensitivity in the rat, although they can be modulated by extrinsic factors including adenosine (852), suggesting that they may have a feedback role to play in modulating cell excitability following, for example, stimulus-induced neurosecretion. In additional contrast to rabbit type I cells, little, if any, spontaneous membrane depolarization is observed in the rat, most likely reflecting the paucity of functional Na^+ channels in the type I cell of this latter species (226, 275, 800).

The initial electrophysiological experiments in rat demonstrated that the partial (*ca.* 50% at 0 mV) inhibition of the whole cell K^+ current by hypoxia could be eliminated by prevention of Ca^{2+} influx through either the modulation of ionic concentrations or by use of Ca^{2+} channel blockers (678) or augmented by Ca^{2+} channel activators (679), suggesting a selective effect of hypoxia on maxiK channels that was supported by a later report showing a lack of direct effect of hypoxia upon Ca^{2+} channel current in the rat type I cell (135), in contrast to the O_2 sensitivity reported for Ca^{2+} channels in rabbit cells (598, 809). However, evidence does support an inhibitory effect of hypoxia upon the Ca^{2+}-sensitive component of the maxiK current, at least in recombinant HEK 293 cells (524), which may act to attenuate the reactivation of maxiK that would otherwise occur at depolarized potentials and thus limit the transduction process. However, at more depolarized potentials, this block of the normal feedback function of maxiK would be lifted and consequent channel activation would return the cell potential toward E_m. In further support for a functional role of maxiK in transduction, hypoxia was shown to induce a depolarization of *ca*. 9 mV in single channel recordings of excised type I cell membranes, an effect largely mimicked by 20 nM application of the selective maxiK inhibitor, charybdotoxin (ChTX) (921). Larger doses of ChTX induced greater depolarization. An important, additional, finding reported in this study was that maxiK channel activity depended upon the presence of cytosolic factors, as channel activity was greatly reduced, by 1 to 2 orders of magnitude, when inside-out patch configurations were compared to either outside-out or, particularly, to perforated patch configurations. This dependency of effect on a cytosolic factor was not, however, observed in a recombinant system coexpressing both α-and β-subunits of the human maxiK channel (524).

The contribution of maxiK to the resting membrane potential of *in situ* carotid body type I cells is central to an appreciation of their role in initiating the membrane depolarization during hypoxia. Early reports utilizing sharp electrodes to measure the resting membrane potential (E_m) of type I cells gave relatively low values, typically between −20 and −30 mV (62), but these are prone to damage-induced artefact, as perhaps suggested by the low membrane resistances recorded with this technique (258). More depolarized potentials are now routinely recorded in isolated cells with patch clamp techniques that indicate the E_m to be below the −30 to −40 mV activation threshold typically observed for type I cell maxiK

channels, although their high conductance and the high input resistance of the type I cell (101) may enable a contribution to the E_m with even just a small, but finite open probability.

If maxiK channels were contributing to the depolarization observed during hypoxia, then the pharmacological block of this channel should also induce a depolarizing effect that should be measurable either at the level of neurosecretion and/or sensory discharge. However, selective blockers of maxiK were without effect upon resting carotid body chemoafferent discharge and/or dopamine (DA) secretion in rat (216) and cat (656). In addition, an effect of ChTX upon chemoafferent discharge could only be observed in the presence of hypoxia, where it acted to shift the Po_2-discharge response curve rightward, implying a significant effect of maxiK only *subsequent* to membrane depolarization (692). These data are all consistent with the findings made at the single cell level, where neither tetraethylammonium (TEA), 4-aminopyridine (4-AP) nor ChTX could evoke Ca^{2+} elevations in single cell recordings of rat carotid body, even when these K^+ channel blockers were used together (101). A more recent study (313) confirms these earlier findings by reporting a lack of effect, in rat type I cells, of blocking doses of TEA and iberiotoxin (IbTX) upon both basal or hypoxia-induced increases of $[Ca^{2+}]_i$ or catecholamine secretion, both common readouts of type I cell depolarization. However, if type I cells were depolarized, not by hypoxia, but by dintrophenol, a stimulatory agent known not to block maxiK, then both TEA and IbTX could now potentiate the cell readouts. The results indicate that the open probability of maxiK at E_m must be too low to be of physiological significance and that blockers of maxiK can only exert an action when the channel is activated. In this schema, maxiK channels will be an important component of the full cell response to hypoxia, with their inhibition contributing to the depolarization required to activate Ca^{2+} channels.

Notwithstanding these results originating from whole carotid body preparations, or from the isolated type I cell in culture, it was observed, by other laboratories, that IbTX inhibition of maxiK channels of type I cells *in vitro* could, just like hypoxia, induce neurosecretion from type I cells maintained in clusters (408) or in thin slice preparations of the carotid body (670), where even drug-induced secretion from quiescent cells was often noted. Thus, when maintained in their more normal anatomical relations with each other, and/or with type II cells and with the nerve endings and blood vessels that encase them, the E_m may be more depolarized than when cells are isolated enabling maxiK channel blockers to have an effect that would not be observable in more hyperpolarized cells. However, it is of note that TEA at a K^+ channel blocking concentration did not prevent a further release of DA when hypoxia was applied (670) which suggests that non-TEA sensitive mechanisms must also be involved in the O_2 sensing pathway.

The membrane resistance and capacitance of type I cells in isolation may also be greater than that of cells in their more natural anatomical arrangement (214). The resistance difference may be accounted for by cellular modulation by endogenous factors that are lost in cell culture and the capacitance difference may be accounted for by a loss of cell processes in cultured type I cells. The use of nonphysiological, 4-(2-hydroxy ethyl)-1-piperazineethane sulfonic acid (HEPES) buffer may also have been a confounding variable, but similar results were obtained when HCO_3^- buffer was used with a perforated patch configuration (148). The main question then is why there remains a discrepancy between the effects of K^+ channel blockers administered to the whole organ or isolated cells with those made from clusters or thin slice preparations?

The possibility of a poor site access of neurotoxins to the transducer elements within whole carotid body preparations has been raised as a possible factor. Whilst not tested directly, this does not, however, seem an entirely satisfactory explanation given the relative ease of penetration of large protein molecules into the carotid body (917) and the finding that type I

cell K^+ currents, measured with a “blind patch” technique, could be inhibited in whole organ preparations by the K^+ channel blockers, TEA and 4-AP, as reported in carotid body slice preparations (670), but without effect upon sinus nerve basal or hypoxia-induced discharge (214). In addition, ChTX had no effect upon discharge in the perfused, superfused whole carotid body preparation of the cat, but did raise carotid body tissue Po_2, so suggesting an intraorgan effect upon blood vessels that may signify site access (656). However, the relative poor lipid solubility of these compounds may have prevented diffusion from the blood vessels to type I cells in perfused preparations or to significant depth when superfused. Nevertheless, the effects observed on isolated cells are still not yet easily discounted, other than to question the E_m in such cells, and so the controversy regarding a primary role for maxiK remains unresolved. It may, however, be pertinent to note here that the coculture preparation and the thin slice preparation both require a much greater time between carotid body excision from donor animals and subsequent recording. In whole organ and isolated cells, recordings are often made within a few hours of the animal’s death whilst in thin slices the delay is two to four days and is even longer in cocultures. This extended time period in culture may affect cell function and underlie some of the differences observed (313, 382, 476).

Inhibition of maxiK, however, may, at least in the rat, be simply a prime requisite for the maintenance and/or amplification of a membrane response to hypoxia rather than for the initiation of the response. If that is so, then closure of another K^+ channel subtype associated with the E_m is required. However, the rat carotid body does not possess O_2-sensitive Kv channels and evidence supports a role for another O_2-sensitive current in the initiation of membrane depolarization in response to hypoxia in this species. These, initially undefined, channels showed a conductance of around 340 pS in the rat type I cell, had no intrinsic voltage sensitivity and were activated at potentials greater than *ca*. −90 mV, which classified them as background, so-called "leak” or K_B channels. Po_2-response curves for channel inactivation correlate well with the cellular response to tissue hypoxia and a $K_{1/2}$ of 12 to 13 mmHg Po_2 has been determined, making them suitable for the initiation of a small, but significant hypoxia-mediated depolarization of around 7 mV and a decrease in resting conductance of 50% to 85% (101). Their sensitivity is appropriate for the detection of tissue Po_2 and correlates well with type I cell Ca^{2+} sensitivity (107). As pointed out by Buckler (102), the small outward K^+ current carried through these channels at the E_m is on the order of 9 pA and this must be balanced, in the steady state, by an uptake mechanism that is most likely to be the Na-K pump. The pump is, however, electrogenic, extruding Na^+ for K^+ at a ratio of 3:2, and so would generate around another 4.5 pA of outward current, giving a total outward current of some 13.5 pA. This must be matched by an inward current of similar magnitude and carried perhaps by a persistent Na^+ current (137). Intriguingly, as Buckler (102) points out, this may be taken to indicate that the persistent Na^+ current is the depolarizing current observed in hypoxia and it is interesting to note that an increase in a similar current was postulated to underlie the spontaneous generation of action potentials in the afferent sinus nerve ending (218).

The characteristics of the background current first identified by Buckler (101) suggested it to be mediated via a member of the voltage-independent, acid sensitive tandem P domain K^+ channel family (65, 232). Of the members of this family, a particular role in the sensing of lowered Po_2 has been identified with the TASK subfamily (112, 911), a function it shares in addition to its more characteristic sensitivity to physiological pH in other cells (233). In the type I cell, the sensitivity of TASK channels to pH is retained, but, interestingly, it has been suggested that this is a particular sensitivity to *extracellular* pH (112), which, if confirmed, would demonstrate that the type I cell has the capacity to sense both intra- and extracellular pH. This K_B channel is highly expressed in the type I cell where its properties support the existence of TASK-1-like (112) and TASK-3-like (911) subunit activities that are likely a

consequence of a TASK1/3 heterodimer that contributes the principal component of the K_B conductance (438, 441). In addition, TASK-2 and TWIK-related arachidonic acid-stimulated K^+ channel (TRAAK) have also been localized by immunohistochemisty to the type I cell (928) and TASK-1 immunoreactivity has also been reported in the mouse carotid body (423) and human carotid body (261) as well as on rat type II cells (485), although their function on these latter cells has not been determined. The channel has a characteristic, low conductance of *ca*. 14 to 16 pS and spontaneous activity runs down quickly to *ca.* 10% of initial activity within 40 s of patch excision. This latter phenomenon suggests an important regulatory role for an intracellular mediator in the maintenance of normal K_B function and Williams et al. (911) suggested this might be a fall in ATP, a suggestion supported by their finding that the inhibitors of oxidative phosphorylation, cyanide and dinitrophenol, could act like hypoxia, to inhibit K_B in cell-attached patches. Feedback regulation of K_B has also been described, with neurosecretion potentially initiating auto/paracrine control via γ-amino butyric acid ($GABA_B$) autoreceptor-mediated, activation of TASK-like channels on type I cells (265) and a phospholipase C (PLC)/PKC-dependent, muscarinic receptor-mediated block of TASK currents (655).

Our understanding of the functional role of K_B in chemotransduction parallels the development of relatively selective inhibitors and activators of the channel and its subtypes. These agents presently include barium, zinc, quinidine, ruthenium red, and methanandamide as well as the volatile anesthetics, including halothane. Thus, barium, in contrast to TEA and 4-AP, increased carotid body afferent discharge frequency in the cat and rat *in vitro* (216, 325). Further studies using these “less reduced” models are required, but caution needs to be applied to any negative findings if site access cannot be guaranteed. More selectively, TASK-1 knockout mice showed a reduced ventilatory response to 10% inspired O_2, although *ca.* 70% of the response remained (844). TASK-3 knockouts, in the same study, showed no impairment of their ventilatory response to hypoxia. The reflex effect was most likely mediated through a fall in chemoreceptor discharge as the afferent response in TASK-1 knockout mice was reduced by a similar amount (*ca*. 50%). Basal discharge was unaffected in TASK-I knockouts, but significantly increased in TASK-1/TASK-3 double knockout animals but in this latter group, the attenuated discharge response to hypoxia was no different to that of the single TASK-1 knockouts. TASK-1 is therefore strongly linked to the mediation of a systemic hypoxia response in mice, although around half of the response to hypoxia still needs to be accounted for. In addition, as TASK-1 and TASK-3 deficient mice would clearly not be able to form TASK1/3 dimers, then the results from mice do not yet fit tidily with rat data where such dimers were held responsible for mediating the most significant component of the O_2 sensitivity of K_B (438). Once again, this could reflect a genuine species difference. Within species, discrepancies also exist, however, as, in another study at the level of single type I mouse cells deficient in either TASK-1, TASK-3, or 1/3, the data indicates that although E_m was less hyperpolarized in TASK1/3 knockouts relative to wild type, with consequently augmented basal catecholamine secretion, the secretory response to hypoxia in these cells was unimpaired, even after block of maxiK channels with IbTX (651). It may be unlikely that catecholamine release cannot be at least an indicator of cell depolarization or chemodischarge and thus the possibility remains of an adapted response in TASK-deleted animals that favours expression of alternate sensor or effector mechanisms. Interestingly, TASK-2 knockout mice show a loss of the centrally mediated, secondary fall in ventilation normally observed in response to hypoxia (309), which suggests amore systemic role for TASK channel subtypes in O_2 sensing.

Despite some of the limitations to the membrane hypothesis as described above, a key role in chemotransduction for O_2-sensitive, plasmalemmal K^+ channels, remains highly likely, if not proven. The species differences, as well as differences reported within a species, need to be reconciled, but appear real and thus may reflect the lack of requirement of a single sensor

and/or effector system for hypoxia sensing with several subtypes of channel acting to ensure a full response is produced and maintained.

Na^+ channels—Regarding other inward currents that may be involved in stimulus-induced depolarization, it is perhaps surprising that a considerable species difference occurs with respect to the expression of tetrodotoxin (TTX)-sensitive Na^+ channels; these being absent in the rat (275) but present in the rabbit (229), where they are activated at *ca.* −40 mV, have a peak current at between 0 and 10 mV and a reversal potential at 32 mV when the extracellular [Na^+] is 140 mM (850). Consequently, spontaneous and stimulus-induced action potentials in type I cells of rabbits (229, 540, 541) are carried by a Na^+ current, whilst in the rat, no spontaneous action potentials are ever observed and stimulus-induced action potentials, albeit much less observed events than in rabbit cells, are here carried by Ca^{2+} currents (106, 107). Whilst such major differences in ionic conductances in two mammalian species, may appear to lessen the evolutionary significance for a role of these channels in chemotransduction, it was noted by Fieber and McCleskey (275) that this may be taken as analogous to the presence of Na^+ channels on pancreatic β-islets of dogs and the absence of such channels on the identical cell type of rats (728) where the difference has only quantitative effects upon the time course of depolarization leading to activation of Ca^{2+} channels in these cells.

The mitochondrial hypothesis

The membrane hypothesis appears in direct contradiction to the proposal of Biscoe and Duchen (76) who, from data gained from rabbit type I cells, claimed that K^+ channel inactivation by hypoxia was most likely secondary to the primary effect of hypoxia upon the mitochondrial membrane potential (Fig. 7), thus relegating the membrane electrophysiological response to a modulatory role in the regulation of voltage-gated, Ca^{2+} influx. Indeed, these authors found that NaCN increased rather than decreased the K^+ current in rabbit type I cells (75). This resurgence of the so-called “mitochondrial hypothesis” a long favored concept in carotid body transduction (42), gained few initial supporters. However, later attempts at recreating O_2 sensitivity using recombinant K^+ channel α and β subunits in cell expression systems, proved inconclusive and, together with studies in other, nonrabbit, species demonstrating the presence of K^+ channel O_2 sensitivity only in patch clamp configurations that would retain cytosolic, or closely associated factors, acted to return the focus to identifying an alternative sensor mechanism that couples to K^+ channels and thus confers upon them an “O_2 sensitivity.” Additionally, the finding that exposure of newborn rats to chronic hypoxia for 9 to 14 days from birth could alter fundamentally the *type* of K^+ channel inhibited by hypoxia suggests that the O_2 sensitivity of any particular type I cell K^+ channel is not intrinsic but instead is conferred by its coupling to an O_2-dependent, extrinsic factor(s) (922). Further, when a known O_2-sensitive K^+ channel (TASK-1) of the rat carotid body was heterologously expressed in an immortalized, chromaffin cell line, the O_2 sensitivity of the channel was lost, suggesting the absolute requirement for cell specific, nonintrinsic sensors (419). These cell-specific sensors could be intrinsic to the channel with redox modulation of cysteine residues in K^+ channel subunits underlying their gating by hypoxia, but equally, rather than be intrinsic to the channel, be simply tightly associated with the K^+ channel in a macromolecular complex that need not be associated with the mitochondria, but could coexist in excised patch preparations. However, they also need not to have to be tightly associated in any physical capacity with a K^+ channel, as long as their product(s) are able to interact with the channel.

Heme proteins as sensors of hypoxia

The acceptance that most K^+ channels of the carotid body type I cell do not possess intrinsic O_2 sensitivity, but can be inhibited by hypoxia, has prompted a search for an upstream

sensor(s) that couples to and regulates channel activity. The present consensus is that this upstream sensor is likely to be an enzyme and a number of candidates have been proposed (435). However, the nature, mechanism of action and physiological relevance of each is still debated. For example, given the close correlation between arterial PO_2, red blood cell hemoglobin saturation, and carotid body afferent discharge, it has long been postulated that a carotid body hypoxia sensor may signal via the desaturation of a prosthetic heme group. Such a concept, whilst attractive, is however, not necessary, as it is likely that a number of nonlinear transforms in the signal between the sensing of the stimulus and the generation of an afferent action potential will alter the stimulus-response relation between sensor and effectors (887). Consequently, not all putative sensors contain heme groups and so the stimulus sensed need not be a heme ligand and thus need not necessarily be molecular O_2. Neither, however, is it necessary to consider the existence of a single sensor and, given the complex stimulus-relation characteristics of the carotid body, it may be more useful to consider several sensors working in concert over different ranges of Po_2 (717).

It is worth considering that a degree of natural redundancy might necessarily be present to ensure the proper functioning of the receptor throughout a lifetime, when sensitivity is known to change and when pathology may affect particular elements of the system. It is also worth recalling the considerable interspecies differences that have been reported and which may relate ultimately to species-specific sensor or channel-coupling mechanisms. Unfortunately, the precise O_2 affinities of all putative sensors of the type I cell is not yet known with any certainty, but what is presumed is that these sensors will have a sufficiently low O_2 affinity to enable them to sense falls in Po_2 from tissue levels that appear too high to affect the flow of electrons through the normal mitochondrial protein transport chain [but see (310)]. Thus, despite the long-recognized finding that all blockers of the mitochondrial electron chain and all uncouplers of oxidative phosphorylation are potent chemoexcitants [(469, 604, 605); but see (652, 748)] whose application occludes hypoxia sensitivity (315, 501), it is not clear how protein complexes in these organelles might act as O_2 sensors unless they, or a subgroup of them in the type I cell, possess a particularly low O_2 affinity. That said, such a low affinity has been observed by a number of groups and this may distinguish type I cells from other cells that respond to hypoxia only at near anoxic levels (227, 228, 806).

The mitochondria of type I cells are different in appearance to those found in both type II cells and sinus nerve endings, with those in the type I cells being the largest with the most cristae and a relatively light matrix (485). Whether these morphological differences are significant for the expression of cell-specific, mitochondrial proteins is not yet known, but mitochondrial function appears closely tied with O_2 sensing and it is apparent that pharmacologically mediated changes in mitochondrial respiration can act like hypoxia to increase Ca^{2+} influx and elevate $[Ca^{2+}]_i$ (77, 109, 918), induce neurosecretion (748), and cause cell depolarization by inhibition of *ca*. 60% (at −70 mV) of K_B (109, 918). Thus, a causal link between mitochondrial depolarization and carotid body function in hypoxia appears established, although it should be noted that many inhibitors of oxidative phosphorylation have nonspecific actions upon K^+ channel activity that can be independent of the effects of hypoxia (652). In addition, partial deletion SdhB (iron-sulfur protein), a component of, a component of succinate dehydrogenase (mitochondrial complex II), in mice, although causing a slight membrane depolarization and an augmented basal release of catecholamine, was without effect upon hypoxia induced catecholamine release from type I cells (706), although here it has to be noted that around 50% of succinate dehydrogenase activity remained intact in these hetetozygous $SDHD^{+/-}$ animals.

Currently, therefore, hypotheses regarding enzyme sensors linked to K^+ channels include those associated with regulating cellular redox state and those concerned with cellular

energy status. In addition, the production of the endogenous gaseous signaling molecules, NO, CO, and hydrogen sulphide (H_2S), enzymes have also, more recently, been implicated in channel regulation during hypoxia (see section on neurotransmitters). Whilst signaling via the redox and energy states of the type I cell are not exclusive of, and may even be dependent upon, the presence of a low-affinity mitochondrial cytochrome, signaling via the gas molecules has no such requirement. Here too, however, it may be prudent to consider a synergistic relation between sensors, as a role for NO in modulating mitochondrial cytochrome *c* oxidase has been proposed that can confer, to a cell, an apparent low O_2 affinity (664, 820), through competition with O_2 at this site. The localization of (presumed) endothelial nitric oxide synthase (eNOS), generated NO to type I cell mitochondrial membranes after exposure to hypoxia suggests this may be a possibility in the carotid body (927), although NOS inhibition seems to augment rather than attenuate the chemoreceptor response to hypoxia (851). Other modulators, including other gas transmitter substances such as H_2S may however, also play a role (see *Neurotransmitter*) and more work is anticipated in this area.

Reactive oxygen species

Assuming that hypoxia sensing arises from a mitochondrial specialism in the carotid body, then a redox change would be consequent to changes in the concentration of reactive oxygen species (ROS), formed by the incomplete transfer of electrons through mitochondrial complexes I and III (314, 848). A variety of ROS may be produced, but most focus has been on the superoxide anion (O_2^-) and its diffusible, product, hydrogen peroxide (H_2O_2), produced by the action of superoxide dismutase located at both intramitochondrial and cytosolic sites. Of these ROS, H_2O_2 is believed the most likely to be functional in signaling due to its greater stability and long diffusion radius (603). ROS may affect K^+ channel function in a number of cell types (391), particularly pulmonary artery smooth muscle cells (551), but their functional role in the coupling of acute (within seconds) hypoxia intensity to the gating of plasma K^+ channels in the carotid body type I cell is contentious and not well established (314, 316).

Much of the contention may be due to technical difficulties in the measurement of ROS production rates (603, 898). However, the concentration of ROS would be expected to be dependent upon cellular O_2 availability, with a fall in cellular Po_2 leading to a more reduced state of those mitochondrial complexes proximal to complex IV and a more reduced cytosolic redox potential, measurable as an equivalent increase in the ratios of reduced to oxidized glutathione (Glutathione (GSH)/Glutathione disulfide (GSSG)) and $NADH/NAD^+$. The effect upon ROS is, however, not so apparent, as a reduced level of ROS due to a reduced amount of O_2 substrate may be countered by an elevation of O_2^- production from "downstream" complexes when "upstream" complexes are blocked (848) and ROS production would ultimately depend upon the expression of the mitochondrial complexes I and III, relative to that of complex IV. In addition, any change in cellular ROS concentration would be dependent upon the availability and activity of ROS scavenger systems, which may themselves be induced by ROS. Global cytosolic redox is therefore complexly regulated, but appears largely unchanged during physiological hypoxia (316) and a role for ROS is less apparent.

Accordingly, a consistent inhibitory effect upon type I background K^+ currents was observed in response to a range of mitochondrial inhibitors but which, individually, would be expected to either elevate or decrease cytosolic ROS (918). In addition, although anoxia and the uncoupler, FCCP had opposite effects upon the $NADH/NAD^+$ ratio, both had similar effects upon background K^+ currents (227, 918). Finally, application of high (mM), transient concentrations of H_2O_2 directly to type I cells had no effect upon resting $[Ca^{2+}]_i$ nor the

effect of hypoxia upon $[Ca^{2+}]_i$ (918), although an earlier report had indicated a depressive effect of H_2O_2 upon chemodischarge (9). Thus, changes in ROS levels are not easily related to chemoreceptor function and any elevations in ROS levels observed during hypoxia may arise only as a consequence of measurement artifact or by addition from an extramitochondrial source. That said, prior application of antioxidant, in man, can prevent the normal reduction in hypoxia ventilatory sensitivity observed in response to volatile anesthetics (825), which could suggest a modulating effect of ROS upon chemosensitivity, mediated via the TASK channel.

It could be argued also that ROS would normally only be expected to rise from a mitochondrial source in pathophysiological or unnatural conditions, such as in the presence of mitochondrial blocking drugs. It is, however, likely that localized changes in ROS may act to control selective functions in microdomains within the cell (667) and that this potential action would be masked by the relatively poor resolution of present technology (17). One such source of ROS localized close to plasmalemmal K^+ channels of type I cells (482) is the extramitochondrial, membrane bound, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox) complex, which produces O_2^- and H_2O_2, in proportion to Po_2 (8). Although proposed as a sensor mechanism, an action of Nox-derived ROS on type I cell K^+ channel inactivation is not proven. Accordingly, Nox inhibitors were without effect upon basal or hypoxia-induced catecholamine release from both rat and rabbit carotid body (640). In addition, mice with a selective ($gp91^{phox}$) gene deletion of Nox activity, retained their normal type I cell and carotid body function in response to hypoxia (353, 756). Further downgrading a necessary role for Nox in sensing hypoxia, is the finding that targeted deletion of the $p47^{phox}$ subunit of Nox led to an enhanced inactivation of type I cell maxiK channels (358), suggesting that ROS may be involved in cell repolarization rather than depolarization. Although an action on other K^+ channels in the carotid body has not been confirmed, a role for ROS in mediating hypoxia chemotransduction is not compelling and some other means of coupling the mitochondria with the cell membrane may be required. On the other hand, Nox-derived ROS have been reported to play an important role in chronic intermittent hypoxia (IH) and induced plasticity of carotid body function (see later section on plasticity and IH).

5′-adenosine monophosphate-activated protein kinase—unifying the mitochondrial and membrane hypotheses?

The carotid body has the particular challenge in hypoxia of increasing its metabolic activity for the purpose of signaling the threat to homeostasis in the face of reduced O_2 substrate. Much of this increased energy consumption in hypoxia, which can be up to 44% above basal (642), appears related to a particularly high activity of Na^+-K^+-ATPase (643), presumably in response to cell membrane depolarization. The rate of ATP utilization by the Na-K pump in type I cells has recently been calculated at 15.5×10^{-6} μmol ATP cm^{-2} s^{-1} (854), a value these authors noted to be 3× greater than that found in cardiac myocytes. An increase in Na-K pump activity during hypoxia may therefore be sufficient to decrease ATP levels, despite the increased metabolic rate and thus an alternate signaling pathway to couple mitochondrial function with K^+ channel inhibition may be via changes in the cytosolic concentration of ATP or factor(s) related to ATP synthesis. This is a long-established hypothesis as described above (42) and is based upon a predicted final common consequence of hypoxia and mitochondrial inhibitors and includes evidence demonstrating an excitatory action upon chemodischarge of a range of mitochondrial electron transport inhibitors and un-couplers (418, 604) and the mitochondrial H^+-ATP-synthase inhibitor, oligomycin, that acts to deplete intracellular high-energy phosphates, but without interrupting electron transport (at least until the proton gradient exceeds a threshold) or Ca^{2+} uptake by mitochondria (605).

Of particular significance, was the finding that, in the presence of oligomycin, neither hypoxia nor cyanide, antimycin A or FCCP were able to stimulate the carotid body discharge any further (605) as would be predicted if ATP production was central to chemoexcitation. It is worth noting that oligomycin, at high concentrations, may also block plasma membrane Na^+-K^+-ATPase and although some caution may therefore be required when interpreting data arising from its use, the finding that ouabain *inhibits* basal chemodischarge (418) may suggest that the results of Mulligan et al. (605) are compatible with an action at the mitochondria. In support of this, inhibition of TASK channels in type I cells by mitochondrial blockade with either carbon monoxide, rotenone, myxothiazol, cyanide, dinitrophenol (DNP), or FCCP have also been reported and provide compelling evidence of a mitochondrial-membrane link. The finding that these inhibitors act upon the same channel that is modulated by hypoxia and that the effects of inhibitors and hypoxia are mutually exclusive (918) supports the concept that the effects of hypoxia are dependent upon metabolism. As reiterated by Wyatt and Buckler (918), the common feature of all of these agents would be a fall in cytosolic ATP synthesis and an increase in ATP consumption, confirming a possible signaling role for cytosolic ATP or a related molecule in the carotid body. Accordingly, a severe hypoxic stimulus would be expected to increase chemodischarge as ATP levels fall and a steady-state level of discharge, lower than peak, may exist via partial recovery of high-energy phosphate levels following stimulation of glycolytic production of ATP. Such adaptation to hypoxia is rarely seen at mild to moderate levels of hypoxia and so one may presume that mitochondrial production of ATP is not limited at these levels of Po_2. As CO_2/pH continues to augment chemoafferent discharge in the presence of oligomycin (605), yet acidosis acts upon type I TASK channels in a similar, inhibitory manner to hypoxia (112), may suggest that severe depletion of ATP is not sufficient to inhibit completely the TASK conductance and additionally, that the channel can also be modulated in a manner independent of ATP.

Unfortunately, making accurate, direct measurements of carotid body ATP content during normoxia and hypoxia has proven difficult and is prone to technical difficulties as ATP is highly labile. Thus, results from whole organs have either described no effect of hypoxia upon total ATP content (15, 866) or a fall of up to 21% (637), although higher falls occur with metabolic inhibition. The degree of hypoxic stimulation will be key to these measurements but is difficult to ascertain precisely in a whole organ, superfused preparation and it is perhaps likely that, global, cellular ATP levels do not fall unless the cell is severely compromised. It can, however, be more readily calculated from measurements of O_2 consumption and glucose utilization that the ATP production by oxidative metabolism in a single type I cell is around 12 to 16 m mol kg^{-1} min^{-1} (854) which will be the rate of fall of ATP when aerobic metabolism ceases, at least until adaptive processes (e.g., glycolyis; reduction in ATP consumption) begin to compensate.

Regarding mechanistic links between $[ATP]_i$ and K^+ channel activity, a direct action of ATP upon TASK channels has been described in isolated membrane patches (911) in a manner that may depend more upon a channel-associated, low-affinity, magnesium-nucleotide receptor rather than an enzyme system (854). The sensitivity of the channel to MgATP is in the millimolar range and it was calculated that falls in Mg-ATP required to inhibit the channel by around 60% appeared commensurate with the falls in ATP recorded by others in response to metabolic inhibition. Whether this might account for the full response seen in the whole cell is not known, but here an indirect action may also occur, via a mechanism attuned to the level of cellular nucleotides but not requiring a fall in ATP. One such mechanism is consequent to the activity of adenylate kinase, which brings about the conversion of two molecules of ADP to produce a molecule each of 5′-adenosine monophosphate (AMP) and ATP and thus elevates the AMP/ATP ratio as a square function of the ADP/ATP (343). Cell function depends upon a ready supply of ATP and cytosolic

ATP levels are defended by the action of the energy sensor protein, AMP-activated protein kinase (AMPK), a serine/threonine kinase that is activated by increases in the cellular AMP/ATP ratio, howsoever this may be brought about (344) and thus can be activated without significant falls in ATP levels.

AMPK, consisting of a catalytic α subunit together with both β and γ regulatory subunits, is ubiquitously expressed in eukaryote cells where it plays a vital role in cellular metabolic regulation and at least 12 different heterotrimeric combinations of subunit isoforms can be formed giving, together with splice variation, a significant potential for within cell and between cell specializations in AMPK function. AMPK activity is tightly regulated by AMP via direct allosteric regulation of its γ subunit (351), enabling increased phosphorylation of its α subunit via an upstream kinase, LKB1 (350) and by decreasing dephosphorylation by protein phosphatases. In addition, calmodulin-dependent protein kinase (CaMKKβ) can also activate AMPK, independently of AMP, via a rise in $[Ca^{2+}]_i$ (916). Further control is provided by increasing ATP concentrations that can directly inhibit AMPK activity by competitive binding at the same allosteric site as AMP (174) and, in the normoxic cell, ATP would be expected to largely occupy these γ subunits. A glycogen-binding domain is also found on the β subunit (559). Thus, a variety of cellular stressors, including a lack of either sufficient oxygen or glucose, will decrease the energy status of the cell and activate AMPK to promote ATP-producing catabolic pathways and suppress ATP-consuming, metabolic pathways.

The AMPKα1 subunit has been immunohistochemically localized within *ca*. 1 μm of the plasma membrane in type I cells (247) and K^+ channels have been identified as a novel downstream target for this kinase (919), leading to the development of a hypothesis that makes AMPK activation both sufficient and necessary for O_2 sensing (245, 246). Thus, subsequent to its [AMP]-independent activation by 5-aminoimidazole-4-carboxamide riboside (AICAR), AMPK can, reversibly, induce both maxiK and TASK channel inhibition and voltage-dependent elevations in intracellular Ca^{2+} in isolated type I cells, as well as an increased afferent neural discharge in the sinus nerve (247, 919). No effect of AICAR was noted upon the O_2-insensitive, rat, K_V, delayed rectifier current in type I cells, but the inhibitory effect of AICAR upon maxiK channels could be replicated on KCa1.1 recombinant channels, expressed in HEK293 cells (919). The effects of AICAR could all also be reversed or inhibited by the AMPK antagonist, compound C and, importantly, a prevention of the effect of hypoxia upon both K^+ currents and chemodischarge was also reported. The attenuation of hypoxia-induced chemodischarge by compound C is not, however, complete, at least in the short term, which suggests that other hypoxia sensing mechanisms may also be operating. This hypothesis is nevertheless, particularly compelling as it unites the mitochondrial and K^+ channel responses to hypoxia without a need to necessarily invoke mitochondrial specialization or decreases in [ATP]. An implication, however, if one assumes that the type I cell has a sensitivity to hypoxia not observed in other non-O_2 sensing tissues, must be that the carotid body either possesses unique isoform complexes of AMPK or that signaling by AMPK is somehow modified for specific function.

Additional support for an action of AMPK on O_2-sensitive currents, comes from HEK293 cells, where AICAR was shown to significantly inhibit outward current via heterologously expressed, rodent TWIK-related K^+ channel, TREK-1 (KCNK2) and TREK-2 (KCNK10), background K^+ channels (468), that have been identified in type I cells (929). Hypoxia (11 mmHg Po_2; 6–7 min) could also, reversibly, inhibit TREK conductance by around 40%. The action of AICAR (AMPK) was mediated via selective serine residues in the C terminals of the channels, which were also targets for protein kinase A (PKA) and PKC. Functional knockdown of AMPK attenuated the inhibitory effect of metabolic stress upon TREK-1 and TREK-2, but not TASK channels, suggesting a more prominent role for TREK than TASK

channels in this system. These data conflict with previous reports where TREK channels expressed in HEK293 cells were unaffected by more intense hypoxia (103) and, although the shorter (*ca.* 3 min) duration of hypoxia in these studies may have precluded observation of a metabolic, indirect effect upon the channel as was suggested by Kreneisz et al. (468), the use by these later workers of non-HCO_3^- buffering and room temperature in their experiments may also have had significant effect upon the findings. Finally, in further support of a key, functional role for AMPK in chemosensing, is the brief description of a reduction in the respiratory frequency response to hypoxia in AMPK-α2 knockout mice (246). A more complete report will, however, be required before the full implications of this important finding can be appreciated.

Neurotransmission in the Carotid Body

Neurosecretion appears fundamental to chemosensory excitation by hypoxia and the carotid body hosts a large number of putative neurotransmitters/modulators as defined, to varying extents, by their presence within type I cells and/or nerve endings, their Ca^{2+}-dependent, stimulus-induced release and the presence of specific receptors. These include an assortment of vesicular-bound, biogenic amines [(ACh), DA, norepinephrine (NE), 5-HT (884, 945)], purines (ATP, adenosine), neuropeptides [enkephalins (884), substance P (439), NPY (462, 713)], calcitonin-gene related peptide (484), cholecystokinin (876), ANP (881), endothelins (354), and amino acids (GABA) (650), and enzymes generating gas transmitters including NO, CO, and H_2S (see sections below for details regarding these three gases). Finally, both histamine (453) and angiotensin II (521) have also been localized to the carotid body where they may be involved in modulating the response to ACh (827) or the pathological changes in chemosensitivity observed in heart failure [(529); see later section], respectively.

Catecholamines, particularly DA, are stored within the dense core vesicles of type I cells and are responsible for their characteristic, chromaffin-like appearance across all species. A high degree of colocalization of these neurochemicals also exists within the type I cells, particularly of biogenic amines with neuropeptides (884). As might be expected, a similarly diverse array of ionotropic and metabotropic receptors has also been identified on both type I cells and nerve endings. Extensive reviews of the role of neurotransmitters in the carotid body have been published previously (269, 279, 315, 401, 627, 715, 939) and thus a general overview only will be presented here.

The neurochemicals play an interacting, complex, and critical role in the sensory transmission process between the presynaptic, type I cells and the postsynaptic afferent nerve terminals, with each type I cell having the potential to generate or inhibit afferent neural discharge depending upon the specific pattern of chemical release and the availability of pre- and postreceptor subtypes in the vicinity of release. The potential to also provide for the longer term plastic changes known to occur with chronic or IH may reside within these chemicals. A consensus has yet to be reached on the precise chemical nature of neurotransmission in the carotid body and it should be noted that species differences, genetic differences and age differences have all been described as having significant effects upon the carotid body neurochemical content and receptor expression (780). It may therefore be unrealistic to attempt a categorization of putative transmitter substances into simple classifications based upon whether they induce excitation or inhibition.

Likewise, caution should be applied to the interpretation of experimental data where exogenous agonist or antagonist drugs have been applied without precise knowledge of their site of action, the affinity of the endogenous receptors or the feedback/forward mechanisms induced by their application. That said, and given that cryodestruction of type I cells abolished chemosensitivity, whilst nerve endings remained intact (864), it is generally

accepted that the nerve endings are not themselves chemosensitive and thus are dependent upon neurotransmitters released from type I cells for the generation of postsynaptic potentials. However, although chemical transmission between type I cell and afferent nerve ending appears to be necessary, it might not be sufficient for the entire transduction process. Thus, it is worth noting that a role for nonchemical, electrical synapses has been forwarded (248) and also that the entire premise of classical neurotransmission in the carotid body has been questioned (218, 811). Donnelly (218) observed that afferent action potentials appear not to be dependent upon synaptic depolarizing potentials in the nerve terminals, as would be expected, but instead, are linked to a process endogenous to the nerve terminals themselves whereby an increased variability in excitability is brought about by channel noise, developed by a persistent Na^+ current (263). In this proposal, chemicals released from type I cells during stimulation would act to modulate rather than generate the nerve terminal excitability and afferent action potentials should therefore persist in the absence of type I cells. Further work is required to confirm or refute this interesting hypothesis.

Source of Ca^{2+} for neurosecretion

Mean, intracellular Ca^{2+} concentration in single type I cells or clusters of type I cells cultured *in vitro* has been measured at 55 and 67 nM, respectively with around half of the cytosolic free Ca^{2+} originating from an intracellular site(s) (416). The source of the elevated Ca^{2+} observed during hypoxia-induced neurosecretion from type I cells must originate from an intracellular and/or an extracellular site and the transduction of hypoxia must be linked to a means of elevating the intracellular concentration of this ion. An extracellular origin is now favored by most as had been suggested by early experiments utilizing Ca^{2+} channel blocking drugs to successfully inhibit the responses to hypoxia with afferent discharge (781), catecholamine release (644), or Ca^{2+} influx (703, 766) used as functional readouts.

In contrast, Biscoe and Duchen proposed an intracellular, mitochondrial, source of Ca^{2+} in a linked series of papers around 1990 (75–78, 227–229). These authors examined mitochondrial function in isolated rabbit type I cells and described 2- to 3-fold elevations in $[Ca^{2+}]_i$ induced by CN^- or anoxia that was more sensitive than that observable in other neural cell types and they calculated that a normal mitochondrial Ca^{2+} pool of 1 to 2 μM would be adequate to generate the 100 to 200 nM increase in Ca^{2+} observed with high stimulation intensities (228). The mitochondrial uncoupler, FCCP also increased $[Ca^{2+}]_i$ and, crucially, occluded any further response to hypoxia, suggesting a common mechanism for both stimuli. They suggested that the response was due to a reduced intracellular sequestration of Ca^{2+} consequent upon impaired, energy-dependent Ca^{2+} homeostasis that could be affected by altering the forward rate of the mitochondrial electron transport chain and thus dissipating the normally highly polarized mitochondrial membrane potential (228). In their interpretation, Ca^{2+} fluxes through voltage-gated channels, that they themselves had reported previously (229), were a reflection of the rise in $[Ca^{2+}]_i$ but not its cause and acted to alter Ca^{2+} loading and thus to modulate the response. The sensitivity of the system would, they argued, reside in the apparent low O_2 affinity of a unique version of the cytochrome a_3 component of mitochondrial cytochrome c oxidase, as had been previously suggested (580, 581) and now further elaborated as cytochrome a_{592} by Streller et al. (806), whereby the P_{50} would not be the typical 1 μM(equivalent to *ca*. 1 mmHg), but would instead be higher at a value around a normal tissue Po_2 level of 40 to 50 mmHg. Thus, the availability of oxygen becomes critical at much higher Po_2 levels enabling the organ to function *actively* in the transduction of hypoxia. Importantly for their interpretation, they showed that the Ca^{2+} response did not disappear with removal of extracellular Ca^{2+}, although it was greatly attenuated by at least 50%. In this latter respect, it has been noted that these authors did not routinely use Ca^{2+} chelators preferring, instead, to use nominally Ca^{2+}-free solutions and this cast serious doubts upon their conclusions (107) as an extracellular source of Ca^{2+} could

not therefore be excluded. In support of this concern, when the chelator, ethylene glycol tetraacetic acid (EGTA) (1 mM), was used by these authors, a greater attenuation in the Ca^{2+} response was reported, although no data was shown (77).

Further supporting the contention of Biscoe and Duchen, however, was the demonstration of response curves obtained between Po_2 and $[Ca^{2+}]_i$ (77), between Po_2 and mitochondrial membrane potential (228), and between Po_2 and the forward rate of mitochondrial electron transport as measured by the NAD(P)H/NAD(P) ratio (227) in type I cells that were all somewhat hyperbolic in shape and positioned over a physiological range matching closely the shape of the chemodischarge response (with a suitable left shift in the curve position to account for the use of single cells). The report that simultaneous application of low pH could augment the response to hypoxia (77) satisfied one further criterion for O_2 sensing. Additionally, the Ca^{2+} response to hypoxia, but not to a depolarizing, 30 mM K^+ solution showed a strong thermal dependency that was interpreted as evidence of a metabolic rather than a membrane-delineated process (77) and it was suggested that a fall in the ATP/ADP ratio rather than a fall in $[ATP]_i$ was a likely stimulus for an additional, extramitochondrial, but still intracellular (likely endoplasmic reticulum), release of Ca^{2+} (228). The response, although seemingly fast, was still on the order of “tens of seconds” (76), however, and thus somewhat slow relative to the much brisker, *in vivo* response to hypoxia. No explanation was given for this difference.

Support for an intracellular source of Ca^{2+} in carotid body neurotransmission has been produced by the Lahiri’s laboratory who showed that $CdCl_2$ block of VGCCs was without effect upon the cytochrome oxidase inhibited elevation of Ca^{2+} in rat type I cells by the use of high tension CO (589) and that the Ca^{2+}-ATPase inhibitor, thapsigargin, could modulate cat chemodischarge in the absence of extracellular Ca^{2+} (500). However, interpretation of their data is complicated by their observation of an unexpected, reversible action of thapsigargin, which could therefore suggest an action independent of the normally irreversible effect of thapsigargin upon sarcoplasmic Ca^{2+}-ATPase and a lack of confirmation of their rat data with other mitochondrial blocking drugs (109). Taken together, however, these findings show that mitochondrial function appears coupled in some way to hypoxia transduction and whilst, perhaps overstating the importance of intracellular Ca^{2+} stores, do demonstrate that multiple sources of Ca^{2+} are potentially available for the type I cell to utilize in the process of neurotransmission.

As stated above, the elevated intracellular $[Ca^{2+}]$ observed during stimulation of the carotid body by hypoxia is now believed to arise almost entirely, but not exclusively, subsequent to Ca^{2+} entry via voltage-gated, membrane channels as identified in the membrane of type I cells (682). Thus, Pietruschka (703) showed a 170% increase in Ca^{2+} influx into type I cells during hypoxic stimulation and Heshcheler et al. (370) and Sato et al. (766) described inward currents and anoxia-induced Ca^{2+} elevations, respectively, in type I cells that could be blocked by the Ca^{2+} channel blocker, D600. Working at the level of the whole organ, Shirahata and Fitzgerald (781) perfused various, micromolar, concentrations of organic and inorganic Ca^{2+} blocking drugs into the carotid body of cats *in vivo* and showed a marked and dose-dependent reduction in the chemoafferent discharge response to hypoxia. In addition, these latter authors showed that application of the VGCC opener, Bay K 8644, could increase the discharge recorded during hyperoxia, but was without further effect upon discharge during severe hypoxia. Thus, the basal discharge in high Po_2 appears to be dependent, or perhaps even regulated, by tonic Ca^{2+} entry, whilst a complete opening of all VGCC seems to occur in severe hypoxia. The use of micromolar concentrations of dihydropyridine antagonists may, however, have caused actions other than block of VGCC that could include block of store-operated channels, voltage-dependent K^+ channels, Na^+ channels (including those of the afferent nerve axons) and ligand-gated ion channels as well

as inhibition of lipid peroxidation (845). Additionally, although a presynaptic (type I cell) site of drug action was implied, it remained possible that drug actions on afferent nerve endings might have impacted on the results through the inhibition of postsynaptic excitatory transmitter release acting on terminal autoreceptors or receptors located on type I cells to reduce the excitability during hypoxia.

Using nanomolar concentrations of dihydropyridines, Obeso et al. (644), confirmed and extended the earlier findings by showing that dopamine release from whole carotid bodies induced by either high K^+ containing or hypoxic solutions could be inhibited by up to 90% to 100% by either exclusion of Ca^{2+} from the bathing media or by application of dihydropyridine antagonists, thus strongly supporting a role for VGCC as the major source of Ca^{2+} entry in type I cells and implicating cell depolarization as an essential requirement of hypoxia sensing. Intriguingly, the response to CO_2/pH was not inhibited by Ca^{2+} channel blocking drugs, suggesting that either a separate pathway might exist for transduction of these stimuli or that the acidic stimulus might impact upon the efficacy of the antagonists. A complication of using pharmacological agents with whole carotid body preparations is that anything other than total block of response may still enable Ca^{2+} responses to occur through the action of endogenous transmitter feedback mechanisms acting presynaptically on type I cells (416) and thus such studies should be interpreted with this caveat in place. However, these data regarding the effect of hypoxia on whole organs were later largely confirmed at the single type I cell level by the use of amperometric techniques in rabbit (849) and rat (349).

Further evidence against a role for an intracellular source of elevated Ca^{2+} during chemotransduction arises from the systematic use of pharmacological agents targeting ryanodine receptors (RyR) and Ca^{2+}-ATPases in rabbit carotid bodies where no effect upon basal or depolarization-induced elevation in $[Ca^{2+}]_i$ or release of catecholamine was observed in the presence of these agents (867). In the rat, however, a small elevation of $[Ca^{2+}]_i$ in response to caffeine is observable in the absence of extracellular Ca^{2+} (106), that at least demonstrates the existence of RyR coupled to Ca^{2+} release in type I cells.

Direct evidence of the importance of VGCC in mediating stimulus transduction in type I cells was produced by Buckler and Vaughan-Jones (106, 107) in rat cells (Fig. 8) and by Urena et al. (849) in rabbit cells who both utilized single cell electrophysiological techniques to demonstrate a necessary requirement for extracellular Ca^{2+}. Stimulus-induced elevations in intracellular $[Ca^{2+}]$ were graded with Po_2, with a cell-dependent, variable threshold between 19 to 35 mmHg, increasing around 10-fold in the presence of severe hypoxia in both species and inhibited almost entirely (98%) in rat type I cells or entirely in rabbit type I cells by the removal of extracellular Ca^{2+} (+EGTA). The elevation in Ca^{2+} with decreasing Po_2 appeared not to be saturable, which implies that failure to sustain chemodischarge in severe hypoxia is most likely due to elements of the transduction process occurring subsequent to Ca^{2+} entry. In rat cells, both 10 μM nicardipine, or 2 mM Ni^{2+} reduced Ca^{2+} entry in anoxia by around 70%, whilst in rabbit cells 0.2 mM Cd^{2+} again was able to inhibit completely the response to hypoxia. Interestingly, in rabbit and rat cells, both removal of extracellular Ca^{2+} or addition of Cd^{2+} or nifedipine did not entirely block the Ca^{2+} response to high, 30 m MK^+ solutions, with a response still discernible in the traces shown (106, 849).

The importance of L-type channels in type I cell neurotransmission in rat cells was confirmed by direct measurement of Ca^{2+} currents where it was shown that although nifedipine blocked just 50% of the total type I cell Ca^{2+} current (681), the inhibition of single cell catecholamine release by the same compound was much greater, at around 80%

(349), exemplifying the nonlinear, power relation between $[Ca^{2+}]_i$ and transmitter release (213) as indicated by a Hill coefficient close to 2 (45).

The definitive evidence for an essential requirement of cell depolarization in the transduction process was, however, provided by Buckler and Vaughan-Jones (107) who noted that Ca^{2+} entry in anoxia was temporally correlated with a membrane depolarization (which they termed a “receptor potential") of 23 mV and performed the critical observation that the elevation in Ca^{2+} could be attenuated by 50% and greatly slowed ($d[Ca^{2+}]_i/dt$ reduced from 254 to just 3 nM s^{-1}) if the anoxia-induced depolarization was prevented by voltage clamping the type I cell near its resting membrane potential of between −40 and −60 mV (Fig. 7). The importance of VGCC is thus confirmed in these studies, but the presence of a residual Ca^{2+} elevation during voltage clamp, coupled with the almost complete block of Ca^{2+} elevation by exclusion of extracellular Ca^{2+}, points to a secondary source of Ca^{2+} entry through a voltage-independent pathway that may play a role in initiating the cell response to hypoxia or it may more simply reflect a nonselective, metabolic effect of anoxia upon Ca^{2+}-ATPases enabling an inward Ca^{2+} leak to exceed the capacity of efflux pathways. Regarding the former possibility, the receptor activated, canonical transient receptor potential (TRPC) channels are expressed in the carotid body, with TRPC1 and TRPC4 selectively identified on the nerve terminals surrounding type I cells and, whilst all TRPC subtypes examined (TRPC1/3/4/5/6/7) were localized to type I cells, TRPC1/7/6 were less obviously associated with the plasma membrane that the other subtypes, with TRPC3 being the most abundantly expressed throughout the cytoplasm and extending to the cell membrane (115). It remains to be established whether these channels may provide the voltage-independent entry pathway for Ca^{2+} during hypoxia as previously identified, although evidence for their role in glucose sensing in the carotid body has been reported whereby removal of extracellular glucose induced a small depolarization in type I cells of 9.6 mV that was not dependent on a reduction of outward K^+ current (303).

The L-type Ca^{2+} channels of the rat type I cell were shown to be unremarkable in their properties (275), activating above −20 mV, peaking in current amplitude at 10 to 20 mV, showing little inactivation with holding voltages below −40 mV and having a typical conductance of 21 pS. In the rabbit, the activation of Ca^{2+} currents occurred at slightly more depolarized membrane potentials of −40 mV (850) but were otherwise similar to that of the rat. In addition to L-type channels, the presence of N, P/Q, and R-type Ca^{2+} channels have also been demonstrated in rat and rabbit type I cells (235, 275, 659, 681, 809). In rabbit cells, L- and P/Q-type channels appear to be the only subtypes whose activation is coupled to neurosecretion, despite carrying, between them, less than 50% of the total Ca^{2+} current (746), although as mentioned above, the relation between $[Ca^{2+}]_i$ and neurotransmission is not a simple one (Fig. 9). Irrespective of subtype, a variable number of Ca^{2+} channels, ranging between 15 and 58 per type I cell, has been reported (414), that would account for natural variation in Ca^{2+} current amplitude between cells and perhaps could account for the range of Po_2 thresholds observed by Buckler and Vaughan-Jones (107) for intracellular Ca^{2+} elevation. It could be speculated that gap junctions may act, in the intact carotid body, to functionally couple type I cells and therefore to attenuate this between-cell variability, reduce intracellular fluctuations and/or provide a means of augmenting sensitivity in cells through propagation of Ca^{2+} oscillations or waves as occurs in many other systems (231).

Acetylcholine

Acetylcholine was the first chemical substance identified as a possible transmitter in the carotid body (770) and appears to fulfill a number of the criteria of a classical excitatory transmitter in this organ (279), although the significance of its role in hypoxia sensing has long been questioned due primarily to the inability of cholinergic blocking drugs to attenuate

the chemoresponse to either anoxia or cyanide (219, 225, 569, 762). In support of its role in transmission, however, ACh is found within the carotid body (273, 440) together with choline acetyltransferase, the rate-limiting enzyme for ACh synthesis, in rat (626), cat and rabbit (883), and its degradative enzyme, cholinesterase, (51, 84). The content of ACh has been estimated at 5.6 p mol/carotid body and was little changed following sinus nerve section, which was taken to indicate that ACh was not significantly localized within afferent nerve endings (273) and is most likely stored within the clear vesicles of type I cells. More recent observations, however, have shown the presence of mRNA and immunoreactivity for the vesicular ACh transporter (VAChT) in microganglion cells of nerve fibers innervating the carotid body and in postganglionic parasympathetic fibers in newborn and adult rats but no coexpression of ACh traits in tyrosine-positive, type I cells (306). The possibility of an alternate splice variant for VAChT in type I cells was considered a remote possibility in these experiments and a neuronal localization for VAChT was subsequently confirmed (131). In response to electrical stimulation (260) or to the more natural stimuli of hypoxia and hypercapnia (283, 286), the cat carotid body releases ACh, although the amount liberated by anoxia would not account for the discharge observed in response to O_2 lack (219). In marked contrast, however, ACh release in rabbits was reversibly *reduced* by up to 68% by hypoxia from a basal level of 5.9 fmol.min^{-1} (440). If, however, blocking concentrations of atropine or domperidone were present, hypoxia then induced ACh release and the data thus suggests that hypoxia inhibits ACh release via the activation of muscarinic (M_2) and dopaminergic (D_2) inhibitory autoreceptors that convert the initial faciltatory effect of hypoxia on ACh release into a sustained inhibition.

Exogenous application of ACh or nicotinic agonists causes a dose-dependent increase in chemodischarge in most species (205, 285, 569, 621, 630) except, however, in the rabbit, where discharge is decreased (212, 257). This species difference appears to be dependent upon the presence of excitatory nicotinic receptors in most species (260) with α3, α4, α5, α7, β2, and β4 subunit mRNA identified in the mouse carotid body (164), with a7 specifically present on nerve terminals of the cat carotid body (783) and α3, α4, and β2 located on type I cells (378), of which pharmacological examination suggests that the α2β4 subunits are the most functionally relevant in the rat (170), although a particularly high sensitivity to α3β4 has been reported in the cat (739).

The inhibitory effect of ACh in rabbits appears mediated via muscarinic receptors (285) and can be antagonized by atropine. Why there should be such a marked difference between rabbits and other species in the action of ACh is not known, but such a difference is also present for DA (see below). ACh acts upon afferent, petrosal ganglion neurons to cause depolarization and action potential generation that can be mimicked by nicotine and blocked by hexamethonium (24, 950). However, neither basal discharge nor the response to hypoxia is fully antagonized by ACh antagonists (630, 951) at concentrations that inhibit the excitatory effect of exogenous ACh. In this respect, the labeling of nicotinic, α-bungarotoxin binding and muscarinic, quinuclidinyl benzylate binding sites on the membranes of type I and type II cells (141, 209, 211) and the lack of effect upon the degree of binding following sinus nerve section would further support the idea that ACh is not a primary chemoexcitatory transmitter acting postsynaptically in the carotid body. However, high doses of the nicotinic antagonist, mecamylamine, or the nonselective, muscarinic antagonist, atropine, when localized to the carotid body in a perfusion circuit *in vivo* significantly attenuated the response to hypoxia (284, 285) particularly if applied together and the conclusions arising from the earlier α-bungarotoxin studies were criticized as α-bungarotoxin binding would not have been selective to the specific nicotinic receptor subtypes subserving neurotransmission (641). Additionally, Fitzgerald et al. (285) showed an excitatory effect of muscarinic M_1 receptor activation and an inhibitory effect of M_2 receptor activation upon hypoxia mediated chemodischarge and thus concluded that the

number, distribution, affinity and site accessibility to pre- and postsynaptically located cholinergic receptors might account for the lack of a consensus regarding a role for ACh in mediating neurotransmission between different carotid body preparations. However, these data were questioned by Donnelly (219) who, using an *in vitro* rat preparation, showed that mecamylamine at low doses (50 μM) *augmented* chemodischarge responses to hypoxia and that the inhibitory effect noted at higher doses (500 μM) were most likely artefacts arising from reduced action potential amplitude and nerve conduction failure. Thus, ACh most likely acts as a neuromodulator rather than as a transmitter in the carotid body.

In support of a presynaptic, modulatory role for ACh, Dasso et al. (183) showed that approximately 50% of isolated rat type I cells tested could respond with an elevation in $[Ca^{2+}]_i$ when challenged with ACh that could be largely blocked by either mecamylamine or atropine, with the majority of cells sensitive to both antagonists. The mechanism underlying the responses were, however, different with nicotinic receptor activation causing only transient elevations in $[Ca^{2+}]_i$ that were dependent upon Ca^{2+} influx, whilst the first phase of muscarinic response was dependent upon intracellular sources of Ca^{2+} with only the secondary, adapted phase dependent upon Ca^{2+} influx. These results are consistent with the inward currents recorded in response to nicotinic stimulation of isolated rat type I cells (920) and thus suggest that ACh plays a modulatory or permissive role in chemotransduction via an action on type I cell autoreceptors. This modulatory role appears to be mediated via the nicotinic receptor-activated release of catecholamines (50, 210, 312, 641, 779) and ATP/adenosine (170) from type I cells with the post synaptic consequence dependent upon the presence of appropriate receptors for these and other transmitter/modulator substances coreleased by the same stimulus, of which ATP appears the most important (947).

Dopamine

The metabotropic, biogenic amine, DA, like ACh, meets many of the criteria associated with a classical neurotransmitter, but, like ACh, its role in neurotransmission in the carotid body is not yet fully established and the presence of multiple receptor subtypes and location as well as a significant species difference may underlie some of the discrepancies noted. Thus, in the rabbit carotid body, DA may be the primary transmitter involved in mediating the hypoxia transduction in type I cells into action potentials in afferent nerve axons, but in other species it most likely acts as a neuromodulator. DA is the predominant catecholamine found in the dense core vesicles of type I cells in cat, (940), rat (868), and rabbit (317) and from where it is released by hypoxia stimulation (49, 271, 868) in much greater quantities than NE (312), as might be predicted from the relative contents of these two catecholamines in the carotid body. The presence of TH and dopamine β-hydroxylase (884) are correspondingly also localized to the type I cell and TH has been further localized to within the dense core vesicles of the dog carotid body (429). Note that markers of TH are often used to identify type I cells, but care should be taken, especially in culture preparations, to ensure that other cell types of similar morphology are not mistakenly identified (924). Carotid body DA synthesis in rabbits is increased by exposure to 3 h of hypoxia (270) but not in rats exposed to much shorter bouts of hypoxia (332). TH is also found in nerve fibers and ganglion cells innervating the carotid body (884), most likely related here to the organ's autonomic innervation.

The release of DA by hypoxia is dependent upon voltage-gated Ca^{2+} influx in the rabbit (644) as well as in rats and cats (743, 868), although the release induced by CO_2/acidosis in the cat and rat appears inconsistent or minimal (400, 868) and in the rabbit, although release in response to CO_2/acidosis was Ca^{2+} influx dependent, it appeared not to be mediated via dihydropyridine-sensitive Ca^{2+} entry (644). It is worth recalling that the effects upon DA release observed with CO_2/acidosis would most likely be affected by a direct inhibitory

effect of protons upon exocytosis and Ca^{2+} channels (745). The release of DA is strongly correlated with the intensity of hypoxia stimulation in the rabbit and with afferent discharge (271) although it was noted by these authors that Ca^{2+}-free media, whilst ablating the release of DA during hypoxia, only reduced chemodischarge by 50%. DA release from the rat type I cell has since been confirmed by quantal resolution, amperometric recordings (849) where a single quantal event had an average charge of 46 fC, correlating to *ca.* 1,40,000 catecholamine molecules per vesicle (670).

Application of exogenous DA in the rabbit induces an excitatory response, yet in other species the release of DA is not so tightly associated with increases in neural discharge and repeated bouts of hypoxia have been applied to show that discharge responses in the rat are maintained despite decreases in the rate of release of catecholamine (215, 402). In addition, the discharge responses to hypoxia in the rat peaked *ca.* 10 s before any rise in catecholamine release was observable and at least 60 s before the peak in catecholamine release (215) and, in the cat, the peak catecholamine response lagged the discharge peak by an even greater amount, of at least 3 min (402) and in both a catecholamine release profile was seen that differed qualitatively in pattern from that of the discharge. Clearly, the relation between catecholamine release and discharge is not a simple, excitatory one in the rat or cat. DA appears therefore to be inhibitory in all but the rabbit and thus, in the cat, a continuous infusion of DA *in vivo* decreases chemoreceptor sensitivity to hypoxia and CO_2 (538) whilst a bolus injection of 10 μg DA will transiently inhibit hypoxia-induced discharge (401). Likewise, domperidone can elevate basal discharge and can block the inhibitory effect of DA on the sensing of natural stimuli, even enhancing the response to hypoxia in some cases (403) and thus the inhibitory action of DA has been attributed to a $G_{\alpha i}$-mediated, postsynaptic action on D_2 receptors (46, 180).

The inhibitory effect of DA is susceptible to desensitization after repeated bouts of DA application and high, repeated doses of DA can eventually lead to an excitatory response in the cat (937) in a manner similar to that observed with D_2 receptor block. However, only 64% of D_2 receptor density was lost 14 days after sinus nerve section (208) and, as confirmed by D_2 receptor mRNA being localized to type I cells, petrosal ganglion neurons, and sympathetic postganglionic neurons of the carotid body (180), the DA receptors are not simply present postsynaptically to type I cells. Additionally, the presence of mRNA for $G_{\alpha s}$-coupled, D_1 receptors has been reported in rabbit, cat, and rat carotid body also on type I cells, petrosal ganglion cells, and autonomic postganglionic fibers (48). Thus the effect of exogenously applied DA may be further complicated, at least if presented to the whole organ, although it remains to be seen if D_1 receptor *protein* is present. If, however, DA is presented selectively to postsynaptic petrosal ganglion cells, no effect is observed in cat (28). Similarly, in cocultures of rat type I cell with petrosal ganglion cells, D_2 receptor antagonists failed to modify either basal discharge or hypoxia stimulated discharge (951). However, in the rabbit a dose-dependent increase in postsynaptic activity can be observed in petrosal ganglion cells that can readily be blocked by the D_2 receptor antagonist, spiperone (27).

A modulatory, rather than necessary role for DAD_2 receptors in nonrabbit species is further exemplified by the finding that, if DA is applied at low dose prior to application of ACh or ATP to cat petrosal ganglion cells, the sensitivity to the ACh is augmented whilst higher doses of DA depress the sensitivity to both ACh (28) and ATP (25) with the effect antagonized by spiroperone. A further consideration is brought into play by the observation that nanomolar concentrations of DA, when applied to isolated rabbit type I cells can, like NE, selectively and reversibly attenuate the inward Ca^{2+} channel current, in this case by around 40% (67), thus perhaps providing a feedback mechanism to inhibit further transmitter release. It is not yet determined whether a similar mechanism may underlie, at

least in part, the inhibitory action of DA observed in other species. Taken together, the results are not confirmatory of an excitatory transmitter role for DA in nonrabbit species but instead indicate a modulatory role, shaping the responses to other transmitter substances. However, DA may act in the rabbit as a neurotransmitter, thus demonstrating a clear species difference in a fundamental process of transduction. It is worth noting, however, that there are indications that the dog carotid body may respond to DA similarly to the rabbit (88, 409), although both of these studies in the dog were performed *in situ* leaving open the possibility of indirect effects mediated via alteration in carotid blood flow.

Regarding other catecholamines, the content of NE is small relative to DA, with most of the carotid body NE localized to sympathetic neurons (268), but an inhibitory action of NE upon basal and hypoxia-induced chemodischarge in the cat *in vivo* has been reported, acting via α_2 receptors (464) mediating inhibition of a voltage-dependent, but resistant (non-L-, N-, or P/Q-type), macroscopic Ca^{2+} current in type I cells (32, 660), in addition to any high-dose effect of β-adrenoceptor mediated vasoconstriction of carotid body blood vessels.

Adenosine

The purine nucleoside, adenosine, is ubiquitously expressed in cells as an intermediary of ATP metabolism, deriving predominantly from the cleavage of AMP by 5′-nucleotidase or by the hydrolysis of S-adenosylhomocysteine. Extracellular adenosine concentration can be raised via facilitated diffusion via nucleoside transporters and/or by the catabolism of extracellular ATP by membrane-bound, 5′-ectonucleotidases. Upon release, adenosine acts on a number of specific, G-protein coupled, P1 purinoceptors located on pre- and postsynaptic membranes. Molecular characterization has identified four adenosine receptor subtypes; the high affinity A_1, A_{2A} subtypes and the lower affinity A_{2B} and A_3 subtypes (292, 774). Degradation of adenosine occurs via the action of adenosine kinase and adenosine deaminase. During hypoxia, the intracellular production and consequent release of adenosine is increased rapidly (514, 915). The release of adenosine from the carotid body during hypoxia arises, unsurprisingly, both from ATP catabolism and nucleoside transporter mechanisms, but appears to have a lower O_2 threshold than other tissues, such that a 44% increase above basal levels was noted in the carotid body at a moderate level of hypoxia at which release was not yet observable from either superior cervical ganglia or carotid artery tissue (169).

A stimulatory effect of adenosine upon carotid body chemodischarge is observable in the cat both *in vivo* (573, 574) and *in vitro* (759) as well as in the rat, *in vitro* (852), demonstrating an effect that must therefore be independent of its known action upon blood flow (699). An excitatory effect of adenosine upon aortic chemoreceptors in the cat, has also been reported (758). Importantly, the addition of the adenosine receptor antagonist, 8 phenyl-theophylline, could significantly reduce the carotid chemoreceptor discharge response to hypoxia in cats (575) and another antagonist, caffeine, had a similar, inhibitory effect upon hypoxia-mediated chemoexcitation in rats (173). The stimulatory effect of adenosine on the carotid body appears sufficient to increase ventilation in both rats (592, 593) and humans (896, 897) and thus demonstrates a “protective” effect of adenosine at a systemic level that complements a similar role at the cellular level where its actions can broadly be taken as aimed at either increasing O_2 delivery or reducing O_2 demand. It should be noted, however, that in a different study, caffeine application to cats had no sustained effect upon carotid body chemodischarge in hypoxia (47).

Pharmacological and histochemical evidence suggests that adenosine most likely activates A_{2A} and A_{2B} receptor subtypes on type I cells (173, 575, 773, 899), as these subtypes have been colocalized with TH in the carotid body (173, 307, 451). However, although it has also been suggested that A_{2A} receptors located postsynaptically, may be of greater functional

consequence than those located on type I cells (173), it appears that both A_{2A} and A_{2B} subtypes contribute almost equally to the modulating effect of adenosine upon chemodischarge in hypoxia. A_1 receptors appear to be present only on rabbit type I cells, where they may modulate Ca^{2+} currents (747) and A_3 receptors are apparently absent from the carotid body (451). Adenosine decreases the type I cell K^+ current amplitude in the rat by an action largely, but not exclusively, upon a Ca^{2+}-insensitive, 4-AP-sensitive component and thus its presynaptic action includes the inhibition of a delayed rectifier component of the outward current (852). Adenosine (largely A_{2B}) receptor activation is known to increase cAMP content in rat carotid bodies (168), an effect that can potentiate the release of catecholamines from the rabbit carotid body in response to hypoxia (696). In addition, the 2- to 3-fold increase in cAMP during hypoxia in rabbit carotid bodies could be entirely prevented by prior application of A_2 receptor antagonists (143). Interestingly, receptor interaction appears to be important in determining the carotid body response to hypoxia as an antagonistic interaction between adenosine A_{2B} and dopamine, D_2 receptors has been reported (168), whereby the augmenting effect of A_2 agonists upon rat carotid body cAMP content could be abolished by D_2 agonists, presumably via actions upon G_s and G_i/G_o proteins, respectively. That the effect of adenosine upon K^+ currents in the rat is mediated via changes in cAMP levels is, however, not supported by direct patch clamp recordings from isolated rat type I cells (348), although in the rabbit, cAMP can decrease K^+ current amplitude (542).

Adenosine triphosphate

Energy rich phosphate compounds, including ATP, are known chemoexcitants (42, 412), with their potency related to the number of energy-rich bonds (224) and even in these early studies, an extracellular site of action was postulated, based on the short latency of effect between intracarotid drug injection and stimulation. A key neurotransmitter role for the purine nucleotide, ATP, has therefore long been considered for the carotid body and we now believe it mediates its main actions primarily through activation of ionotropic P2X receptors, located on the afferent terminals of sinus nerve petrosal ganglion neurons. As with ACh and DA (see above), however, it appears likely that released ATP is not, by itself, solely responsible for mediating the excitatory effect of natural stimuli and is therefore best considered as a cotransmitter, acting in most likelihood with ACh (947). In addition, a possible feedback, modulatory role for metabotropic, P2Y auto inhibitory receptors has also been postulated.

In accordance with its putative transmitter role, ATP is taken up by type I and type II cells and concentrated in the cell nuclei (450), adenine nucleotides are found in large quantity and associated with catecholamines and neuropeptides within dense-core, secretory granules of type I cells (94, 95), and ATP is released, in concentrations up to 500 nM, from the carotid body during hypoxia in a manner dependent upon both cell depolarization and voltage-gated Ca^{2+} entry (122). The stimulus-induced release of ATP has negligible effect upon the total carotid body content of this nucleotide (639). Although exogenously applied ATP or adenosine can evoke a dose-dependent increase in discharge in the cat carotid body (571, 574) and reflex hyperventilation (571), these early functional studies, *in vivo* indicated that the effect of ATP was most likely subsequent to its ectonucleotidase-mediated, hydrolysis to AMP/adenosine as its effect could be potentiated by the adenosine uptake inhibitor, dipyridamole and depression of basal discharge, rather than excitation, was observed in response to the stable ATP analogue, α-β methylene ATP. Whilst this latter finding led McQueen and Ribeiro (574) to postulate the existence of purinergic P2 receptors in the carotid body, in addition to the, by then, established P1 receptors, it was subsequently concluded that the depressive effect of α-β methylene ATP applied *in vivo* was possibly consequent upon the concomitant fall in arterial blood pressure, as *in vitro* application of

ATP, in the same species, always generated chemoexcitation (794). In addition, it is possible that α-β methylene ATP may have induced significant receptor desensitization and/or induced inhibition via a possible presynaptic, hyperpolarizing effect on type I cells (588). The study by McQueen and colleagues (571) failed to repeat the inhibitory effect of α-β methylene ATP on chemoreceptor discharge, showing instead a significant excitation but which, additionally, could not be blocked by the P2 antagonist, suramin. In contrast, the actions of adenosine could be readily be replicated *in vitro* (759) and a specific role for adenosine in chemotransmission remains viable with actions identified both at presynaptic and postsynaptic locations in the carotid body (171). It should be noted, however, that suramin does not block P2X4 or P2X7 receptors (625), leaving the possibility of some residual effect being mediated via these receptor subtypes.

Utilising a unique functional coculture preparation of experimentally accessible, presynaptic type I cells and postsynaptic petrosal ganglion neurons (951), Nurse and colleagues demonstrated that ATP could induce a fast depolarizing, suramin-sensitive and slowly desensitizing inward current in rat petrosal neurons (947). Most significantly, however, they also showed that total inhibition of spontaneous and hypoxia-induced postsynaptic membrane activity could be achieved, in around two-thirds of trials, only with the coapplication of ATP and ACh antagonists (947). They thus provided the first convincing evidence of ATP-ACh cotransmission, at least in the carotid body (771) and, in so doing, also incidentally provided a possible explanation for the discrepancies often noted in simpler, single drug, studies on the carotid body.

Immunohistochemical and pharmacological approaches were used to conclude that the postsynaptic effect of ATP was mediated via $P2X_2/P2X_3$ heteromultimeric purinoceptors as found in other sensory neurons (525). A later study by the same group utilized single cell RT-PCR to identify mRNA for both $P2X_2$ and $P2X_3$ subunits in the same postsynaptic neurons and showed also colocalization of the two proteins, with confocal microscopy, in petrosal ganglion cell somas and afferent nerve endings (727). These authors suggested that varying amounts of coexpression of these two subunits might account for intra- and interspecies variability in the responses to ATP brought about by the generation of heterogeneous populations of P2X receptors (535).

A genetic proof of the importance of ATP and its particular receptor subtypes for carotid chemotransmission was provided subsequently by the demonstration of loss of function in mice engineered to be deficient of $P2X_2$, $P2X_3$, or double-knockout of $P2X_{2/3}$ subunits (752). In this study, only mice deficient in $P2X_2$, whether alone or in combination with the $P2X_3$ subunit, showed impairment in both carotid chemosensory discharge and ventilatory response to hypoxia whilst $P2X_3$-deficient animals were no different to the wild-type phenotype in these two readouts. Thus, a crucial role for $P2X_2$ purinoceptors appears established for hypoxia sensing mediated via the carotid body.

Ventilatory sensitivity to elevated PCO_2 was, however, unchanged in all $P2X_2$ and $P2X_3$-deficient animals, which suggests either that the gain of central chemoreceptors is less dependent upon peripheral chemoreceptor input in mice relative to, for example, dogs (92) or that a compensatory mechanism has been invoked in these animals or that $P2X_2/P2X_3$ receptors are not involved in CO_2/acid transmission at the carotid body. This latter possibility is, however, not apparent in the coculture recordings of Zhang and Nurse (946) who showed that the petrosal ganglion response to CO_2/acidosis required the Ca^{2+}-dependent release of ATP with ACh (946) with acidosis possibly acting to potentiate the sensitivity of P2X receptors (805) and that the same afferent neuron could respond to both hypoxia and CO_2 in a greater than additive and ATP-dependent manner (727), as might have been predicted from the synergistic effects of these two stimuli on type I cell intracellular

Ca^{2+} (184). It should be noted, however, that the resolution of current plethysmographic methods to measure hypercapnic responses as utilized by Rong et al. 752), is not sufficient to separate peripheral and central CO_2 sensitivities.

A direct chemoexcitant effect of ATP upon P2X receptors of the afferent sinus nerve terminals, rather than an indirect effect via modulation of type I cell neurotransmission was further confirmed by Alcayaga et al. (22) who demonstrated a dose-dependent increase in chemodischarge induced in isolated petrosal ganglion cells by ATP that persisted after application of the P2Y receptor antagonist, Reactive Blue 2. ACh-ATP cotransmission was also later confirmed in the cat where a combination of hexamethonium and suramin transiently inhibited the discharge response to acid or stop-flow stimulation of identified petrosal neurons, superfused *in vitro* (853). However, in a contradiction of the earlier studies, Reyes et al. (740) showed that neither basal nor hypoxia stimulated chemodischarge, in a perfused-superfused, cat carotid body preparation (where site access and effective drug concentrations are less an issue than in superfused-only preparations), were *not* inhibited significantly by a combination of hexamethanium and suramin at the concentrations used by Zhang et al. (947) in their rat coculture preparation and also that the sensitivity to ATP was relatively low; its ED_{50} for discharge of 20 μg being 10 to 20× greater than for ACh or nicotine (739). Whether this is a(nother) species difference or whether it reflects the methodology used is not known, but it does mean that a key role for ATP-ACh cotransmission is not yet fully established. It should be appreciated that cotransmission as a term does not, of course, imply costorage or corelease (939). As a less-important, related aside, it is perhaps unfortunate that Sun and Reis (811) in their rebuttal of the necessity for chemotransmission in the carotid body, utilized Reactive Blue 2 rather than, for example, a nonselective P2 antagonist such as suramin or pyridoxalphosphate-6-azophenyl-2′-4′-disulphonic acid (PPADS) and so would have failed to antagonise P2X receptors and consequently, at least part, of the excitatory effect of NaCN.

Although certain P2X receptor subtypes can desensitize rapidly, little desensitization was noted in petrosal ganglion discharge responses to repeated, closely spaced (20 s), bolus injections of ATP (22), which contrasts with the marked desensitization observed when ATP is continuously applied for 2 min (794). This does not appear consistent with the sustained chemoafferent discharge observed during a period of hypoxia and it has been suggested that the $P2X_2$ subtype located on the postsynaptic site may reduce desensitization (947). Alternatively, or additionally, an interplay between both excitatory and inhibitory transmitters might need to exist to prolong excitation and a “push-pull” hypothesis has been raised to account for this phenomenon (717). Whether this sustained response may in some way be related to the identified presence of $P2Y_1$ autoreceptors on type I cells (924) or $P2Y_2$ receptors on type II cells of the rat carotid body (923) is not known, but is a possibility and suggests that the responses mediated by ATP will not be simple, postsynaptic ones. Thus, agonist activation of $P2Y_2$ receptors in type II cells initiates an intracellular Ca^{2+} elevation (923) that could be involved in carotid body paracrine signalling. Additionally, whilst exogenous ATP had no effect upon basal Ca^{2+} levels in type I cells, it was able to prevent or reverse the intracellular Ca^{2+} elevation induced in type I cells by hypoxia (924) at high concentrations of up to 100 μM, but even at 500 nM, it could reduce the stimulus-induced Ca^{2+} elevation by up to 40% via an unspecified, membrane hyperpolarising effect. This negative feedback mechanism could account for the inhibitory action of ATP noted by others upon chemodischarge (588). It was suggested by Campannuci et al. (132) that this may arise from the inhibitory action of NO formed subsequent to the P2X receptor activation of efferent GPN, following ATP release from type I cells or from red blood cells (241) during hypoxia.

Neuropeptides

Carotid bodies express a variety of neuropeptides that serve as transmitters or modulators elsewhere in the nervous system.

Substance P—Substance P (SP)-like immunoreactivity is expressed in type I cells as well as nerve fibers of sensory and autonomic origin in the carotid body (140, 305, 489, 721). type I cells express carboxypeptidase-E (CPE), an enzyme necessary for processing of SP (317, 470) and the enzyme neutral endopeptidase (NEP), responsible for terminating the actions of SP, is also found in the interstial spaces surrounding the type I cells and the nerve terminals (471). Kim et al. (439) reported that hypoxia releases SP from rabbit carotid bodies in a stimulus-dependent manner. Hypoxia-evoked SP release requires extracellular Ca^{2+} and activation of N- and L-type Ca^{2+} channels. On the other hand, hypercapnia has no effect on SP release from the carotid body.

Exogenous administration of SP augments the sensory discharge of the carotid body in a number of species (175, 472, 570, 595, 721) with the exception of goats (707). SP potentiates the hypoxic sensory response (721) and, whereas SP receptor antagonists, when given in nanomolar concentrations, prevent the excitatory effects of SP, and abolish the sensory response to hypoxia while leaving the sensory response to hypercapnia unaffected in cats (718, 722, 726) as well as in rats (175). In goats SP reduced ventilation (707). However, carotid body sensory activity was not recorded in this study. It is likely that the reduced ventilation by SP in goats is independent of the integrity of the carotid body and may conceivably be due to either broncho constriction and/or blood pressure changes.

Autoradiographic analysis has also revealed SP-binding sites in the cat carotid body [see (715) for references]. Blockade of NK-1 receptors in the cat carotid body prevented hypoxia-evoked sensory excitation (718) suggesting that SP, acting on NK-1 receptors, participates in the sensory transmission in this species. In rats, although SP stimulates the carotid body activity (175), there is no evidence for NK-1 receptor mRNA either in glomus cells or in the petrosal neurons (305). In rats, the actions of SP therefore may be mediated by other neurokinin receptors (for example, NK-2 or NK-3). SP may directly influence mitochondrial metabolism by acting as a protonophore (725). Therefore, in rats, a dual mechanism, one involving receptor activation (via NK-2 or NK-3) and the other via an action on mitochondria may mediate the sensory excitation by SP. Thus, SP-like peptide(s) may participate in the sensory transmission during hypoxia by mechanisms not strictly adherent to the criteria proposed for conventional synaptic transmission. Interactions of SP with other neurotransmitters/modulators in the carotid body remain to be elucidated.

Endothelin—Endothelin (ET-1) expression is barely detectable in type I cells under basal conditions; whereas prolonged hypoxia markedly increases its expression (354). ET-1 stimulates carotid body activity by acting on ETA receptors (145). Whilst ET-1 by itself had little or no effect on basal $[Ca^{2+}]_i$ in glomus cells, it markedly potentiates hypoxia-evoked elevations in $[Ca^{2+}]_i$ which was attributed to phosphorylation of Ca^{2+} channel proteins (144). ET-1 also increases cAMP levels in the carotid body. These studies suggest that ET is a potent modulator of the sensory response to hypoxia. Recent studies (675) have shown that ET-1 contributes to sensitization of the hypoxic sensory response of the carotid body during prolonged sustained as well as IH (see later sections on plasticity).

Enkephalins and other peptides—Nearly 98% of type I cells express Enkephalins (ENK)-like immunoreactivity (269, 884). Hanson et al. (337) reported a decrease in met-ENK content following 30 min of hypoxia in rabbit carotid bodies indicating release of ENK by hypoxia. ENK inhibits the carotid body activity, and naloxone and delta-opiate receptor

antagonist augment the baseline carotid body activity as well as the sensory response to hypoxia (709). The functional significance of ENK in hypoxic sensory transmission of the carotid body is not known.

Type I cells also express ANP. Wang et al. (881) reported that ANP inhibits the carotid body activity and its actions are mediated by cyclic guanosine monophosphate (cGMP). Galanin, NPY, and calcitonin-gene-related peptide (CGRP)-like immunoreactivities were also reported in the nerve fibers innervating the glomus tissue (397, 444). However, the significance of these peptides in sensory transmission has not been established.

Gasotransmitters

It is being increasingly appreciated that endogenously generated gas molecules [e.g., NO, CO, and H_2S] function as transmitters in the central and peripheral nervous system. Unlike classical neurotransmitters or modulators, gasotransmitters are not stored in vesicles. Once generated, they rapidly diffuse to neighboring cells and are inactivated with a half-life of only a few seconds. More importantly, NO and CO share some common properties with molecular oxygen. For instance, like oxygen, both NO and CO are gases and bind to heme, perhaps with greater affinity than oxygen (3, 577). Many of their biological actions are coupled to activation of heme containing proteins and are regulated by the redox state of the cell (591, 792). Similarities with molecular oxygen prompted several investigators to examine their roles in carotid body function.

Nitric oxide—NO is generated during the enzymatic conversion of L-arginine to -L-citrulline and this reaction is catalyzed by the eNOS. Three isoforms of NOS have been isolated: neuronal, endothelial, and inducible (591). In the carotid body the neuronal isoform of NOS is found in the nerve plexuses innervating the chemoreceptor tissue (720, 878, 885). Chronic ablation of sinus nerve abolishes NOS-positive fibers in the carotid body (885). NOS containing neurons are present in the petrosal ganglion, which provides sensory innervation to the carotid body (131, 885). Some ganglion cells in the carotid body also express neuronal NOS (322, 878). The endothelial isoform of NOS is expressed in blood vessels of the carotid body (885). It is uncertain whether glomus cells express NO synthase. Prolonged hypoxia induces inducible NOS expression in carotid bodies, an effect that was more pronounced in younger compared to older rats (202).

The NO synthase activity was measured by [^{3}H] citrulline assay in the cat (720) and rat carotid bodies (880). Omission of Ca^{2+} inhibited the enzyme activity suggesting that NO synthase in the carotid body is primarily of the constitutive type. Acute hypoxia inhibited NO synthase activity in the carotid body (720).

Inhibitors of NO synthase augment the carotid body activity (153, 720, 846) and nitrosyl compunds that liberate NO (NO donors) reduced carotid body activity (153, 880). The role of endogenous NO was further evaluated by using mice with selective disruption of neuronal NO synthase (446). Neuronal NO synthase knockout mice exhibit enhanced carotid body activity evidenced by more pronounced respiratory depression by brief hyperoxia (Dejour's test; an index of peripheral chemoreceptor sensitivity) and augmented ventilatory response to hypoxia. Interestingly, mice deficient in endothelial NO synthase exhibited reduced peripheral chemoreceptor sensitivity and a blunted ventilatory response to hypoxia (447). Since endothelial NO synthase knockout mice had higher blood pressure compared to wild type controls, it is possible that chronic vasoconstriction might have rendered the carotid bodies insensitive to hypoxia.

There are a number of pathways through which NO can exert its actions in the carotid body. One such pathway includes activation of the heme containing guanylate cyclase and

subsequent elevation in cellular levels of cGMP (792). NO synthase inhibitors reduce, whereas NO donors increase cGMP levels in the carotid body (885). Actions of NO may also involve naturally occurring NO derivatives, such as S-nitrosothiols, whose actions depend on the redox environment of the cell (133, 795). Summers et al. (808) reported that NO inhibits L-type Ca^{2+} channel activity of type I cells and Campanucci et al. (132) showed that released NO could hyperpolarize type I cells. These effects may be mediated through S-nitrosothiols but more data is required.

Paraganglia near the carotid body contain nNOS (720) and the VAChT and are intrinsically chemosensitive (129, 130, 306) as well as possessing P2X purinoceptors sensitive to ATP (132) and are presumably the source of the endogenous NO released from the hypoxia-stimulated carotid body (298, 880, 885). As well as inhibiting chemoafferent discharge (21, 23, 720), NO would also induce vasodilation of the carotid body microvasculature and correspondingly, increase total blood flow through the carotid body (615). Accordingly, nNOS deficient mice show both an elevated basal chemodischarge and an augmented sensitivity to hypoxia (446), although the contribution of an altered blood flow to this response was not determined. Utilising data derived from a novel GPN-type I cell coculture preparation, Campanucci et al. (132) proposed that the paraganglia were depolarized directly by hypoxic inhibition of background (TWIK-related) K^+ channels and indirectly by ATP released during hypoxia from either nearby type I cells and/or circulating red blood cells and which activated P2X receptors located on the paraganglia, leading to raised intracellular Ca^{2+} and the nNOS-mediated release of NO. The full physiological significance of these effects has not yet been determined but they do offer a form of local feedback during chemostimulation that may be modulated by pathological states

Thus, Ye et al. (930) reported that chronic hypoxia (10% O_2 for four weeks) upregulates inducible NO synthase expression in the carotid body and acute hypoxia increases NO levels and S-methylisothiourea (SMT), a specific blocker of inducible NOS prevented this effect. These authors suggested that NO generation might play a physiological role in blunting the hypoxic chemosensitivity during chronic hypoxia. Ding et al. (206) reported that decreased NO levels in the carotid body account in part to the augmented peripheral chemoreflex in heart failure rabbits (see later section on heart failure).

Carbon monoxide—CO is synthesized in mammalian cells during degradation of heme by the enzyme heme oxygenase (HO) and the reaction requires molecular oxygen. NADPH and cytochrome P-450 reductase are essential cofactors (548). Two isoforms of heme oxygenase have been characterized including an inducible HO-1 and a constitutively expressed HO-2 and the later is predominantly expressed in neuronal cells. HO-2 is expressed in type I cells of the cat and rat carotid bodies (719). HO-2 immunoreactivity was not seen in nerve fibers and supporting cells.

Zn-protoporphyrin-9 (ZnPP-9), a potent inhibitor of HO, augments carotid body sensory activity in a dose-dependent manner and exogenous administration of CO reversed this effect (719), suggesting that endogenously generated CO, like NO and oxygen, inhibit the carotid body sensory activity. Williams et al. (913) reported that hypoxia evoked inhibition of calcium-sensitive potassium (BK) channels is absent after knockdown of HO-2 expression and suggested that HO-2 is an oxygen sensor that controls channel activity during oxygen deprivation. However, hypoxia-evoked catecholamine secretion from type I cells is preserved in HO-2 null mice (653).

The studies described thus far indicate that NO generated by the nerve endings and blood vessels, and CO produced by type I cells exert tonic inhibitory influence on carotid body activity. How might NO and CO contribute to O_2 chemoreception in the carotid body?

Synthesis of NO and CO requires molecular oxygen (548, 591). Biochemical studies indicate that NO synthase activity is sensitive to hypoxia over a physiologically relevant range in the carotid body (720). The affinity of the heme-hemeoxygenase complex for oxygen is 30- to 90-fold greater than myoglobin (577). Since NOS and HO operate over a wide range of oxygen, it is likely that endogenously generated NO and CO keep the carotid body activity low under normoxic condition, and sensory excitation by hypoxia might in part due to decreased enzymatic generation of NO and CO and thus releasing this inhibition (716).

Hydrogen sulfide—Like NO and CO, H_2S is a gasotransmitter physiologically regulating neuronal transmission (443). Cystathionine γ-lyase (CSE; EC 4.4.1.1) and cystathionine β-synthase (CBS; 4.2.1.22) are the major enzymes associated with generation of endogenous H_2S (299, 606). CBS is the predominant H_2S synthesizing enzyme in the brain, while CSE preponderates in the peripheral tissues whose H_2S levels are reduced 90% in $CSE^{-/-}$ mice (299). Li et al. (526) reported that CBS is the major enzyme expressed in the mouse carotid body and inhibitors of CBS but not CSE impairs carotid body sensory response to acute hypoxia. On the other hand, a study by Peng et al. (684) showed that CSE is localized to type I cells of mouse and rat carotid bodies as evidenced by colocalization with TH a marker of type I cells (Fig. 10). More importantly, hypoxia increased H_2S generation in a stimulus-dependent manner in the carotid body in wild type but not in CSE knockout mice. Peng et al. (684) further demonstrated that low H_2S generation during normoxia is due to inhibitory effects of CO generated by heme oxygenase. Furthermore, CSE knockout mice displayed selective impairment of carotid body response to hypoxia but not to CO_2 (684). Despite the differences in the enzymatic source of H_2S generation, these studies (526, 684) demonstrate that H_2S is an excitatory gasotransmitter in the carotid body.

The excitatory effects of H_2S on carotid body activity seem to require Ca^{2+} influx (526, 684) and may involve inhibition of maxiK currents (526, 821), but at a site of action different to that proposed for the action of CO (98). Interestingly, it has been proposed that H_2S also mediates O_2 sensing by trout gill chemoreceptors indicating it is ancient and well conserved system across phyla (647). It has also been reported that H_2S is involved in hypoxic pulmonary vasoconstriction (646, 648), lending further support to its role in O_2 sensing.

Clearly, the carotid body possesses multiple transmitters and modulators, acting in concert to both excite and inhibit chemodischarge depending upon their specific concentrations and receptor affinities, their temporal and spatial relations with regard to the sites and timing of their release and even the maturational state of the animal. The temporal relations regarding release are particularly important when considering transduction mechanisms. Thus, an initial, stimulus-induced, release of excitatory postsynaptic transmitter could, via feedback modulation on autoreceptors, lead subsequently either to release of more of the same or another excitatory transmitter, cause release of an inhibitory transmitter and/or attenuate of release of the initial transmitter. The permutations are considerable given the complexity of the organ's repertoire of transmitters and receptors. In addition, species differences appear to be a real phenomenon in terms of transmitter utilization in carotid body chemosensory transduction and the different methodologies and functional readouts used in different laboratories appears to be a potential source of discrepancy. As the response to natural stimuli is almost always sustained, albeit with adaptation in some cases, it is apparent that the feedback modulation pathways described cannot exceed the forward gain of any excitatory pathway. These modulatory pathways may therefore play a role in the acute sensing of stimuli but probably play a greater role in mediating the plastic changes in chemoreceptor function noted with the adaptation to chronic hypoxia in health and disease (see later section on plasticity). A greater understanding of their functional roles may thus be

of potential clinical interest for the future development of selective therapeutic agents aimed at relieving the adverse consequences of O_2 lack.

Other Physiological Stimuli of the Carotid Body

Hyercapnia and acidosis

Carotid body chemoafferents are stimulated by rises in $PaCO_2$ which may account, even in hyperoxia, for up to 30% to 50% of the respiratory drive induced by systemic hypercapnia (100, 365, 611), with some of the reflex response due to a peripheral chemoreflex-mediated sensitization of central chemoreceptors (92, 181). In addition, the time for a ventilatory response to a hypercapnic stimulus is increased in carotid body denervated animals (18, 788). This is not an insignificant contribution by the carotid body and it is therefore perhaps surprising that the study of CO_2 transduction in the carotid body has received relatively little attention over the years, especially given the potential role this may play in the prevention of sleep disordered breathing (788).

The carotid body response to CO_2 differs from of that to O_2 in a number of ways. Thus, in contrast to O_2, there is a threshold for CO_2 excitation of the carotid body, and afferent discharge in normoxia can be silenced if $PaCO_2$ falls below 18 to 25 mmHg (63, 254). The threshold can be varied by hypoxia: if Pao_2 is reduced below *ca.* 60 mmHg, the $PaCO_2$ threshold reduces in an approximately linear fashion to be less than 10 mmHg from below a Pao_2 of *ca*. 30 mmHg (491, 493). There appears to be no level of hypoxia-induced discharge that cannot be abolished by appropriate reduction in PCO_2. Whilst this may be taken to imply that the hypoxia sensing mechanism has an absolute requirement for CO_2/acidosis, it may more simply reflect the necessary, but nonselective, need for an optimal pH range for normal physiology. Above the threshold, at a fixed Po_2, the chemoafferent response to PCO_2 increases linearly through the physiological range until *ca.* 65 mHg $PaCO_2$ (80) after which the rate of rise decreases until discharge plateaus when $PaCO_2$ tensions reach a value in excess of 70 to 80 mmHg (80, 254, 280). This differs from increasing hypoxia, where single-fiber discharge increases until it fails, with little indication of a plateau. It is worth noting that, at the plateau level of discharge, whilst further increases in PCO_2 do not increase discharge further, the discharge is not “saturated” as lowering the Po_2 can further increase it. Remarkably, discharge can be maintained steady even at a $PaCO_2$ >200 mmHg, if Pao_2 is held normoxic (80). However, the amplitude of chemoafferent response to CO_2 over physiological ranges of stimulus intensities is small when compared to the response to physiological levels hypoxia. Thus, even at peak stimulation intensity, the afferent discharge response to hypercapnia is often only, at best, less than 50% of the peak response to hypoxia (282, 493, 693) and this reduced potency of CO_2 has been ascribed to direct, inhibitory effects of hypercapnic acidosis on both VGCCs and the exocytotic processes of type I cells (745).

If a step increase in $PaCO_2$ is applied to the carotid body, the response is characterised by an initial fast overshoot in discharge, peaking within 1 s, followed by an adaptation phase with a half time of 5 to 10 s before a new a steady level is achieved (90). Similarly, an undershooting response in afferent discharge, with adaptation, is observable on return to a lower $PaCO_2$. Thus, in contrast to the response to Pao_2, the response to $PaCO_2$ is more rapid and has both a dynamic and a steady-state component. This speed of response makes it more likely that natural fluctuations in $PaCO_2$ rather than Pao_2 might generate proportional oscillations in chemodischarge, independent of the mean level of blood gas tensions and which could provide feed forward information to the CNS regarding exercise intensity (57, 58, 480), perhaps with an augmenting influence from some other blood-borne factor (71, 553). Certainly, oscillations in blood gas tensions and pH can modulate ventilation in anesthetized dogs and cats (177, 822), but some concerns remain regarding the fidelity of

the oscillating signal transmitted in blood to the carotid bodies of conscious, free-breathing mammals (631). However, the speed of response of related, intrapulmonary CO_2 receptors in birds does appear able to provide natural breath by breath control of breathing, at least in this class of animals (266).

Chemoreceptor discharge can be altered by changes in blood pH (253, 872, 954) and the question of whether CO_2 stimulates the carotid body via its molecular form and/or whether through alteration in pH, has long been debated. In addition, as alterations in PCO_2 and/or pH *in vivo* can influence O_2 delivery via shifts in the position of the O_2-hemoglobin dissociation curve, the interaction of these three stimuli need also be considered when interpreting data generated *in vivo*. Thus, if, during normoxia *in vivo* arterial pH is held constant by the systemic infusion of HCO_3^- whilst $PaCO_2$ is elevated, chemodischarge is still able to increase (80) and at any fixed level of $PaCO_2$, an increase in pHa decreases discharge frequency (710). In addition, superfusing the *in vitro* carotid body with isohydric hypercapnic solutions elicits an increased rate of synthesis and release of dopamine and an augmentation of sinus nerve discharge (733, 742), thus providing evidence contrary to the concept of pH/CO_2 acting upon local vasculature of the carotid body (612).

The uncatalysed hydration of CO_2 to equilibrium is quick, with a half time of 3.7 s in strongly buffered solutions, or even less if H^+ is not held constant (494), but this reaction can be accelerated by 0.5 to 1.0×10^6 times via the action of the zinc metalloenzyme, carbonic anhydrase, with the rate-limiting factor being the transfer time of products and substrates between the environment and the zinc atom located within the enzyme. The localization of this enzyme in the carotid body may therefore be of functional consequence and indirect experiments to address this showed that the dynamic carotid body response to PCO_2 both *in vivo* and *in vitro* could be reduced or abolished by the sulphonamides, acetazolamide (90, 321) or methazolamide (404), membrane-permeant inhibitors of carbonic anhydrase, but was not affected by the membrane-impermeant isoform, benzolamide (340). Carbonic anhydrase inhibition reduced basal discharge around 5-fold with a half time of 19 s (404) and this may account for the significantly reduced peripheral CO_2 ventilatory sensitivity observed when low-dose acetazolamide was administered to anesthetized cats (873). Caution should however, be applied when interpreting data when acetazolamide has been used as a carbonic anhydrase inhibitor, as the compound is only very slowly permeating and can also directly affect maxiK channels (700, 701). Methazolamide is more selective for carbonic anhydrase. Carbonic anhydrase is localized within the cytosol and is also membrane bound in type I cells (741) and thus, whilst molecular CO_2 *per se* cannot be entirely excluded as a signaling molecule, the evidence suggests that the carotid body response to CO_2 is not mediated via variation in extracellular pH but is subsequent to the intracellular acidification of type I cells. The carotid body also responds to nonrespiratory acidosis, generating chemoafferent response curves to non-CO_2-dependent variations in pH that are similar to the response to changes in CO_2 (80, 90, 320) with near-linear responses to pH between *ca*. 7.7 and 6.9 (494), from where it tends to plateau. Whist this may provide a unique signal for respiratory drive during the acute metabolic acidosis of exercise (908), the stimulus most likely acts no differently to variations in pH induced by changing PCO_2. However, by determining the chemoafferent response to increases in arterial PCO_2 before and after infusing HCO_3^- to increase the pH, Pokorski and Lahiri (710) could show in the cat *in vivo* that, the response to any level of PCO_2 was greater than the response at a lower PCO_2 but at the same arterial $[H^+]$, suggesting that there may be an, as yet undetermined, effect of CO_2 that is independent of its effect on pH.

These findings at the level of the whole organ are in broad agreement with direct fluorometric measurements of intracellular pH (pHi) in isolated type I cells, where at normal levels of PCO_2, the type I cell pHi in HCO_3^- buffered solutions is typically held at a fairly

unremarkable value of between 7.2 and 7.3 (111, 910). Although other values for pHi have been reported, these may have been unduly low due to the cell injury induced by the invasive use of H^+-selective ion electrodes (360) or by the use of non-HCO_3^--buffered solutions (78, 110). The type I cell pHi is well regulated, both by its cytoplasmic buffering capacity, of which CO_2/HCO_3^- buffering makes a considerable contribution to any non-CO_2 acid load (108) and by a number of acid/alkaline regulating mechanisms present in the membrane of type I cells. These include the amiloride-sensitive, Na^+-H^+ exchanger (104, 110, 910) and the Na^+-HCO_3^- symporter (110) as well as a 4,4′-Diisothiocyano-2,2′-stilbenedisulfonic acid (DIDS) sensitive, Cl^-/HCO_3^- antiporter (110) and perhaps an anion permeable channel (799). Consequently, a fall in pHi is met by a regulatory increase in acid (or acid equivalent) extrusion via transmembrane transport.

Irrespective of the measurement technique used or the buffer solution applied, the type I cell steady-state pHi varies proportionally with pHo (104, 110, 111, 360, 910) as was predicted from earlier studies on whole organs and in accordance with expectations from many other cell types (Fig. 11). As the rate of change of pHi differed when pHo was altered by varying PCO_2 rather than when pHo was altered in non-HCO_3^- solutions, the presence of HCO_3^- appears important to the normal physiological function of these cells as also demonstrated by the reduced basal discharge and sensitivity to hypoxia observed in HEPES buffered solutions (951). In themselves, however, these findings are not remarkable and provide little insight into the type I cell's particular ability to transduce a CO_2/pH stimulus. That ability appears to be due to an unusually high correlation of 0.63 to 0.85 between pHi and pHo in these cells (110, 910); a value two to three times greater in magnitude than typically found in most other cells including various types of muscle (19, 20, 240), CNS neurons (837) and neutrophils (787), but more similar to that observed in red blood cells (295). Thus, despite the various pHi regulatory mechanisms described above, the type I cell acts as if it has little ability to resist a change in pHo. The particular sensitivity to CO_2/pH of the carotid body appears therefore not to lie with any specialized proton extrusion mechanism or intrinsic (non-CO_2) buffering power but, rather, more with its tight coupling of pHi to extracellular pH.

The mechanism underlying this tight coupling is not established in type I cells, but a role for a pHo/pHi-dependent modulation of the Na^+-H^+ exchanger has been proposed (110) and partly rejected (910), but in either case would still require this exchanger in the type I cell to possess a particular acid sensitivity. It is worth noting that the aortic chemoreceptors of the cat are barely able to transduce an hypercapnic stimulus, at least if Po_2 is held above a level of mild hypoxia and so must have a considerably lower CO_2 sensitivity than that of carotid chemoreceptors in the same species (495). What the reason for this reduced sensitivity may be, is not known but it may perhaps lie with a lower dependency of pHi upon pHo in the aortic body when compared to the carotid body. This has yet to be confirmed.

The mammalian carotid body possesses an ability to transduce a carbonic anhydrase-mediated change in pHi brought about by natural variations in arterial PCO_2 or pH that is independent of its sensing of hypoxia and with a uniqueness that may have its origins within the particularly high dependency of pHi upon pHo in type I cells. From an evolutionary perspective, it is interesting to note that many of these various findings in the mammalian carotid body have direct correlate with the sensing of CO_2 in neuroepithelial cells of the zebra fish gill (734), where the sensitivity to CO_2 is much greater than that of the mammal.

Interactions between hypoxia and CO_2—Each chemoafferent fiber of the sinus nerve can convey frequency-coded information regarding carotid body hypoxia and hypercapnic stimulus intensity. Although the stimuli and their transduction processes appear to be independent, it appears that they might interact positively in the adult to produce discharge

frequencies that are greater than additive and thus partly account for the so-called "multiplicative" interaction observable at the level of ventilation (618). Accordingly, although there may be additional CNS interaction between peripheral and central chemoreceptors (744), it has long been recognized that when hypoxia is applied together with hypercapnia to present an "asphyxial" stimulus to the carotid body, the resulting chemoafferent discharge frequency is greater than the sum of the two responses when applied independently and this is seen in multifiber and single fiber recordings both *in vivo* and *in vitro* (493, 693). Thus, it is perfectly straightforward to determine a level of relatively high $PaCO_2$ in which chemodischarge is almost abolished by a concurrent high Pao_2 and a level of relatively low Pao_2 where discharge is similarly almost absent due to a reduced $PaCO_2$. If, however, the higher $PaCO_2$ and the lower Pao_2 are applied together, the resulting afferent discharge is greatly increased to many times more than the sum of the frequency responses to the original pairs of stimuli. As a consequence, increasing PCO_2 might be said to augment the sensitivity of the carotid body to hypoxia but it may be equally be stated that hypoxia increases the carotid body sensitivity to CO_2. The plainest way to show this is by determination of a "fan" of linear CO_2 stimulus responses with slopes that increase with increasing hypoxia that can equally be expressed as rightward and upward shifts in the position of the steady state exponential Po_2 stimulus-response curves with increasing hypercapnia.

In contrast, the dynamic sensitivity to PCO_2 appears independent of Po_2 (126, 840) and thus it follows that the degree of adaptation must be Po_2 dependent with the maximum steady-state PCO_2 stimulus response gradients at low Po_2 perhaps representing the dynamic sensitivity without adaptation. Conversely, a transient response to hypercapnia with full and brisk adaptation would appear as a weak CO_2 sensitivity as was proposed to account for the aortic chemoreceptor response to this stimulus (342). It is also worth noting here that stimulus interaction in discharge *in vivo* is not seen if $PaCO_2$ is varied without change in pHo by blood injection of HCO_3^- (390). In addition, the *in vivo* chemoafferent sensitivity to hypoxia appears critically dependent upon the presence of CO_2/HCO_3^- (782). Thus, the assertion postulated by Torrance and colleagues (341, 840) that the hypoxic response of the carotid body is an acidic response that is modulated by Po_2 cannot yet be countered directly, although mechanistic evidence for this is not forthcoming. Indeed, the attractive possibility that variation in pHi may have been a common stimulus for both CO_2 *and* hypoxia, appears untenable as direct measure of type I cell pHi shows little change during stimulation either by hypoxia or metabolic uncouplers and where change does occur, this is not prevented by intracellular alkalosis (109).

Many newborn animals show little carotid body hypoxia sensitivity at birth and a greater than additive interaction between Po_2 and PCO_2 in chemoreceptor discharge is correspondingly not observable in newborn rats from birth until hypoxia sensitivity has become established which is usually around the time of weaning (693). As CO_2-O_2 interaction can be prevented from maturing naturally by artificially resisting the natural rise in arterial Po_2 that occurs postnatally with the onset of air breathing (511), the development of O_2 sensing appears itself to be an O_2-dependent process. This "resetting" of chemoreceptor afferents is translated into a maturational augmentation of the reflex ventilatory responses to hypoxia in animals (339) and humans (127, 912) and it has been suggested that a failure to re-set adequately may predispose infants to cardiorespiratory disturbances that could increased morbidity or even mortality (128). Thus, without sensitivity to hypoxia, the carotid body sensitivity to hypercapnia seems set at a minimal, hyperoxic level, such that decreasing steady state Po_2 simply shifts the PCO_2 stimulus response curve upward without measurable change in slope. In this respect, the newborn carotid body response is somewhat similar to the response of the adult aortic chemoreceptor, where the relatively reduced Po_2 sensitivity of this organ (495) may account for the greatly

reduced degree of multiplicative interaction at this receptor observed from and above arterial normocapnia and over physiological ranges of Po_2. A greater O_2-CO_2 interaction has been reported in hypocapnia (497) which may, perhaps, be the result of a plateau in the discharge response to increased CO_2 being achieved at lower stimulus intensities here than in the carotid body. The mechanism for this measurable difference between the two organs in the adult is not known but may reflect differences in blood flow, the regulation tissue Po_2 or variation in sensor type or sensitivity.

Whether the development of multiplicative interaction in the carotid body might, even in part, account for the augmented hypoxia chemoafferent sensitivity (re-setting) at the carotid (93, 437, 693) and aortic bodies (479) is not known. As resetting can be measured both *in vivo* and *in vitro* this suggests it to be an O_2-dependent process inherent to chemoreceptor tissue and as there is little evidence to suggest gross morphological changes in this organ over this time period (161, 600), the mechanism for the development of interaction most likely occurs with changes at the level of the sensor and/or other elements of the transduction process including Ca^{2+}-dependent, neurotransmitter release (680) and/or ionic conductances of type I cells (347, 890, 891). Alternatively, this maturation may arise from postnatal changes in the degree of coupling between pHi and pHo as Teppema and colleagues have shown that ventilatory CO_2-O_2 interaction in the cat (824) and human (823) could be abolished by low dose acetazolamide acting at the carotid body and postulated that this might represent an alkalizing effect upon type I cells mediated through inhibition of a plasmalemmal Cl^-/HCO_3^- exchanger. Whilst this may also have been, at least in part, an effect of acetazolamide upon maxiK channels (700), there has been no indication of such CO_2-O_2 interaction occurring at the level of maxiK channels in type I cells (691), although a potentiating effect of hypercapnia upon hypoxia has been noted at the level of Ca^{2+} signaling (184). The effect of acetazolamide on CO_2-O_2 interaction in the reflex studies of Teppema and colleagues was less in hyperoxia than hypoxia, which may reflect a functional consequence of the O_2 sensitivity of carotid body carbonic anhydrase (97).

The presence of stimulus interaction in the carotid body need not require a common transduction mechanism for O_2 and CO_2, as the two pathways need only to share common elements, but wherever this interaction does occur, any mechanism(s) proposed for hypoxia chemotransduction should be able to account for its modification by pH/CO_2. In this respect, it may be of note that whilst increasing temperature, a recognized chemoexcitant stimulus, augmented CO_2 sensitivity (512), hyperkalaemia, another chemoexcitant, did not (694).

Hypercapnia and Ca^{2+}—Decreases in pHi, induced by hypercapnic acidosis, isocapnic acidosis, or isohydric hypercapnia can all elevate $[Ca^{2+}]_i$ in type I cells (105). The elevation was not sustained in hypercapnic acidosis or isocapnic acidosis, with the secondary fall in $[Ca^{2+}]_i$ being almost completely adapting in the latter form of stimulation. In contrast, the response to isohydric hypercapnia was more gradual and sustained. These temporal variations in $[Ca^{2+}]_i$ were noted by Buckler and Vaughan-Jones (105) to be remarkably similar to the neural discharge responses to the same stimuli as observed previously (90, 320) and could be abolished in type I cells by removal of extracellular Ca^{2+} or greatly attenuated by application of either the nonselective Ca^{2+} channel blocker Ni^{2+} or by the L-type channel blocker, nicardipine (105, 106, 108). These data support an extracellular source of Ca^{2+} for CO_2/pH-induced neurosecretion as reported also for hypoxia mediated elevations in Ca^{2+} and, similarly, the elevation can be prevented if membrane potential is clamped during hypercapnic stimulation (106). Accordingly, catecholamine secretion in response to acidosis is also dependent on extracellular Ca^{2+} (748). Thus, a common source of stimulus-induced Ca^{2+} elevation is found for both hypoxia and hypercapnia, with voltage-gated, L-type Ca^{2+} channels providing most, but not all of the entry pathway. Summers et al. (808) reported that isohydric hypercapnia augmented, whereas decreasing either extra or

pHi, inhibited L-type Ca^{2+} current in rabbit glomus cells. Isohydric hypercapnia increased cyclic AMP levels in carotid bodies and 8-Br-cAMP mimicked the effect of CO_2 on glomus cell Ca^{2+} current. A PKA inhibitor prevented hypercapniaevoked augmentation of Ca^{2+} currents. Thus, the results suggested that the effect of CO_2 on the L-type Ca^{2+} current is not secondary to changes in pH and seems to be mediated by a PKA-dependent mechanism. Furthermore, the same authors showed that hypercapnia and hypoxia act additively in stimulating Ca^{2+} current in rabbit type I cells.

However, a role for falls in pH leading to reverse mode Na^+-Ca^{2+}-exchange subsequent to activation of Na^+-dependent acid extrusion mechanisms as suggested for the rabbit carotid body (748) appears not essential for rat acid transduction as the Ca^{2+} response to acid in this species is independent of extracellular [Na^+]. Voltage-gated Ca^{2+} entry can be evoked by hypoxia and hypercapnia within a single cell and the response to the two stimuli summate, at least additively, with the response to hypoxia potentiated by concurrent acidosis and vice versa (184). This elegantly demonstrates that convergence of stimuli must occur prior to Ca^{2+} channel activation and supports the concept that stimulus interaction is an inherent property of type I cells. However, unlike the afferent chemodischarge response to a combination of the two stimuli, the degree of interaction in type I cells is relatively variable and weak. Consequently, the hyperbolic intracellular Ca^{2+} response to graded changes in Po_2 was not significantly right-shifted (P_{50} 2.73 and 2.66 mmHg in 5% and 20% CO_2, respectively) by hypercapnia, which could be taken to show that CO_2 is not sensed via alteration in the affinity of a Po_2 sensor.

An alternate view for the relatively weak stimulus interaction at the level of the single cell may, however, lie in the more recent findings of Rocher et al. (745). These authors aimed to ascertain why the catecholamine release and the afferent chemodischarge responses to hypercapnic stimuli were almost always considerably smaller than these responses to hypoxia, despite the acidic stimuli causing, on average, a 2-fold greater degree of membrane depolarization and similar elevations of $[Ca^{2+}]_i$ in type I cells for comparable level of stimulus intensities. Their data provide the convincing evidence of a reversible, direct inhibitory effect of high H^+ concentrations upon both the Ca^{2+} currents induced by acidosis and upon the exocytotic process itself, with a reduction to pH 6.6 from control reducing these currents by 63% and the catecholamine release in response to a Ca^{2+} ionophore by between 45% to 98%.

In addition the hypoxic, but not the hypercapnic, exocytotic response was potentiated by a cAMP-dependent activation of an EPAC (exchange-protein activated by cAMP)-mediated, PKA-independent, mechanism. Although the data was not shown, these authors also describe an essential role for cAMP in mediating the stimulus interaction between hypoxia and hypercapnia. These findings offer a new avenue for carotid body research and the finding of a nonspecific effect of pH on cellular function does not diminish their importance. Thus, the earlier viewpoint that the two major stimuli of the carotid body might differ only in the sensor mechanism(s) for each, appears increasingly less tenable and greater consideration of the methodology used to measure a chemoresponse to either and/or their interaction at the cellular or organ level will need to occur if interpretations of function are not to be confused.

Is the carotid body a glucosensor?

Systemic metabolic regulation requires a link between the active sensing of plasma glucose concentration and a set of regulatory, reflex neuroendocrine responses that act to maintain plasma glucose in the normal range of 4 to 6 mM (*ca*. 70–110 mg dl^{-1}). Whilst a number of central neural sites, including the ventromedial and lateral hypothalamus, are glucose sensitive (119), the main receptor sites for initiating counter regulatory responses to falls in

plasma glucose levels lie in the periphery and are found in β cells of the pancreas (117) as well as in liver (223) and hepatic portal vein (374).

In addition to the above established sites, a potential low glucose-sensing role for cells of the carotid body has also been proposed and its particular blood flow and metabolism would appear to make it a likely candidate receptor for the sensing of a metabolic substrate. However, at present, the various pieces of evidence supporting this novel sensory role appear conflicting. Thus, contrary to expectation, given an initial report that showed no discharge sensitivity to low glucose in an isolated cat carotid body preparation (31), *in vivo* glucose infusion into the vascularly isolated carotid sinus region in the same species was found to reduce carotid body activity by 20% and increase the threshold to hypoxia (35). In addition, direct carotid chemoreceptor stimulation by sodium cyanide was shown to increase hepatic glucose output and increase brain glucose retention within 2 to 8 min, via an adrenal gland- and sinus nerve-dependent manner (35). This same group subsequently demonstrated that the increase in plasma glucose following chemostimulation was mediated via activation of hepatic arginine vasopressin V1a receptors (594) and have since identified both an nNOS-activated mechanism in the hypothalamopituitary axis (125) and a $GABA_B$-mediated mechanism in the nucleus tractus solitarius (518) that may be involved in the regulation of brain and plasma glucose levels following chemoreceptor stimulation. Similarly, intracarotid injections of glucose could significantly attenuate the normal hormonal response to hypoglycaemia (293), although these authors did not consider the reflex to originate from peripheral receptors in the carotid sinus region.

The inference that low glucose could be a stimulus at the carotid body, was subsequently tested further in conscious dogs, where the response to insulin-mediated, low plasma glucose concentration was shown, in a glycaemic clamp protocol, to be less effectively counter regulated in bilateral carotid chemo- and barodenevated animals than in sham controls (466, 786), through an impairment in the normal elevation of plasma glucagon and cortisol levels. However, although the presumed adequate carotid body stimulus in these dog experiments was hypoglycaemia, a role for insulin-induced, counter regulatory hormones could not be excluded. The authors also noted an expected fall in arterial Po_2 in chemodenervated dogs of *ca.* 20 mmHg from a control level of 102 mmHg, but had previously shown (953) that Pao_2 would need to fall to <60 mmHg before an hypoxic effect on glucose metabolism could be noted and so excluded this as a stimulus in their experiments, although did note that they could not discount the possibility that hyperinsulinaemia may have interacted positively with hypoxia. In a subsequent study (465), it was demonstrated that maintenance of plasma glucose during exercise in dogs, was also sinus nerve dependent. Irrespective of the stimulus, no role for aortic chemoreceptors was discernible in these experiments.

Given the polymodal nature of chemoafferents, it may be expected that carotid body stimulation by insulin-mediated hypoglycaemia should induce, like hypoxia, a hyperventilation and hypocapnia. Although not considered by Koyama et al. (466), this was tested directly by Kumar and colleagues (70) who measured ventilation and O_2 consumption in anesthetized rats and found a reflex increase in alveolar ventilation induced by insulin infusion that was correlated to the fall in plasma glucose concentration and which could be prevented by euglycaemic clamp or sinus nerve denervation. However, and importantly, there was no corresponding fall in $PaCO_2$ induced by the reflex increase in ventilation and this was presumed a consequence of the measured and expected hypermetabolism, making the reflex response to insulin infusion, an appropriate isocapnic hyperpnea induced by the elevated metabolic rate rather than a hypocapnic hyperventilation. These workers subsequently proposed that the hyperpnea was mediated by a hormone-mediated increase in carotid body CO_2 sensitivity (71). Entirely consistent findings were made in an experimental

rat model of type I diabetes, where insulin infusion decreased the plasma glucose to euglycaemic levels and which was correlated to a proportional increase in both ventilation and metabolic rate such that the ratio of the two was unchanged (761). Further confirmatory studies measuring ventilatory responses in humans who had been made hypoglycaemic for 30 min, showed that the expected elevation in ventilation also occurred here without change in arterial PCO_2, and which may have been mediated through an action of a number of counter regulatory hormones, including glucagon and cortisol (886). In addition, a role for adrenaline, has been proposed and which may act to increase carotid body and ventilatory CO_2 sensitivity (553).

A recent study performed on the *in vitro* cat carotid body (283) showed that removal of glucose for 12 min had no effect on the *absolute* release of either ACh or ATP from *in vitro* cat carotid bodies, thus providing circumstantial evidence against a direct role for reduced plasma glucose as an acute chemostimulus. They are therefore in accordance with earlier measurements made from the whole rat carotid body *in vitro* where no elevation, and often a decrease in type I cell catecholamine or ATP release or single fiber chemoafferent discharge frequency (31, 172, 198), was observed with glucopenia, for up to 1 to 2 h of incubation, after which time an irreversible and relatively small (*cf* hypoxia) increase in discharge could sometimes be observed, that preceded organ death.

However, the results described above using intact carotid bodies are in direct contradiction with findings made using the novel, reduced carotid body preparations of petrosal ganglion neuron-type I cell cocultures (951) and carotid body thin slice preparations (670). These latter preparations offer a powerful methodological link between studies performed on the isolated type I cell and studies performed on the whole organ and have the advantage of enabling a greater level of investigation than is technically feasible in the connective tissue encased, whole organ coupled with the greater integrity conferred by retaining a degree of intact intercellular communication (Fig. 12). Thus, superfusion with zero/low glucose solutions could stimulate a, dose dependent, up to a 20-fold increase in catecholamine secretion (in an intracellular ATP-independent manner) from type I cells in slices (539, 669), beginning within a minute of application; a level equivalent to that observed in response to anoxia. Low glucose could also excite rat petrosal neurons cocultured and in postsynaptic contact with type I cells (944), through the presynaptic corelease of ATP and ACh. Together, these data were consistent with electrophysiological recordings of ion channel activity made subsequently from isolated type I cells (303) who also demonstrated the presence of the facilitated glucose transporters, GLUT1, GLU3, and GLUT4 in these cells but not GLUT2, as may have been expected from its role in other glucose sensing systems (829, 830).

If low glucose is detected by the carotid body, the sensed variable appears most likely to be a metabolite of glucose, evidenced by the finding that glucose analogs do not prevent the catecholamine response to 0 mM glucose in thin carotid body slices (303). On examining the mechanism of glucose sensing in these preparations, it appears that, although the transduction process shares some common downstream elements, such as raised intracellular Ca^{2+} concentration and neurotransmitter release, these are triggered through a quite different membrane delimited process than that currently ascribed for hypoxia sensing. Thus, low glucose activates an inward Na^+ permeable cationic conductance leading to a reduction, rather than an increase, in membrane resistance in cocultured cells (944) that, at least in single cells appears to induce a membrane depolarization of *ca*. 10 mV due to the opening of type 1 cell TRPC channels (303) that had previously been described in this organ (115), but ascribed no specific function. In addition, a selective inhibition of voltage-gated K^+ channels at membrane potentials above −40 mV had also been reported that was of similar magnitude to that observed in response to severe hypoxia (669) and which would presumably

counteract, or at least attenuate, the fall in input resistance induced by the opening of TRPC channel. Again, the carotid body appears to differ here from more-recognized glucose sensors where background "leak" K^+ channel inhibition to low glucose has as described for orexin-sensitive CNS neurons (118).

The reason for the various differences between species, between carotid body preparations and between carotid body tissue and other glucose-sensing systems, is an important one to determine as any sensor role for the carotid body in the neuroendocrine axis, if vindicated, could perhaps prove to be of greater physiological importance than its ability to sense hypoxia. Any such role should require the carotid body to detect, quickly, small (<1 mM) falls in plasma glucose levels in otherwise normal conditions and initiate corrective reflexes. Zhang et al. (944) suggested that in the experiments of Bin-Jaliah et al. (70), the relatively high Po_2 deliberately used by these workers to exclude hypoxia-induced excitation, may have prevented the full glucose response from being observed, given the positive interaction previously described between hypoxia and glucopenia (283, 669). Although, this might reasonably lead to the question of whether low glucose is acting as an adequate stimulus in its own right or simply facilitating an hypoxic sensitivity, it is also necessary to appreciate that the level of tissue Po_2 at the site of postsynaptic site of afferent action potential initiation in the whole carotid body *in vitro* although not known in most cases, will be much lower as a consequence of diffusion and metabolism than the superfusate level and could even be equivalent to the stimulatory level of Po_2 used in the, essentially monolayer, coculture experiments of Zhang et al. (944). Thus, in both cases, the Po_2 is likely to have been higher than *in vivo* tissue levels. The interaction with oxygen was tested directly (172, 382), by measuring the response to low glucose at varying levels of Po_2, between hyperoxia and hypoxia. In both studies, hypoxia did not induce glucose sensitivity as measured by ATP or catecholamine release or afferent nerve discharge. Indeed, the sensitivity to hypoxia appeared reduced by glucopenia. How the disparate findings are to be reconciled regarding glucose sensitivity in this organ is not yet known but the answer may yet lie in the preparations being utilized and their metabolic status (476), especially given the greatly increased metabolism known to be required to maintain carotid body function during hypoxia (638, 643).

Immune functions of the carotid body

Histologists have long noticed the presence of macrophages in the carotid body, but have either largely disregarded them or presumed them incidental to the high organ blood flow. Although a functional role for macrophages as a source of cytochrome b558 has now been described (234), the last few years have seen a growing realization that the carotid body may possess an immununlogical function, both as a means of communicating peripheral systemic immune status to the CNS (645) and as a means of instigating the plastic changes observed within the carotid body during exposure to the chronic hypoxia of altitude or disease states such as COPD. Thus, the interleukin, cytokine receptors, IL-1R1 and IL-6Rα were first identified in carotid body tissue, localized within the cytoplasm, nucleus, and cell membrane of type I cells even in normoxia (874, 875) findings described previously in pain sensing primary neurons (895).

Sensitivity of the carotid body to hypoxia could also be modulated by the proinflammatory cytokines, IL-1β, IL-6, and TNF-α (509, 536, 785). In addition, the systemic injection of IL-1β in rats, increased the expression of its receptor in type I cells (948) as did chronic hypoxia (509, 536), with tissue hypoxia most likely triggering the elevation of cytokines. Liu et al. (536) additionally showed that chronic hypoxia induced macrophage invasion in the carotid body that peaked at three days of stimulation and only a transient rise in carotid body IL-1β and TNF-α expression on both immune cells and type I cells but a more

sustained increase in IL-6 expression on these cells that persisted for more than 28 days. Importantly, these authors showed that the common antiinflammatory drugs, ibuprofen and dexamethasone, could reduce macrophage invasion, cytokine expression, and block the augmented sinus nerve discharge induced by chronic hypoxia.

In a related study, Fernandez et al. (267) showed that systemic infusion or topical application of the bacterial endotoxin, lipopolysaccharide (LPS) could induce carotid body inflammation, as expected in the cat, but was also capable of inducing a tachypnoea via a significant augmentation of basal sinus nerve discharge. The tachypnoea was prevented by bilateral sinus nerve and aortic nerve section. Anatomically, these latter authors also described a “disorganization” of carotid body glomoids, an increase in carotid body connective tissue and recruitment of polymorphonuclear cells (granuloyctes) within 4 h of i.v. LPS administration. No apoptosis was observed, thus negating this as the mechanism for enhanced basal function. Intriguingly, the ventilatory responses to hypoxia or hyperoxia of animals exposed to LPS, although intact, were not enhanced and might even have been reduced. Accordingly, TNF-α when applied to an *in vitro* carotid body preparation reduced the discharge induced by hypoxia in a dose-dependent manner. The authors suggest that the reduced cytokine effect during hypoxia may reflect the common observation that the degree of enhancement to stimulation is typically reduced as the basal effect is increased (239), despite they themselves having seen no effect of TNF-α upon basal discharge in their *in vitro* carotid body preparation. Such an effect may therefore not explain why no effect upon peripheral chemoreceptor function, as assessed indirectly by reflex ventilatory responses to an alternating blood gas stimulus, had been observed in an earlier study (894) when IL-1β was administered intraperitoneally in cats to raise metabolic rate.

Mechanistically, the activation of cytokine receptors appears to initiate a sequence of cellular events bearing remarkably similar features to the hypoxia transduction process. Thus, IL-1β could, reversibly, inhibit outward K^+ currents in rat type I cells in a concentration-dependent manner (785) as well as induce a rise in intracellular Ca^{2+} in these cells (509, 785), as could IL-6 and TNF-α (509). No release of catecholamine could be detected in the study of Shu et al. (785), but they were able to demonstrate that IL-1β could increase discharge rate in the whole CSN *in vivo*, albeit it after a relatively long latency of around 6 min and which could be blocked by ATP but not dopamine receptor antagonists. Subsequent studies, by the same group demonstrated that IL-6 application could induce catecholamine release in a dose-dependent manner via a Cd^{2+}-sensitive elevation in intracellular Ca^{2+} (262). The question remains as to how the cytokine receptor activation induces K^+ channel inactivation and whether this is direct or via interaction with the cellular machinery directed toward transduction of blood gas and pH stimuli. Additionally, the reason why IL-6 could induce catecholamine release but IL-1β could not, may warrant further investigation.

Thus, the possibility that cytokines, poorly transferred across the blood brain barrier, may act via the csn chemoafferent-mediated excitation of brainstem neurons, as a means of informing the CNS of peripheral inflammation and thus enable initiation of appropriate systemic responses including fever, inactivity, and appetite loss. In addition, the adaptation of the carotid body to chronic hypoxia may be, at least in part, a consequence of local inflammatory responses rather than any intrinsic feature of type I cells and its reversibility by antiinflammatory agents offers an exciting avenue for carotid body research and, although relatively little studied to date, it is expected that more attention may be paid to this key area in the near future.

The carotid body—a polymodal receptor?

In addition to blood gas tensions and/or pH, glucose and proinflammatory cytokines the carotid body is also sensitive to a number of other blood-borne, physicochemical stimuli (478). These include various circulating hormones including catecholamines (537, 553), but see (534), angiotensin (30, 296, 413, 506, 521), adenosine (573, 759, 852), and extracellular ions, particularly K^+ (54, 412, 533) as well as changes in osmolarity (136, 300, 301, 590) and temperature (26, 61, 62, 69, 572). The carotid body responses to these stimuli are robust and reproducible and can in most cases also be observable at the level of cardiorespiratory reflexes (602, 672, 777, 942), which has led to the notion that the carotid body might act as a polymodal receptor within the stress axis of the body (478).

Consequently, these “other” stimuli may yet prove to be as relevant for human health as the response to hypoxia and more complete descriptions of stimulus-dependent chemoreceptor transduction mechanisms are required to establish whether the carotid body contains multiple, stimulus-specific, transducing pathways or whether the various stimuli simply modify some element of the O_2 transduction pathway. In addition, recent studies have shown that the contribution of the carotid body basal discharge to eupnic ventilation in the intact and awake animal (Fig. 2B) may previously have been grossly underestimated in some studies through a failure to completely silence the receptor and suggests that the carotid body may contribute in excess of 50% of the drive to breathe in normoxia (91) and also play a major role in determining CNS CO_2 chemosensitivity (92). Interestingly, the finding of Blain et al. (91) is not too dissimilar to the original postulation of a 34% carotid body contribution to tonic respiration by Bernthal and Weeks (69) who used hypothermia to decrease carotid body discharge. However, despite a long history of such knowledge, such considerations of the importance of the carotid body are still effectively in their infancy and it may be time for a reconsideration of how we view the role of this receptor system in health and disease.

Plasticity of Carotid Body Chemoreceptors

Physiological responses of the carotid body to acute hypoxia are dynamically altered by chronic perturbations in systemic and/or environmental O_2. This section summarizes the plasticity of the carotid body function under the physiological conditions of exercise, high altitude, pregnancy, as well as pathophysiological situations including congestive heart failure (CHF), sleep-disordered breathing, and hypertension.

The Carotid Bodies and Exercise

Reflexes arising from carotid bodies have been implicated in ventilatory stimulation during exercise (672, 888, 892, 907). Exercise hyperpnea was depressed in carotid body-resected subjects compared with subjects with intact carotid bodies (384, 893). Stulbarg et al. (807) noted a decreased ventilatory response to exercise in three subjects with carotid body resection who became severely hypoxemic during exercise. It should, however, be noted that some carotid body-resected subjects had a history of asthma, which by itself depresses the hypoxic ventilatory response (HVR) (394). Nonetheless, these studies indicate the potential importance of carotid body reflexes in exercise-induced hyperpnea. Ventilatory changes during exercise are categorized into three phases: phase I, the rapid increase in ventilation that occurs at the start of the exercise; phase II, the exponential increase in ventilation during exercise; and phase III, the steady-state ventilation. It was proposed that the carotid body chemoreflex contributes significantly to ventilatory stimulation during phases II and III (888, 907). Thus, it maintains arterial PCO_2 and prevents hypoxemia during phase II and contributes up to 20% of the ventilatory drive during phase III. The relative contribution from the chemoreflex may increase as much as 50% to ventilatory stimulation in phase III

during exercise in an hypoxic environment, with an arterial Po_2 of 50 mmHg (888, 907). Although the studies on human subjects suggest a role for peripheral chemoreceptors in exercise-induced hyperventilation, observations from experimental animals are not entirely consistent with such a possibility. Based on the data from carotid body-denervated ponies and experiments on awake dogs and goats, Dempsey and Smith (200) concluded that carotid body chemoreceptors exert an inhibitory influence to the respiratory motor output during heavy exercise. Forster and Pan (291) studied the role of carotid bodies in the control of breathing during submaximal exercise in humans and ponies and concluded that the carotid bodies “fine tune” alveolar ventilation during submaximal exercise but do not provide the primary drive for exercise hyperpnea. It should be noted that much of the evidence for a role of carotid bodies in exercise hyperpnea is based on indirect assessment of peripheral chemoreceptor function. Direct monitoring of carotid body activity in humans, and exercising animals is technically problematic. Thus, some of the controversies surrounding the role of peripheral chemoreceptors in exercise-induced hyperventilation are likely due to inherent technical limitations for assessing chemoreceptor function.

Besides ventilation, exercise also affects the cardiovascular system. There is evidence suggesting that the carotid bodies contribute to exercise-induced cardiovascular changes in healthy humans (803) and the increase in sympathetic vasoconstrictor outflow (804). In addition to their role in cardiorespiratory stimulation, carotid body reflexes play a role in neuroendocrine responses to exercise (465), where circulating glucagon and NE were markedly attenuated in exercising dogs whose carotid sinus nerves had been bilaterally sectioned 16 days before the experiment.

Mechanisms underlying carotid body stimulation by exercise

Arterial Po_2 and PCO_2 are maintained within the physiological range during exercise in a normoxic environment. How then might the carotid bodies become stimulated during exercise? Exercise increases circulating catecholamine levels, which may stimulate chemoreceptors. Although metabolic acidosis (lactic acid) has been thought to be a stimulus for peripheral chemoreceptors, especially during heavy exercise (892), patients with McArdle’s syndrome (myophophorylase deficiency) who cannot generate lactic acid, respond with an increase in ventilation during heavy exercise (673), suggesting that lactic acid may not be a major stimulant. As K^+ is released from contracting muscle (576), hyperkalemia has been proposed as a stimulus to the carotid body during exercise (672). The following findings support this possibility: (i) systemic administration of K^+ augments breathing, and sinoaortic denervation prevents this response (55, 56) and (ii) exogenous K^+ stimulates the carotid body activity in experimental animals, and this effect was potentiated by hypoxia and depressed by hyperoxia (55, 120); whereas hypercapnia had no effect on K^+-induced augmentation of the carotid body activity (120). It is likely that K^+ stimulates the carotid body activity by directly acting either on the sensory nerve ending and/or on type I cells. Although some studies question the role of K^+ (791, 889), a majority of the evidence seems to support K^+ as a major stimulus to chemoreceptors during exercise. It has also been suggested that a circulating factor(s) during hyper metabolic states may augment the carotid body sensitivity to arterial PCO_2 and that this may contribute to the hyperpnea of exercise (71). However, although much supporting evidence exists, a definitive role for the carotid bodies in exercise has yet to be determined.

Carotid Body Function in Pregnancy

It has long been known that normal pregnancy is associated with increased resting ventilation and an augmented ventilatory response to hypoxia and hypercapnia (901). The increased chemosensitive drive to ventilation is evident after week 20 of gestation, is correlated with elevated levels of progesterone and estradiol and precedes an increase in

metabolic rate (599). Enhanced hypoxic ventilatory chemosensitivity can be elicited in nonpregnant women by combined administration of progesterone and estrogen (736). Hannhart et al. (334) reported a marked enhancement of carotid body sensory response to hypoxia in pregnant compared to nonpregnant control cats. These observations suggest that increased carotid body sensitivity to hypoxia contributes in part to elevated basal ventilation and augmented hypoxic ventilatory sensitivity in pregnant animals. Estrogen affects neuronal excitability by two mechanisms. One, the “genomic” effect, is slow in onset; the other is rapid and of short duration, the “nongenomic” effect (567). Nuclear estrogen receptors mediate the slow actions of estrogen, whereas the rapid actions (nongenomic actions) are associated with activation of G-proteins, cAMP, mitogen-activated protein kinases, Ca^{2+} channels, and Ca^{2+} entry [see (567)]. Determining which of these mechanisms contributes to the estrogen-induced enhanced hypoxic sensitivity of the carotid body would provide a better understanding of chemoreceptor function during pregnancy.

Carotid Bodies and the Acclimatization to Altitude

Sojourns at high altitude lead to a series of physiological adaptations, which are critical for overcoming the deleterious effects of hypoxia. One such adaptation is the ventilatory acclimatization to hypoxia (VAH) (714). VAH is characterized by a progressive increase in baseline ventilation (Fig. 13), which ensures adequate oxygen supply. Failure to hyperventilate sufficiently during the ascent to altitude can have adverse physiological consequences and is associated with, for example, acute mountain sickness, pulmonary edema, and cerebral edema. VAH is associated with augmented HVR humans (199, 290, 767) and experimental animals (871).

There is a large body of evidence suggesting that carotid bodies are critical for VAH (86). In experimental animals, bilateral denervation of carotid bodies attenuates or abolishes VAH (86, 289). Direct recording of carotid body sensory activity showed progressive increase in afferent discharge in response to prolonged hypoxia (617). Following several days of hypobaric hypoxia, carotid body response to acute hypoxia is enhanced in cats (59, 871) and rats (147). Human subjects who have undergone carotid surgery adapt rather poorly to high altitude (750). Prolonged exposure to several weeks of hypoxia may eventually desensitize the carotid body response to acute hypoxia (817, 900).

Mechanisms associated with the carotid body response to chronic hypoxia

Chronic hypoxia causes hyperplasia of the type I cells and hypertrophy as well as neovascularization in the carotid body (201, 363, 422, 504, 695). Growth factor (VEGF and PDECGF) expression is increased in carotid bodies exposed to chronic hypoxia. VEGF expression is increased in type I cells and platelet-derived endothelial cell growth factor (PDECGF) expression in the extracellular stroma (422). Carotid bodies also express receptors associated with VEGF actions, that is, Fit-1 and Flk-1 (422). VEGF acting on Flk-1 might mediate hyperplasia of the type I cells and acting via Flit-1 might promote neovascularization. In addition, PD-ECGF might also contribute to angiogenesis, as it does elsewhere in the body including the lung. These findings might of relevance in explaining the high incidence of carotid body tumors in high altitude residents.

Kusakabe et al. (487) examined the reversibility of the effects of prolonged hypoxia on carotid body morphology. These authors reported that after eight weeks of hypoxia, rat carotid bodies were enlarged and showed pronounced neovascularization. One to two weeks after terminating hypoxia, vascularization decreased but the size of the carotid bodies still remained larger. However, after eight weeks of reoxygenation, carotid body morphology appeared normal compared to controls, suggesting that the effects of chronic hypoxia on carotid body morphology are reversible after reoxygenation.

K^+ channels are down regulated in adult rat carotid bodies exposed to 3 to 4 weeks of hypoxia. Ortiz et al. (654) reported that acute hypoxia decreases the open probability of TASK-like currents in glomus cells of the normoxic carotid body and this response was markedly augmented in type I cells cultured under an hypoxic environment for 48 h. Caceres et al. (124) reported that mRNA encoding Na(v)1.1, Na(v)1.2, Na(v)1.3, Na(v)1.6, and Na(v)1.7 isoforms are expressed in normoxic rat carotid bodies and expression of three isoforms, Na(v)1.1, Na(v)1.3, and Na(v) 1.6 was seen in TH expressing type I cells. The Na(v)1.1 isoform was upregulated following one week of hypoxia (10% O_2). It was suggested that Na(v) upregulation represents an adaptive mechanism to increase chemoreceptor sensitivity during acclimatization to prolonged hypoxia.

Voltage gated Ca^{2+} channel density increased in type I cells of carotid bodies treated with prolonged hypoxia (368) and c-AMP has been implicated in this response (797, 798). A recent study by Cáceres et al. (123) reported that the carotid body expresses α1G, α1H, and α1I isoforms of the T-type Ca^{2+} channels and hypoxic treatment for eight days decreased α1G expression and hypoxia evoked catecholamine secretion was inhibited by mibefradil, a purported inhibitor of T-type Ca^{2+} channels.

Studies with heterologous expression systems provided some insights into the effects of prolonged hypoxia on ion channel expression. Hartness et al. (345) reported that three days of hypoxia increased maxi-K^+ current density and markedly altered Ca^{2+} sensitivity in HEK293 cells stably coexpressing α and β subunits. The increased maxi-K^+ current density was due to a 3-fold increase in the β-subunit expression, which was attributed to posttranslational mechanisms. On the other hand, the α-subunit expression was unaltered by prolonged hypoxia. Del Toro et al. (197) reported upregulation of T-type Ca^{2+} channels by prolonged hypoxia in PC12 cells which was attributed to hypoxia-inducible transcriptional factor (HIF-1)-mediated transcription. In addition to ion channels, chronic hypoxia also increases gap junction-forming protein connexin43 protein in the carotid body and petrosal ganglion in rats (146). While these studies provide evidence for altered ion channel expression, their relevance to the sensitization or desensitization of carotid body response to chronic hypoxia need to be established.

Chronic hypoxia and neurotransmitters

Catecholamines—Chronic hypoxia increases TH mRNA, protein, as well as the enzyme activity in the carotid body (179, 331, 578, 877). Exposure to two days of hypoxia increased dopamine levels and turnover in the type I cell (333). The D_2 receptor mediates the inhibitory actions of DA and hypoxia has a biphasic effect on D_2 receptor expression in the carotid body, an initial decrease followed by increase (395). It was proposed that the chronic hypoxia-induced enhanced carotid body response involves a down regulation of the dopaminergic system, which normally exerts an inhibitory influence on the chemoreceptor response to hypoxia (503, 649, 818).

Acetylcholine—ACh stimulates carotid body sensory activity via neuronal nicotinic cholinergic receptors (nAChRs). Following 14 days of hypoxia, the sensory response to acute hypoxia and ACh are markedly augmented. However, mecamylamine, a potent blocker of nAChRs, prevented the enhanced ACh but not the hypoxia-induced sensory excitation in carotid bodies treated with two weeks of chronic hypoxia (357), suggesting that ACh does not contribute to the enhanced hypoxic sensitivity. The failure of mecamylamine to reduce the chronic hypoxia-induced increase in carotid body sensory activity might, however, be related to its ability to completely inhibit basal dopamine release (357).

Peptidergic transmitters/modulators—Two weeks of chronic hypoxia reduces SP-like immunoreactivity in type I cells of the cat carotid body (486, 879). Likewise, prolonged hypoxia for eight weeks decreases NPY-, and CGRP-like immunoreactivity and increases VIP-like immunoreactivity in nerve fibers innervating the rat carotid body and these effects are reversed following eight weeks of reoxygenation (487). The significance of altered SP, NPY, CGRP, and VIP remains to be established.

ET-1 is present in either low or undetectable levels in type I cells under normoxic conditions (147). The effects of ET-1 are mediated by ET_A receptors (144). Chronic hypoxia markedly increases the expression of ET-1 and ET_A receptors in the carotid body (147). More importantly, blockade of ET_A receptors prevented the enhanced hypoxic sensory response in animals conditioned with chronic hypoxia (144, 147). It has been suggested that ET, by promoting the phosphorylation of Ca^{2+} channel proteins, leads to potentiation of the sensory response to hypoxia (144). More interestingly, an ET receptor antagonist prevented proliferation of type I cells in hypoxia treated carotid bodies (147). Thus, ET-1 signaling seems to be a promising candidate for mediating the sensitization of the hypoxic sensory response and morphological changes in response to chronic hypoxia, at least in the rat.

The renin-angiotensin system (RAS) is expessed in the carotid bodies and chronic hypoxia: (i) increases angiotensin I (AT-1) receptors along with an enhanced chemosensory response to the exogenous application of angiotensin II (521); (ii) upregulates angiotensinogen mRNA in type I cells (508); and (c) increases angiotensin converting enzyme (ACE) activity (506) and the metabolite, angiotensin-4 as well as AT-4 receptors in type I cells of the rat carotid body (297). Exogenous AT-4 elevated $[Ca^{2+}]_i$ in type I cells and this response was more pronounced in type I cells from chronic hypoxia treated carotid bodies (297). The functional significance of RAS in chemoreceptor adaptation to prolonged hypoxia requires further studies.

Tjong et al. (836) reported that melatonin enhances the type I cell $[Ca^{2+}]_i$ response to hypoxia in rats treated with four weeks of 10% O_2 and that these effects were prevented by application of putative melatonin receptor antagonist. The effects of chronic hypoxia were associated with upregulation of melatonin 2 receptor mRNA in the carotid body. While these studies indicate that melatonin modulates Ca^{2+} signaling in the type I cells, its functional significance to carotid body adaptations to chronic hypoxia is not clear.

Purinergic transmission—He et al. (355) examined the effects of chronic hypobaric hypoxia (0.38 ATM; 9–16 d) on the expression of P2X2 receptor expression and their contribution to the sensitization of the hypoxic sensory response. P2X2-like immunoreactivity was seen in nerve plexus and type I cells in normal carotid bodies and chronic hypoxia had no effect on P2X2 expression. P2X2 receptor antagonists had no apparent effect on chronichypoxia evoked sensitization of the carotid body response to low O_2.

Nitric oxide and carbon monoxide—Di Giulio et al. (204) reported upregulation of nNOS protein in the carotid body after 12 days of hypoxia. However, Ye et al. (930) found no changes in nNOS expression in the carotid body with four weeks of hypoxia; whereas iNOS expression increased markedly, which was not expressed in normoxic tissue. These investigators further showed that chronic hypoxia increases NO production in the carotid body and elevated iNOS levels contribute at least 30% to this response. Because NO exerts inhibitory actions on the carotid body sensory activity, these authors suggested that increased NO production contributes to the blunting of the hypoxic sensitivity that occurs with several weeks of hypoxia. He et al. 356) reported that sensory response to S-nitroso-N-acetyl-penicillamine (SNAP), a NO donor was enhanced in carotid bodies from chronic

hypoxia exposed rats and this response was associated with decreased response of soluble guanylate cyclase (sGC) and downregulation of sGC mRNA.

Inflammatory cytokines—Liu et al. (536) reported that carotid bodies exposed to chronic hypoxia showed robust invasion of ED1(+) macrophages, upregulation of proinflammatory cytokines, especially IL-6 in type I and in type II cells. Antiinflammatory drugs (ibuprofen or dexamethasone) blocked immune cell invasion and severely reduced CH-induced cytokine expression as well as prevented hypersensitivity of the carotid bodies to acute hypoxia. These findings indicate that chemoreceptor adaptation involves novel neuroimmune mechanisms, which may alter the functional phenotypes of type I cells and chemoafferent neurons.

NADPH oxidases—Type I cells express Nox enzymes, especially Nox2 and 4 (685). He et al. (359) examined the roles of Nox and the ensuing generation of ROS in the enhanced hypoxic sensory response of carotid bodies induced by chronic hypoxia. Carotid bodies from rats exposed to chronic hypoxia (two weeks; 0.38 ATM) showed higher levels of ROS as measured by dihydroethidium fluorescence and AEBSF, an inhibitor of Nox, prevented the response. AEBSF caused a ~3-fold increase in carotid body sensory response to hypoxia in rats exposed to chronic hypoxia as opposed to small increase in controls. Nox2, Nox4, and p47 (regulatory subunit) mRNAs increased in hypoxia-treated carotid bodies. From these observations, the authors concluded that ROS generated by Nox dampens chronic hypoxia-induced hypersensitivity of the carotid body to acute hypoxia.

Molecular mechanisms underlying the carotid body response to chronic hypoxia

Increasing evidence suggest that transcriptional regulation of genes is one of the pivotal mechanism that underlies the long-term adaptations to chronic hypoxia (116, 776). The following section summarizes studies addressing the role of transcription factors in mediating carotid body adaptations to chronic hypoxia.

Hypoxia-inducible factor-1—Hypoxia-inducible factor-1 (HIF-1) is a heterodimeric protein composed of a constitutively expressed HIF-1β subunit and an O_2-regulated HIF-1α subunit. HIF-1 is a global transcriptional regulator of oxygen homeostasis that controls multiple physiological processes (776). Type I cells express low levels of HIF-1α under normoxia, and hypoxia upregulates the HIF-1α protein (510, 755). Complete HIF-1α deficiency results in embryonic lethality at midge station with major malformations of the heart and vasculature (405). *Hif1a*$^{+/-}$ heterozygous mice, which are partially deficient in HIF-1α develop normally and are indistinguishable from wild type controls under normoxic conditions. Although the carotid body morphology was normal, the sensory response to hypoxia but not to cyanide was markedly impaired in *Hif1a*$^{+/-}$ mice (445). Moreover, wild type mice displayed VAH in response to prolonged hypoxia, and this response was absent in *Hif1a*$^{+/-}$ mice (445). The absence of VAH in *Hif1a*$^{+/-}$ mice was attributed to impaired hypoxic sensing of the carotid body (Fig. 14). Several genes are regulated by HIF-1 including those encoding endothelins, TH, VEGF, and ion channels (776), which are also affected by prolonged hypoxia in the carotid body. It is likely that HIF-1 contributes to carotid body adaptations to prolonged hypoxia by altering the transcription of one or more of these genes.

Immediate early genes and activator protein-1—Activator protein-1 or AP-1 is a transcriptional activator composed of either a heterodimer of Fos and Jun family of proteins or a homodimer of Jun family of proteins (40). Members of the *fos* family of genes include *c-fos, fos B*, and *fra-1* (369). *c-fos* and *fos B* are expressed in the nervous system and share 72% homology in DNA binding sequence. Malik et al. (550) reported that mice lacking the

fos B gene when exposed to prolonged hypoxia exhibit an impaired VAH, which was attributed to the impaired carotid body response to acute hypoxia.

The studies outlined above suggest that VAH is impaired in mice deficient in transcriptional activators *fos B* and HIF-1. It likely that the magnitude and the time course of carotid body adaptations to chronic hypoxia and the ensuing VAH depend on complex interactions between more than one transcription factor resulting in the coordinated regulation of several downstream genes and their protein products.

Carotid Bodies and Intermittent Hypoxia: Relevance to Sleep-Disordered Breathing

For people who reside at or near sea level, recurrent episodes of hypoxia are encountered more frequently than continuous hypoxia in life. Transient episodes of hypoxia are associated with many pathophysiological situations, including sleep-disordered breathing with recurrent apneas (obstructive or central apneas). Recurrent apneas are prevalent in premature infants (708), 5% of middle-aged men and 2% of women after menopause (619, 778) and the incidence of recurrent apneas is increasing in adults. In severely affected patients, the frequency of apneas may exceed as many as 60 episodes/h and arterial blood O_2 saturation can be reduced to as low as 50%. As a consequence of periodic cessations of breathing, patients with recurrent apneas experience not only chronic intermittent hypoxia (IH) but also chronic intermittent hypercapnia (i.e., elevations in arterial CO_2). A major advance in the field of apnea research is the demonstration that exposing experimental animals to chronic IH alone is sufficient to evoke physiological changes similar to that described in recurrent apnea patients (287). Although both continuous and IH results in decreases in arterial O_2, physiological responses to both forms of hypoxia differ quite considerably. Whilst continuous hypoxia leads to adaptations of the physiological systems, chronic IH associated with recurrent apneas result in morbidities including development of hypertension, elevated sympathetic nerve activity, myocardial infarctions, and stroke (619, 778).

Much of the earlier information on the role of carotid bodies during chronic IH has come from studies on patients with recurrent apneas (obstructive or central apneas). It has been suggested that the carotid bodies constitute a “frontline” defense system for detecting systemic hypoxia associated with apneas (156). Patients with recurrent apneas exhibit augmented HVR (610), which was attributed to enhanced peripheral chemoreceptor sensitivity (428). Consistent with such a possibility is the finding that ventilatory depression with brief hyperoxic challenge (Dejour’s test, a measure of peripheral chemoreceptor sensitivity) was more pronounced in obstructive sleep apnea patients than in control subjects (428, 815). Furthermore, glomectomized subjects with sleep apneas do not develop hypertension [see discussion in (793)]. Rats exposed to 30 days of IH (20 s of 5% inspired O_2; 9 episodes/h; and 8 h/day) develop hypertension similar to that seen in patients with recurrent apneas, and chronic bilateral sectioning of sinus nerves prevented this response (520). These studies thus provide indirect evidence for altered carotid body function both in humans and rodents experiencing chronic IH.

IH and adult carotid body activity

Direct evidence for altered carotid body function in response to chronic IH has come from studies on experimental models including rats (686, 723), cats (737, 738), and mice (690). Peng & Prabhakar (687) examined the effects of graded isocapnic hypoxia on carotid body sensory activity in anesthetized adult rats that were exposed to 10 days of IH (15 s of 5% inspired O_2 followed by 5 min of 21% O_2/episode; 9 episodes/h; and 8 h/day). These

investigators found that the sensory response to hypoxia (Pao_2 35 mmHg) was significantly augmented in IH compared to control rats (Fig. 15). Similar sensitization of the hypoxic sensory response was also reported in cats (737) and mice (690). Thus, chronic IH sensitizes carotid body response to acute hypoxia in three species studied thus far. The augmented hypoxic response could be reversed by reexposing IH treated rats to normoxia for 10 days. In contrast, the carotid body response to hypercapnia, albeit weak in rats, was unaffected following chronic IH. These findings support the notion that chronic IH resulting from recurrent apneas does indeed affect the O_2 sensing ability of the carotid bodies.

Chronic IH in addition to sensitizing the carotid body to hypoxia also induces hitherto uncharacterized form of plasticity manifested as sensory long-term facilitation of the carotid body [LTF; (686, 690)]. When anesthetized rats were exposed to acute intermittent hypoxia (AIH; 15 s of hypoxia followed by 5 min of reoxygenation, 10 episodes), sensory activity increased with each episode and promptly returned to baseline after terminating AIH. In striking contrast, in chronic IH-exposed animals, AIH produced long-lasting increase in baseline activity that persisted for at least 60 min after terminating AIH stimulus (Fig. 16). This long-lasting increase in baseline sensory activity has been termed sensory LTF (686), because it resembled the time course of LTF of breathing elicited by repetitive hypoxia (584). On the other hand, control carotid bodies do not exhibit (or have a weak) sensory LTF in response to repetitive hypoxia (178).

Unlike AIH, acute intermittent hypercapnia (10 episodes of 15 s of 7% CO_2 + 93% O_2 interspersed with 5 min of 100% O_2), another “physiological” stimulus to the carotid body was ineffective in evoking sensory LTF in IH-treated rats (686). The induction of sensory LTF is a time-dependent phenomenon, in that it was apparent after three days of IH, and the magnitude further increased following 10 days of IH. More interestingly, the magnitude of sensory LTF was not dependent on the severity of hypoxia used for IH conditioning, because it was indistinguishable with either FiO_2 of 5% or 10% O_2 used for IH conditioning (686). Like the sensitization of the hypoxic response, sensory LTF was also reversible after reexposing chronic IH treated rats to 10 days of normoxia.

The studies outlined above suggest that chronic IH exerts two major effects on the carotid body including (i) selective sensitization of the hypoxic sensory response and (ii) induction of sensory LTF. The total duration of hypoxia accumulated over 10 days of IH corresponds to 4 h of exposure to low O_2. However, a single episode of 4 h of hypoxia or 4 h of hypoxia/day for 10 days were ineffective in eliciting sensitization of the hypoxic sensory response and sensory LTF of the carotid body (686, 723). These findings suggest that for a given duration of hypoxic exposure, an intermittent pattern of stimulation is more effective than continuous hypoxia in altering carotid body function.

IH and neonatal carotid body function

Clinical studies have shown that depending on gestational age, nearly 90% of infants born preterm experience IH as a consequence of recurrent apneas (708). Carotid bodies are immature at birth and respond poorly to hypoxia compared with adults (93, 138, 217, 437, 693). However, preterm infants exhibiting greater number of apneas display augmented peripheral chemoreceptor reflex as evidenced by more pronounced ventilatory depression in response to brief hyperoxia (Dejour’s test) than those having lesser incidence of apneas (134, 624). To test whether IH induces hypoxic sensitivity in neonatal carotid body, Peng et al. (688) exposed rat pups at age P2 to 16 h of IH. The carotid body response to hypoxia in age matched control pups was weak and slow in onset (100 s). In striking contrast, the carotid body sensory response to hypoxia was greater and the time course of the response was faster (30 s) in IH treated pups. These observations suggest that exposure to IH, as short as few hours, induces hypoxic sensitivity of the carotid bodies in neonates, which otherwise

are insensitive to hypoxia. The enhanced hypoxic sensitivity of the carotid body was also reflected in augmented HVR in neonatal rat pups exposed to IH (688). Thus, these studies suggest that IH sensitizes carotid body response to hypoxia in neonates as it does in adults. However, whether IH affects the carotid body response to CO_2 has not been examined.

Although IH affects carotid body function in both adults and neonates, some notable differences were reported (676). Unlike adult carotid bodies, chronic IH was ineffective in inducing sensory LTF in neonatal carotid bodies. In contrast to adults, sensitization of the carotid body response to hypoxia was not reversed in neonates after reexposure to normoxia. Rather they persisted in juvenile life. Finally, carotid bodies from neonates appear more sensitive to IH than in adults because even few hours of IH was sufficient for eliciting sensitization of the hypoxic response in neonates, whereas a minimum of three days of IH was required in adults to elicit a similar effect. Although IH augments carotid body sensitivity to hypoxia in both adults and neonates, sensory LTF was seen only in the former but not the later suggesting that these two effects are distinct from each other.

Mechanisms associated with the effects of chronic IH on carotid bodies

Chronic IH-induced sensitization of the hypoxic response as well as the sensory LTF could be elicited in *ex vivo* superfused carotid bodies of rats and mice (686, 690) suggesting that the effects of IH were not secondary to elevated blood pressures seen in IH-exposed animals (473, 520, 690). Morphometric analysis revealed no significant differences in the total volume of the carotid body, number of type I cells, or type I cell volume between control and chronic IH exposed adult rats (686). However, IH caused hyperplasia of the type I cells of neonatal carotid bodies (675).

Cellular mechanism(s—Chronic IH increased ROS in adult (686) and neonatal carotid bodies (675) as evidenced by decreased aconitase enzyme activity, as well as increased in malondialdehyde levels, robust biochemical markers of ROS. Adult rats treated with a stable, membrane permeant superoxide dismutase mimetic [manganese(III) tetrakis (1-methyl-4-pyridyl) porphyrin pentachloride (MnTMPyP), 5 mg/kg, i.p.], a potent scavenger of ROS each day for 10 days before subjecting them to IH prevented sensitization of the hypoxic response as well as the sensory LTF of the carotid body (686, 723). Interestingly, a single application of superoxide dismutase mimetic on the tenth day of IH treatment, instead of daily administration, failed to prevent IH-evoked changes in the carotid body function (686), implying that a ROS-mediated signaling cascade rather than ROS *per se* is critical for chronic IH-induced functional alterations in the carotid body. Antioxidant treatment also prevented IH-induced sensitization of the carotid body response to hypoxia in neonates (675). Thus, ROS seem to play an important role in altering the carotid body response to hypoxia in response to IH.

Cellular sources of ROS can arise from the inhibition of complexes I and III of the mitochondrial electron transport chain (36), as well as from the activation of several oxidases (330). Biochemical measurements of carotid bodies from chronic IH exposed rats showed marked downregulation of the mitochondrial complex I but not the complex III (686). A recent study showed that chronic IH upregulates Nox2 and Nox4, NADPH oxidase isoforms, and Nox activity in the adult carotid bodies (685). Furthermore, chronic IH-induced sensory LTF was absent in mice deficient in Nox2 (685). Nox2 activation mediates IH-induced complex I inhibition via s-glutathionylation of complex I subunits in cell cultures (436), suggesting interactions between two prooxidant systems resulting in ROS-induced ROS. Whilst the role of Nox in acute response to hypoxia is debated, it appears that activation Nox and the ensuing ROS seems critical in altering the carotid body function under pathophysiological situations involving IH.

Neurotransmitters/modulators—As described in the preceding section, neurotransmitters/ modulators play important roles in sensory excitation of the carotid body by hypoxia. As the effects of IH on carotid body are reversible in adults, it has been proposed that IH recruits additional neurotransmitters are modulators that play minor or negligible role(s) in mediating sensory excitation under normal conditions. To date, a role has been proposed for ET-1 and 5-HT in IH-induced sensitization of the hypoxic response and sensory LTF of the carotid body.

Endothelin-1—Kanagy et al. (427) reported elevated plasma endothelin-1 levels in chronic IH-treated rats. Similar elevations in serum endothelin-1 levels were also reported in obstructive sleep-apnea patients (760). ET-1 expression increased in type I cells of carotid bodies from IH-treated adult cats (738) and neonatal rats (675). Studies on neonatal rats further showed enhanced basal release of ET-1 from the carotid body under normoxia and upregulation of ET_A but not ET_B receptors (675). An ET_A but not ET_B receptor antagonist (675) prevented the enhanced hypoxic sensory response of the IH-treated neonatal carotid body. Likewise, Rey et al. (738) reported that ET-receptor antagonists could also block (738) sensitization of the hypoxic sensory response in IH-treated adult cats. Antioxidant treatment prevent ET-1 release, upregulation of ET_A mRNA, and augmented hypoxic sensory response, but not the hyperplasia of glomus cells in IH-treated neonatal carotid bodies (675). These studies suggest that ROS by affecting ET-1 signaling mechanisms seem to mediate in part carotid body sensitization caused by IH.

5-hydroxytryptamine—5-hydroxytryptamine (5-HT) leads to long-term neuronal activation elsewhere in the nervous system (547, 558). The carotid body expresses substantial amounts of 5-HT (410). Repetitive applications of 5-HT evokes sensory LTF of the carotid body via PKC-dependent activation of NADPH oxidase (689). A recent study reported that acute hypoxia releases 5-HT from IH but not from control rat carotid body (685). Furthermore, the same study also showed an absence of an IH-induced sensory LTF of the carotid body by a 5-HT2 receptor antagonist and in mice deficient in 5-HT. Thus, although the role of 5-HT in carotid body response to acute hypoxia is uncertain, the above studies suggest that it plays a role in eliciting plasticity of the carotid body under the conditions of IH.

Molecular mechanisms—HIF-1 and HIF-2 (also known as endothelial (per-arnt-sim, PAS) domain protein-1, EPAS-1) are the two well-studied members of the HIF family of transcriptional activators and they share 80% sequence homology as well as interact with HIF-1β (832). Similar to continuous hypoxia (see the earlier section on altitude acclimatization), IH activates HIF-1-mediated transcription. However, IH-evoked HIF-1 requires signaling mechanisms distinct from chronic hypoxia and includes Nox/ROS-dependent accumulation of HIF-1α and a novel Ca^{2+}/CaMK-mediated recruitment of transcriptional coactivator CREB-binding protein (CBP) (935, 936). In contrast, IH downregulates HIF-2α in the rat carotid body via proteolytic degradation by Ca^{2+}-activated proteases, calpains (608).

The carotid body responses to chronic IH were examined mice with heterozygous deficiency of HIF-1α (690). Carotid bodies from wild type mice exposed to chronic IH exhibited sensitization of the hypoxic response and sensory LTF and these responses were strikingly absent in IH exposed *Hif-1a*$^{+/-}$ mice, suggesting that HIF-1-mediated transcriptional activation is an important molecular mechanism underpinning the carotid body changes by chronic IH. How might HIF-1 contribute to altered carotid body function by IH? Intriguingly, IH-induced increases in ROS were absent in *Hif-1a*$^{+/-}$ mice (690), indicating that HIF-1 plays a role in ROS generation by IH. Indeed, IH-induced upregulation of

Nox2mRNA in the carotid body was absent in *Hif-1a*$^{+/-}$ mice (934), suggesting that HIF-1 activation mediates transcriptional upregulation of Nox2 by IH. Given that Nox2 is required for HIF-1 activation by IH (936) and HIF-1 regulates Nox2 expression suggests a positive feed-forward interaction between Nox2 and HIF-1. On the other hand, IH downregulates superoxide dismutase-2 (Sod-2), a mitochondrial antioxidant enzyme and preventing HIF-2α degradation by systemic treatment of calpain inhibitor rescues downregulation of Sod-2 (608). Downregulation of HIF-2 therefore contributes to elevation of ROS by IH via insufficient transcription of antioxidative enzymes, such as Sod-2. Thus, an imbalance between HIF-1 and HIF-2 contributes to IH-induced changes in the carotid body via increasing oxidative stress and ensuing recruitment of additional transmitter/modulators. However, as delineated in the preceding sections, the sensory response to hypoxia involves a variety of K^+ channels, Ca^{2+} signaling pathways, changes in tissue O_2 consumption, and metabolism. Undoubtedly, studies are needed to investigate further the effects of IH on these signaling pathways for better understanding of altered carotid body function under the setting of IH.

Cardiorespiratory consequences of chronic intermittent hypoxia induced changes

The chronic IH-induced sensitization of the hypoxic sensory response in neonates is of considerable physiological significance in that in its absence, hypoxia resulting from apneas may have deleterious effects on the central nervous system resulting in ventilatory depression. In the adults, on the other hand, in the early stages of apnea syndrome, carotid body sensitization might be beneficial in that it aids to maintain oxygenation via augmented HVR, and increases in blood pressure. However, if the apneas are untreated, the augmented hypoxic sensitivity of the carotid body might lead to unstable breathing resulting in an increased number of apneas. Moreover, patients with sleep-disordered breathing display persistent increase in sympathetic nerve activity and often exhibit hypertension (428). Activation of the carotid body reflexly activates sympathetic nervous system and elevates blood pressures. It is likely that chronic IH-induced sensory LTF contribute to persistent elevation of sympathetic nerve activity in patients with recurrent apnea (Fig. 17).

Hyperoxia and Carotid Body Function

Whilst the preceding sections describe the impact of prolonged sustained and IH on carotid body function and their functional consequences, this section outlines the impact of too much oxygen (hyperoxia) on carotid body function and its consequences on breathing. The effects of ~70 h of exposures to normobaric (100% O_2) and hyperbaric (5 ATM) hyperoxia were examined cat carotid body function (496, 839). Normobaric as well as hyperbaric hyperoxia selectively impaired the carotid body response to hypoxia and cyanide; whereas it augmented the hypercapnic sensory response. However, the carotid body response to nicotine and dopamine were unaltered. Electronmicroscopic analysis of carotid bodies from hyperbaric hypoxia treated cats showed significantly increased mean volume-density of mitochondria and decreased cristated area per mitochondrion, whereas the distribution of dense-cored vesicles was unaltered in type I cells (839). These observations suggest that the impaired hypoxic response is due to changes in oxidative metabolism resulting from altered mitochondrial structure rather than availability of neourotransmitters.

A majority of preterm infants exhibit recurrent apneas and supplemental O_2 is a common therapy to improve arterial blood O_2 in these subjects. Katz-Salamon and Lagercrantz (431) reported that preterm infants on long-term supplemental O_2 display attenuated ventilatory response to hypoxia. Ling et al. (532) reported that perinatal hyperoxia does not impair central integration of carotid chemoreceptor afferent input. On the other hand, several studies reported severely impaired carotid body responses to acute hypoxia in rats treated with chronic hyperoxia (89, 221, 338, 729, 869). A recent study by Donnelly et al. (220)

showed enhanced carotid body response to hypoxia in P7 rats treated with 60% O_2 for a day; whereas extending hyperoxic exposure to three to five days significantly reduced the hypoxic response. The attenuated sensory response was associated with reduced $[Ca^{2+}]_i$ responses to hypoxia in type I cells as well as decreased afferent nerve conduction. Furthermore, the effects of hyperoxia persisted into adult life. Thus, the carotid bodies of adult rats treated with perinatal hyperoxia showed blunted sensory response to hypoxia, decreased dopamine release by hypoxia, decreased dopamine synthesis, reduced $[Ca^{2+}]_i$ responses to hypoxia of type I cells (729).

Chronic perinatal hyperoxia caused hypoplasia of the carotid body (242, 729). Ultrastructural analysis revealed necrosis of type I cells, increased endoplasmic reticulum, Golgi appratus, and mitochondrial volume with loss of cristae in young compared to older rats that were treated with perinatal hyperoxia (203). A study by Erickson et al. (242) showed loss of unmyelinated but not myelinated axons in the carotid sinus nerves and degenerative changes in chemoafferent neurons in the glossopharyngeal petrosal ganglion in prenatal hyperoxia exposed rats. These authors suggested that the chemoafferent degeneration following perinatal hyperoxia is due specific loss of trophic factor(s) from the carotid body. Kwak et al. (490) found that hyperoxia elevates ROS levels and increased cell death in organotypic slices of petrosal-nodose ganglia from neonatal rats and antioxidants prevented this response, suggesting that ROS mediates hyperoxia-induced loss of chemoafferent neurons. However, Bavis et al. (64) reported that the carotid body response to hypoxia was not restored by treating hyperoxic perinatal rats with vitamin E (1000 IU vitamin E/kg) or a superoxide dismutase mimetic, MnTMPyP (daily intraperitoneal injection of 5–10 mg/kg), two established antioxidants. Thus, these studies show that perinatal hyperoxia, when given in a critical time window after birth, leads to long-lasting impairment of hypoxic sensing by the carotid body and associated with structural changes including those in mitochondria, hypoplasia of type I cells and loss of chemoafferent neurons in petrosal ganglion. Further studies addressing the mechanisms linking the structural and functional changes in the carotid body to hyperoxia are needed.

The Carotid Body and Congestive Heart Failure

CHF is a major health care problem affecting one in 10 men and women after age 60 in the USA alone (753). Sympathohumoral activation is a hallmark of CHF, which contributes to the progression and ultimate mortality of the disease (243). The role of chemoreflexes arising from the carotid body in sympathetic activation in CHF has received considerable attention in recent years (154, 769). The following section summarizes how CHF affects carotid body function and its impact on autonomic functions.

CHF patients, especially those in more severe stages of heart failure display exaggerated peripheral chemoreflex function as evidenced by augmented ventilatory response to hypoxia (152, 155). The augmented chemoreflex function, however, is not obvious in patients with mild CHF (609). Studies on CHF patients are complicated by several factors including etiology, duration, severity, and treatment and animal models may thus provide some further insight. Rapid pacing of the heart (360–380 beats/min) in rabbits causes dilated cardiomyopathy with progressive deterioration of left ventricular function over the course of three to four weeks (813) and studies on this rabbit model provided clear evidence for the contribution of the carotid chemoreflex to sympathetic activation in CHF (528, 531, 813). Direct recording of carotid body sensory activity (Fig. 18) showed elevated baseline activity and enhanced sensory response to hypoxia in CHF compared to control rabbits (812).

Mechanisms associated with enhanced carotid body sensitivity to hypoxia in congestive heart failure

Angiotensin II (Ang II)—Circulating and local tissue Ang II levels are increased in CHF patients (751) and in CHF rabbits (531). Since carotid bodies express the enzymatic machinery for local generation of Ang II (296, 507), recent studies have examined the role of Ang II in CHF-induced sensitization of the carotid body function. Ang II levels and AT1 receptor mRNA and protein expressions were elevated in carotid bodies from CHF rabbits (768). An AT1 receptor antagonist prevented CHF-evoked sensitization of the carotid body response to hypoxia (531). Recent studies explored the mechanisms by which Ang II contributes to the enhanced hypoxic sensitivity in CHF carotid bodies. Hypoxia-induced K^+ channel inhibition in type I cells was more pronounced in CHF rabbits and ATI receptor blockade prevented this effect (529). Furthermore, NADPH oxidase subunit mRNA and protein are elevated in carotid bodies from CHF rabbits, and a NADPH oxidase inhibitor or a superoxide dismutase mimetic prevented the effect of Ang II on the sensitivity of the K^+ current to hypoxia (527). Furthermore, blockade of AT1R by L-158,809 attenuated or normalized the exaggerated hypoxia-induced chemoreflex responses in CHF rabbits (531). Based on these findings it has been suggested that Ang II/AT1R signaling via NADPH oxidase/ROS enhance O_2 sensitivity of K^+ channels in type I cells, which might account for the enhanced hypoxic sensitivity of the carotid body in CHF rabbits.

Nitric oxide—NO-synthase activity and NO production were decreased in various tissues from CHF rabbits (955). The role NO in carotid body function in CHF rabbits was examined. Type I cells from rabbit carotid bodies, in contrast to rat and cat type I cells seem to express neuronal NO synthase (812). Carotid bodies from CHF rabbits showed a decreased basal production of NO (528, 812) and a reduced number of NADPH-diaphorase positive cells, a marker of neuronal NO synthase. The NO synthase inhibitior, N-nitro-l-arginine (L-NNA) increased baseline discharge and augmented the hypoxic sensory response in control rabbits, whereas it had very little effect on carotid bodies from CHF rabbits (812). Furthermore, gene transfer of nNOS using an adenoviral vector (Ad·nNOS) to the carotid body in CHF rabbits enhanced protein expression and NO production in the carotid body and reversed the augmented chemoreceptor activity in the CHF state (528). Ad·nNOS treatment of the carotid body restored normal chemoreflex function in CHF rabbits (528).

The cellular mechanisms underlying the effects of NO in CHF carotid bodies were also examined. NO donors enhanced K^+ currents in type I cells in sham and CHF rabbits (530); whereas the NOS-inhibitor, L-NNA blunted I_K in sham rabbits but not in CHF rabbits (768). It was suggested that blunted K^+ currents in type I cells under normoxic conditions is a consequence of NO depletion in the carotid body. These observations indicate an absence of tonic inhibitory influence from endogenous NO generated by nNOS in carotid bodies from CHF rabbits. The enhanced carotid body sensitivity to hypoxia in CHF appears in part due to upregulation Ang II/superoxide anion pathway and downregulation of NOS/NO pathways in the carotid body (768).

The Carotid Body and Hypertension

Patients with essential hypertension exhibit enhanced sympathetic nerve and ventilatory response to hypoxia, and these responses were attributed to an exaggerated carotid body response to low O_2 (428, 847). Fukuda et al. (294) recorded carotid body sensory activity in spontaneously hypertensive rats (SHR) and found augmented sensory response to hypoxia but not to CO_2 in SHR rats compared to normotensive controls. Carotid body sensitivity to hypoxia, however, was unaltered in renal hypertension (41). Enlargement of the carotid body was reported in SHR rats and humans with essential but not with renal hypertension

(328, 329). Catecholamine levels were elevated in carotid bodies from hypertensive rats (328). Although these studies demonstrate that certain forms of hypertension lead to enhanced carotid body sensitivity to hypoxia, little is known about the underlying mechanisms. Recently Tan et al. (816) studied the responses of amiloride-sensitive acid-sensing sodium channel (ASIC3) and TASK-1 responses to acidic pH in type I cells from young SHRs. They found that both ASIC3 and TASK-1 channel expressions were elevated and depolarizing effects of low pH on type I cells were pronounced in SHR compared to normotensive controls and have suggested that these changes may contribute to the elevated sympathetic activity that precedes the full expression of hypertension.

Conclusion

Since the discovery of carotid bodies as sensory organs for detecting arterial blood O_2, much attention has been focused on elucidating the mechanisms of transduction and transmission. A substantial body of evidence points to the type I cell and the opposing afferent nerve endings as the primary site of sensory transduction. The past couple of decades has witnessed a rapid progress in our understanding of the cellular and molecular mechanisms associated with the transduction process. We have attempted to summarize the current concepts on the transduction and transmission mechanisms with a particular emphasis on integrating cellular responses with the sensory nerve activity of the whole organ in response to hypoxia. The suggestion that type I cells have neurosecretory function is now well supported by evidence of an absolute requirement for Ca^{2+}-dependent neurotransmission between type I cells and afferent nerve endings. The nature of the O_2 sensor mediating cellular response to acute hypoxia is a key to the understanding of transduction. Whilst a number of hypotheses (e.g., membrane and mitochondrial hypotheses, as well as heme proteins), have been advanced, none appear to describe the chemosensory response to hypoxia in full. Part of the difficulty arises from a lack of detailed descriptions of O_2 affinity of the purported "O_2 sensor." It would be of great value in future studies for greater attention to be paid to the stimulus-response curves of PO_2 in cellular models, similar to those described for the whole organ. Furthermore, any hypothesis regarding the transduction mechanism(s) should take in to account (i) the particularly high sensitivity of the organ for changes in Po_2, (ii) its rapid, within seconds, speed of response, and (iii) for the modulating actions of other natural stimuli such as CO_2. Given these caveats, there is little, if any, compelling evidence to suggest that a single O_2 sensor can be responsible for the entire stimulus-response characteristics of the carotid body. Alternatively, given the complex stimulus-relation characteristics of the carotid body, it may be more useful to consider several sensors working in concert over different ranges of Po_2 rather than a single sensor. Such a multi-O_2 sensor concept may provide an important failsafe redundancy for a vital homeostatic process.

Whilst progress on the transduction mechanism has been substantial, understanding the sensory transmission has been relatively slow. One reason may be the species-, gender-, and age-dependent variations apparent in the neurochemical content and receptor expression of the carotid body and another is the inherent limitations associated with pharmacological approaches. Recent studies on transgenic animal models appear promising in resolving some of the controversies and limitations associated with pharmacological approaches. Given the complexity of the organ's repertoire of transmitters and receptors, it is likely that the excitatory and inhibitory transmitters work in concert with a "push-pull" mechanism conferring the sustained excitation during acute hypoxia and probably playing a greater role in mediating the plastic changes in chemoreceptor function in health and disease.

The carotid body, in addition to hypoxia, also responds to a wide variety of other respiratory and nonrespiratory stimuli including CO_2, pH, glucose, proinflammatory cytokines,

circulating hormones, K^+, osmolarity, and temperature. These observations raise the question whether carotid body is a “polymodal” sensory receptor or whether these stimuli simply modify some elements of the O_2 transduction pathway. Future studies addressing the carotid body response to these “other” stimuli may prove to be as relevant for human health as the response to hypoxia.

It is becoming increasingly evident that the carotid body response to acute hypoxia is dynamically altered in response to chronic perturbations in systemic and/or environmental O_2. The plasticity of the sensory response of the carotid body and ensuing alterations in reflex responses are critical for ventilatory adaptations to hypoxia at high altitude as well as during exercise. Further studies addressing cellular and molecular mechanisms underlying carotid body adaptations to chronic hypoxia are needed. Although it is appreciated pregnancy leads to hyperactive carotid body, neither the underlying mechanisms nor its functional consequences are delineated. Recent studies provide rather more compelling evidence for carotid body reflexes triggering autonomic morbidities associated with pathological situations such as recurrent apnea syndrome, CHF and hypertension. A greater understanding of key signaling pathways associated with carotid body plasticity under pathological conditions is of potential clinical interest for the future development of selective therapeutic agents aimed at relieving the adverse consequences associated with these diseases.

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The original drawing from Fernando de Castro reproduced in Figure 1A belongs to the Fernando De Castro Archives, and we are indebted with Fernando-Guillermo de Castro to authorize us for the use of this in the current work.

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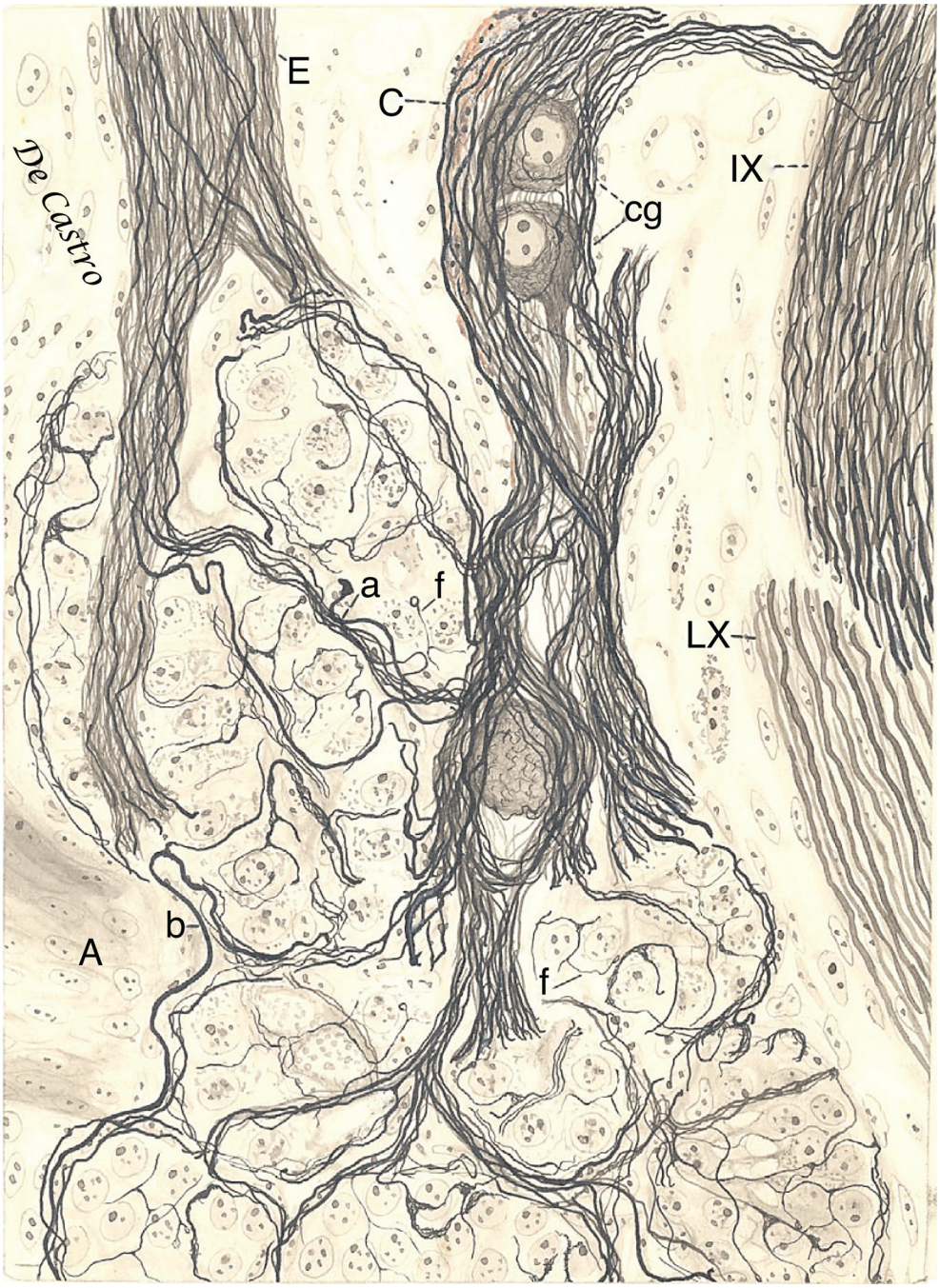

Figure 1.
Ultrastructure of the carotid body. (A) Reproduction of the original drawing of Fernando de Castro published in De Castro (1926), part of the Fernando de Castro Archives. Different glomeruli are shown close to the carotid artery (A). Incoming sympathetic nerve from the superior cervical ganglion (E) is a minor contribution to the innervation of the carotid body. The same can be said about the vagus nerve (LX) in the vicinity of the carotid body. By contrast, the most relevant contingent of afferents comes from the intercarotid (sinus) nerve branch of the glossopharyngeal nerve (IX). A sympathetic microganglion can be seen within the latter nerve (cg). Adapted, with permission, from (191) by de Castro.

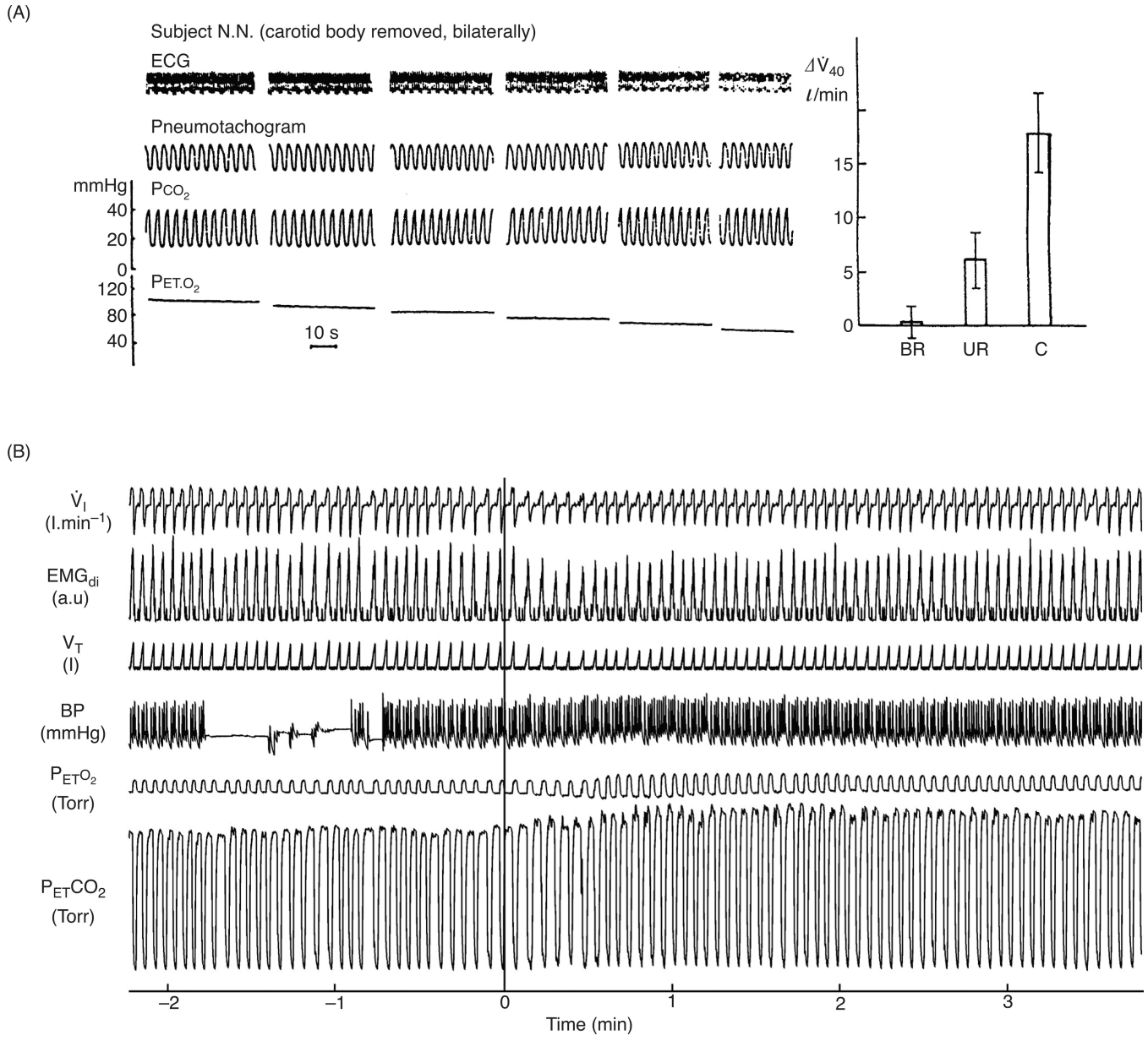

Figure 2.

Role of carotid body in ventilatory response to hypoxia. (A) Ventilatory response to progressive hypoxia in subjects after bilateral carotid body resection (BR). End tidal P_{O2} (P_{ETO2}) was measured by a rapid-response O_2 electrode housed in an end-tidal sampler. P_{ETO2} was progressively decreased at *ca*. 10 Torr/min, from 100 to 40 Torr. Despite advancing hypoxia, no change in ventilation was seen, as clearly reflected by unaltered profile of airway PCO_2 measured by infrared CO_2 analyser. Right: hypoxic ventilatory response in terms of ΔV_{40} (mean ± SE), that is, increment in ventilation as P_{ETO2} decreased from 100 to 40 Torr, while P_{ET}, CO_2 was kept at resting level. UR, patients with unilateral carotid body resection; C, controls. ΔV_{40} of BR group is statistically not different from zero, whereas that of UR group is between BR and C groups. Adapted, with permission, from (385) Honda. (B) Polygraph record of a representative trial of carotid body (CB) inhibition in a dog. The left CB was denervated prior to experiment. Perfusion of the isolated right

carotid sinus region with hyperoxic (>500 mmHg) and hypocapnic (*ca.* 20 mmHg) blood begins at time 0 (solid vertical line). VI, ventilation; EMGdi, moving-time-averaged electromyogram of the costal diaphragm; VT, tidal volume; BP, blood pressure; $P_{ET}CO_2$ end tidal PCO_2; $P_{ET}O2$, end-tidal P_{O_2}; and ua, arbitrary units. Interruption in the BP trace is due to blood sampling. Note that the immediate hypoventilation and $P_{ET}CO2$ increase with CB inhibition persists for the duration of the trial. Adapted, with permission, from (91) Blain et al.

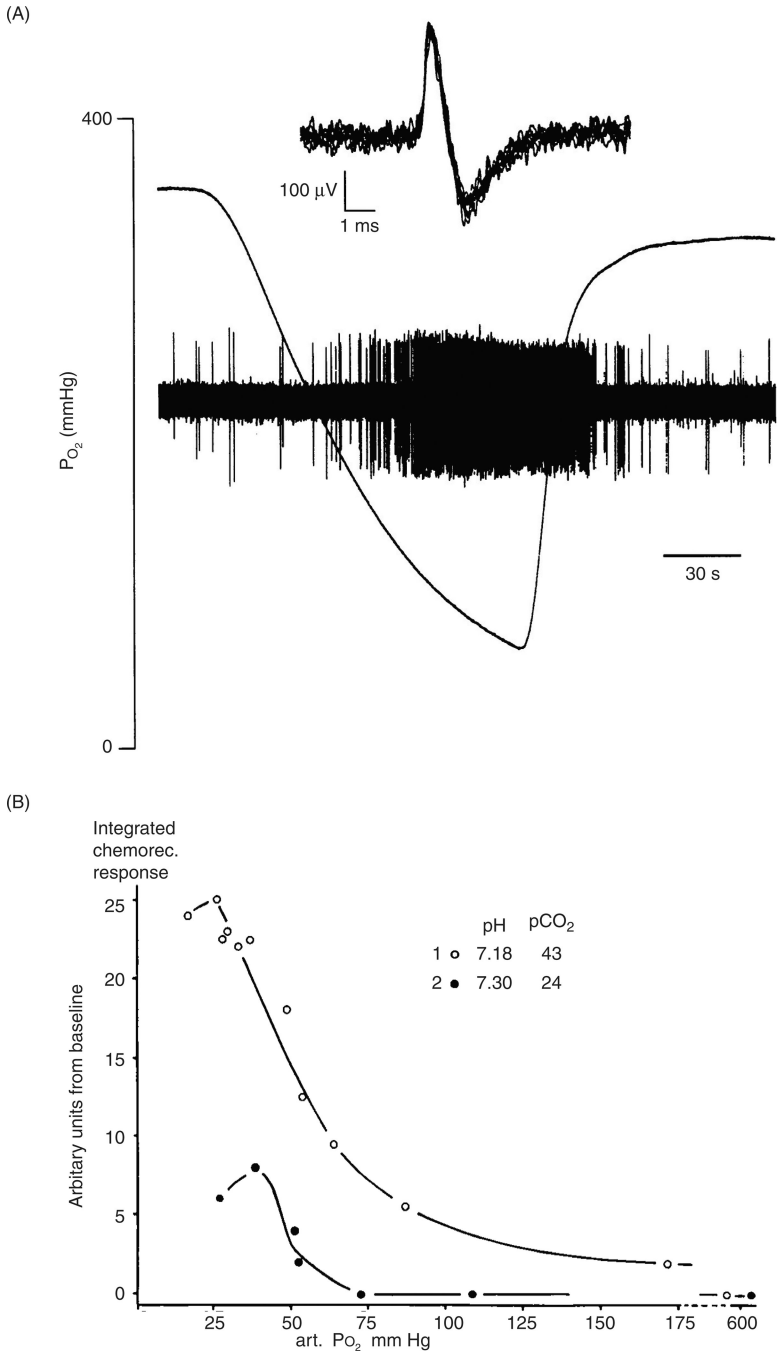

Figure 3.

Chemoafferent response to hypoxia. (A) The central, superimposed traces show an example of the increase in the frequency of action potentials, recorded from a slip of the rat carotid sinus nerve as P_{O2} was reduced from hyperoxia to hypoxia and then back to hyperoxia. The inset at top shows, on a different timescale, a composite of eight, superimposed action potentials. PCO_2 = 38 mmHg. Adapted, with permission, from (693) from John Wiley and Sons, Pepper et al. *B.* Integrated chemoreceptor response to changing arterial P_{O2} at one level of H^+-PCO_2. Curve 1: mean arterial B.P. 130 mmHg, increasing to 160 mmHg at lowest PaO_2. Adapted, with permission, from (389) Hornbein et al.

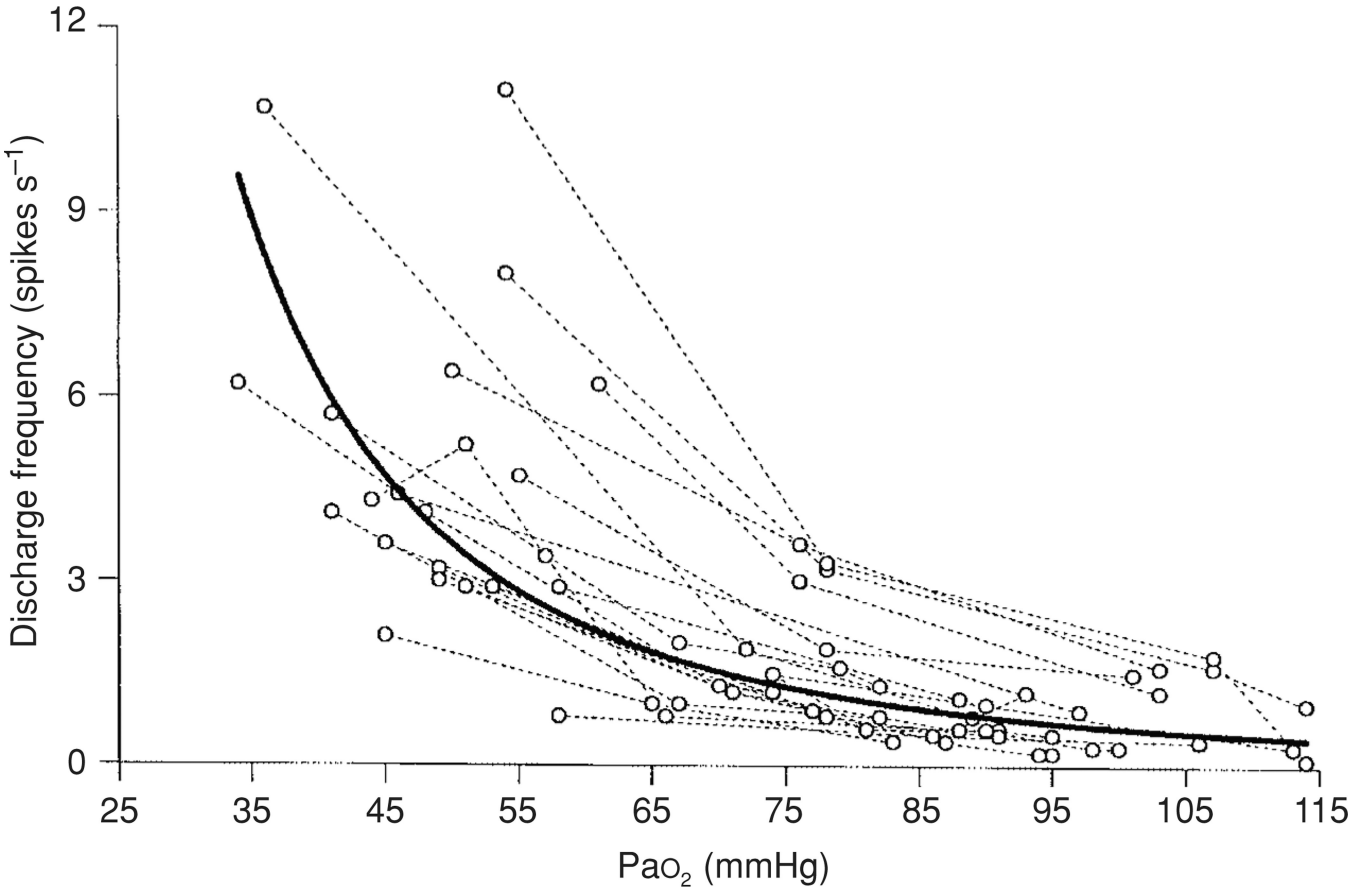

Figure 4.

Effect of different levels of oxygenation upon single-unit chemoreceptor activity in the rat *in vivo*. Each of the dashed lines connects the data points for a given unit (n = 20 units). Note the variation in basal discharge in normoxia, the Pa_{O2} at the point of inflexion and the sensitivity at any PaO_2, between units. The continuous line is the best fit (r^2 = 0.6) for all points and represents a function in which $f_{dis} = 74010\ (PaO_2)^{-2.5}$, where f_{dis} is the discharge frequency (spikes s^{-1}) and PaO_2 is recorded in mmHg. r^2 is the correlation coefficient. Adapted, with permission, from (870) John Wiley and Sons, Vidruk et al.

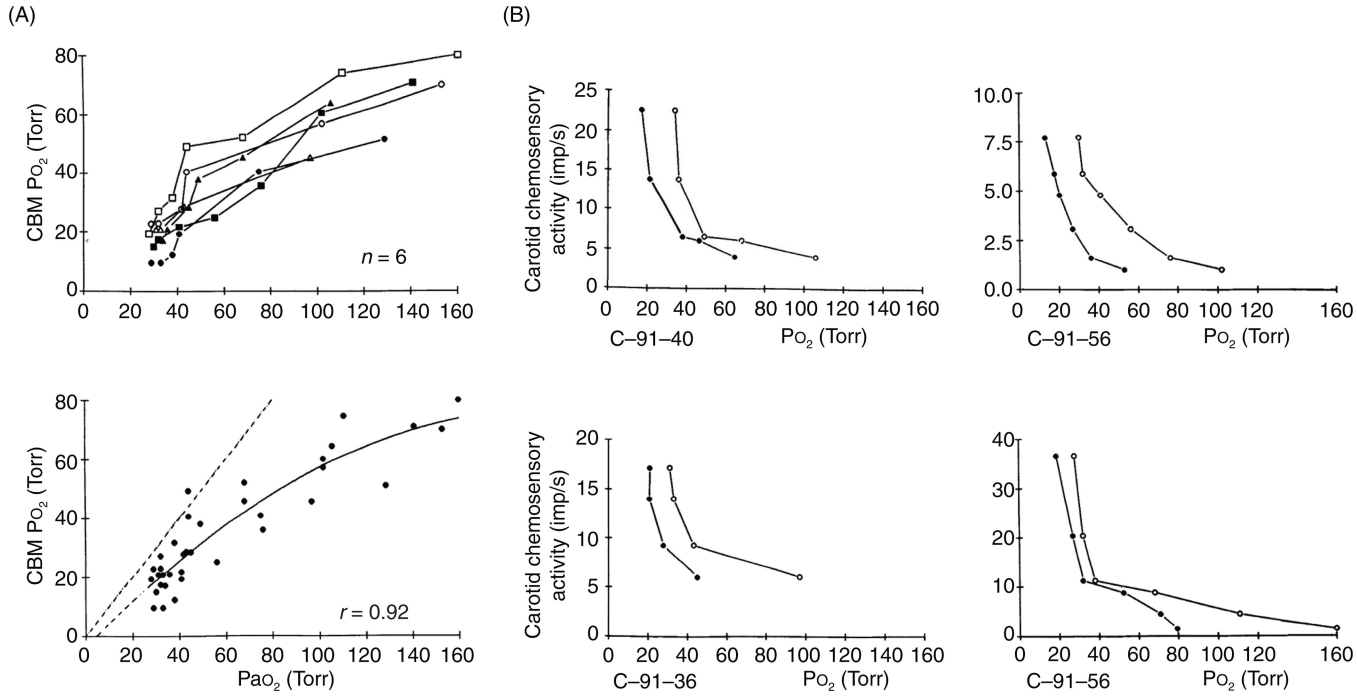

Figure 5.

Relationship between arterial and microvascular P_{O_2} and consequence upon chemodischarge response curves. (A) Relationship between Pa_{O_2} and carotid body microvascular (CBM) P_{O_2}. O_2 pressure in inspired gas was lowered in steps. At each step, Pa_{O_2}, chemosensory nerve activity and phosphorescence images were measured *ca*. 3 min after end-tidal gas values stabilized. Average CBM P_{O_2} was calculated for central region of O_2 pressure map of carotid body and this is plotted against measured Pa_{O_2}. Top: data from six cats are presented. Each cat has a different symbol. Below: data from all six cats are fitted to single curve (line of identity also shown). (B) Relationship between P_{O_2} (arterial and CBM) and chemosensory nerve activity. Measured values of chemosensory nerve activity in four different cats are plotted against Pa_{O_2} (open circles) and CBM P_{O_2} (filled circles). imp/s, impulses per second. Modified, with permission, from (502) Lahiri et al.

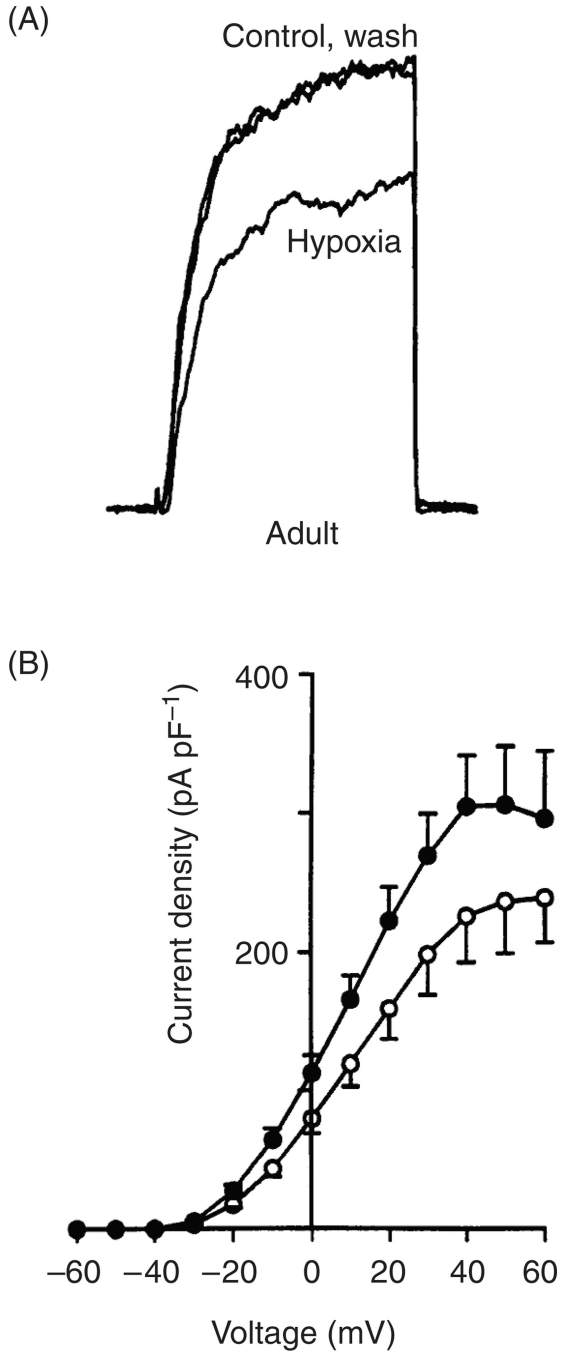

Figure 6.
Inhibition of type I cell K^+ currents by hypoxia. (A) Currents from type I cells evoked by step depolarizations from −70 to +20 mV before (control), during (hypoxia), and after (wash) lowering of perfusate P_{O2} to 16 to 23 mmHg. The traces are representative recordings from type I cells of adult rats. (B) Mean + SEM current density-voltage relationships before (filled circles) and during (open circles) application of hypoxic perfusate (*n* = 14). Modified, with permission, from (347) John Wiley and Sons, Hatton et al.

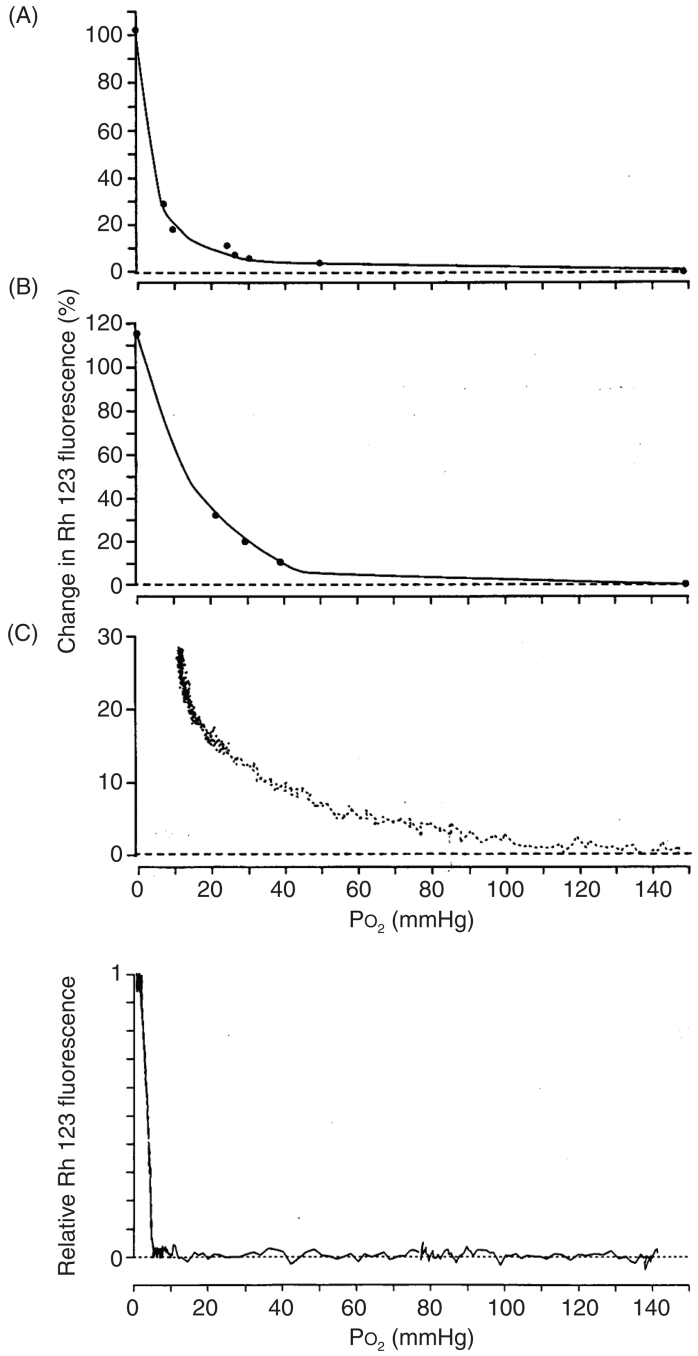

Figure 7.

Effect of hypoxia upon mitochondrial membrane potential as measured by Rh 123. Top three traces: Stimulus-response curves for three different rabbit type I cell groups. In A and B, the steady-state maximal increase in Rh 123 fluorescence is plotted against the minimum P_{O_2} achieved during superfusion. The trace in C shows a continuous stimulus-response curve, as the Rh 123 fluorescence is plotted directly against the trace from a P_{O_2} electrode placed close to the cells. Bottom trace: A continuous plot of Rh 123 fluorescence as a function of P_{O_2} obtained from a freshly dissociated rat chromaffin cell. There was no measurable change in signal until the P_{O_2} fell below about 5 mmHg. Modified, with permission, from (228) John Wiley and Sons, Duchen and Biscoe.

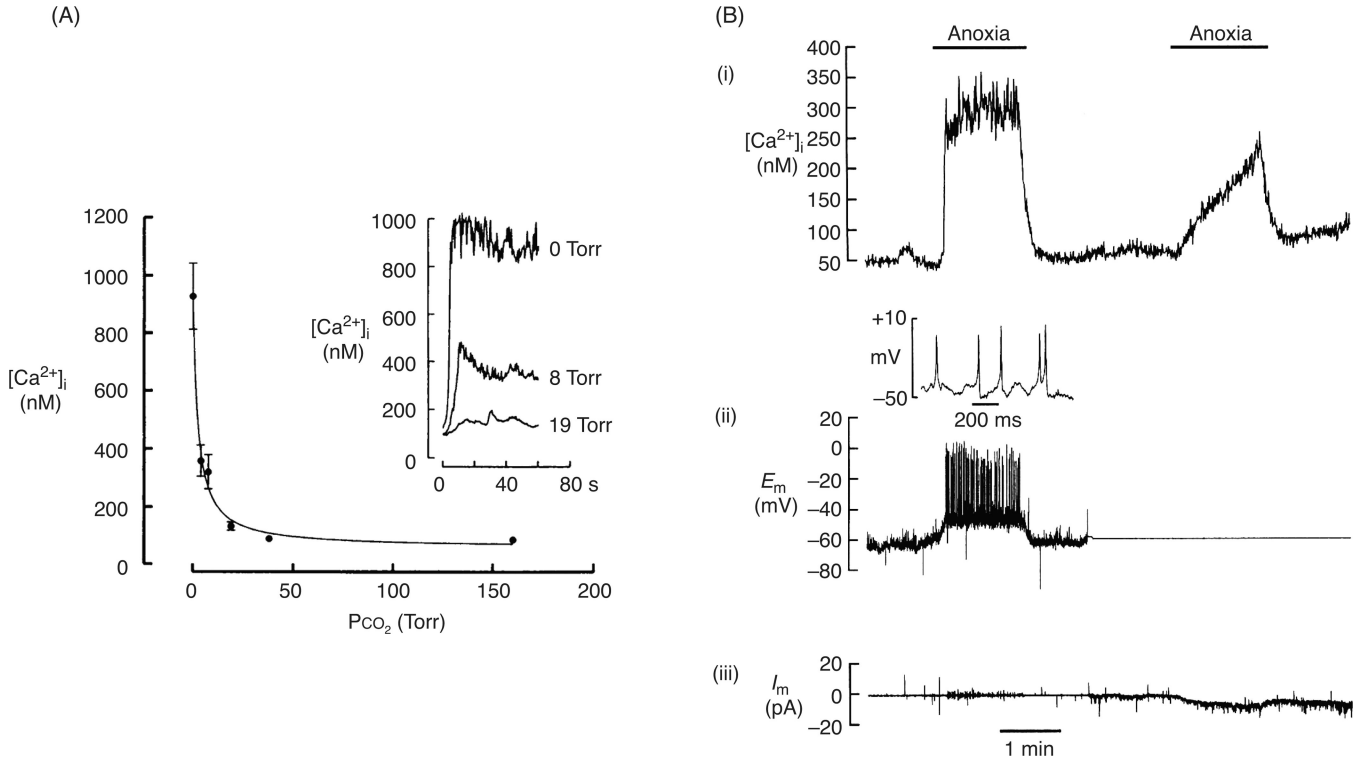

Figure 8.

Relation between hypoxia and intracellular $[Ca^{2+}]$ in rat type I cells. (A) Effects of hypoxia on $[Ca^{2+}]_i$ in rat type I cells. Plot of the mean $[Ca^{2+}]_i$ against P_{O_2} for 15 recordings.; error bars are ± SEM (for each level of P_{O_2} in each recording $[Ca^{2+}]_i$ was averaged over a 1 min period). Continuous line is the best fit to a hyperbolic function of form $[Ca^{2+}]_i = (a + c) - [(a\ P_{O_2})/(b + P_{O_2})]$; where a = 820 nM, b = 2.47 Torr, and c = 54.8 nM. Inset shows averaged (n = 15) $[Ca^{2+}]_i$ responses to three levels of hypoxia. (B) Simultaneous recordings of membrane potential and current (using the perforated-patch recording technique) and $[Ca^{2+}]_I$ in a single, isolated type I cell. (i) Shows $[Ca^{2+}]_i$, (ii) membrane potential (E_m), and (iii) current (I_m; current is averaged over 10 ms intervals). The experiment begins in voltage recording mode (i.e., current clamp with I = 0; noise in current trace during voltage recording is artefactual). Inset shows action potentials recorded during anoxia on a faster time base. After the first exposure to anoxia the cell is voltage clamped at approximately −60 mV (see change in voltage trace). A second exposure to anoxia is then given. Modified, with permission, from (107) John Wiley and Sons, Buckler and Vaughan-Jones.

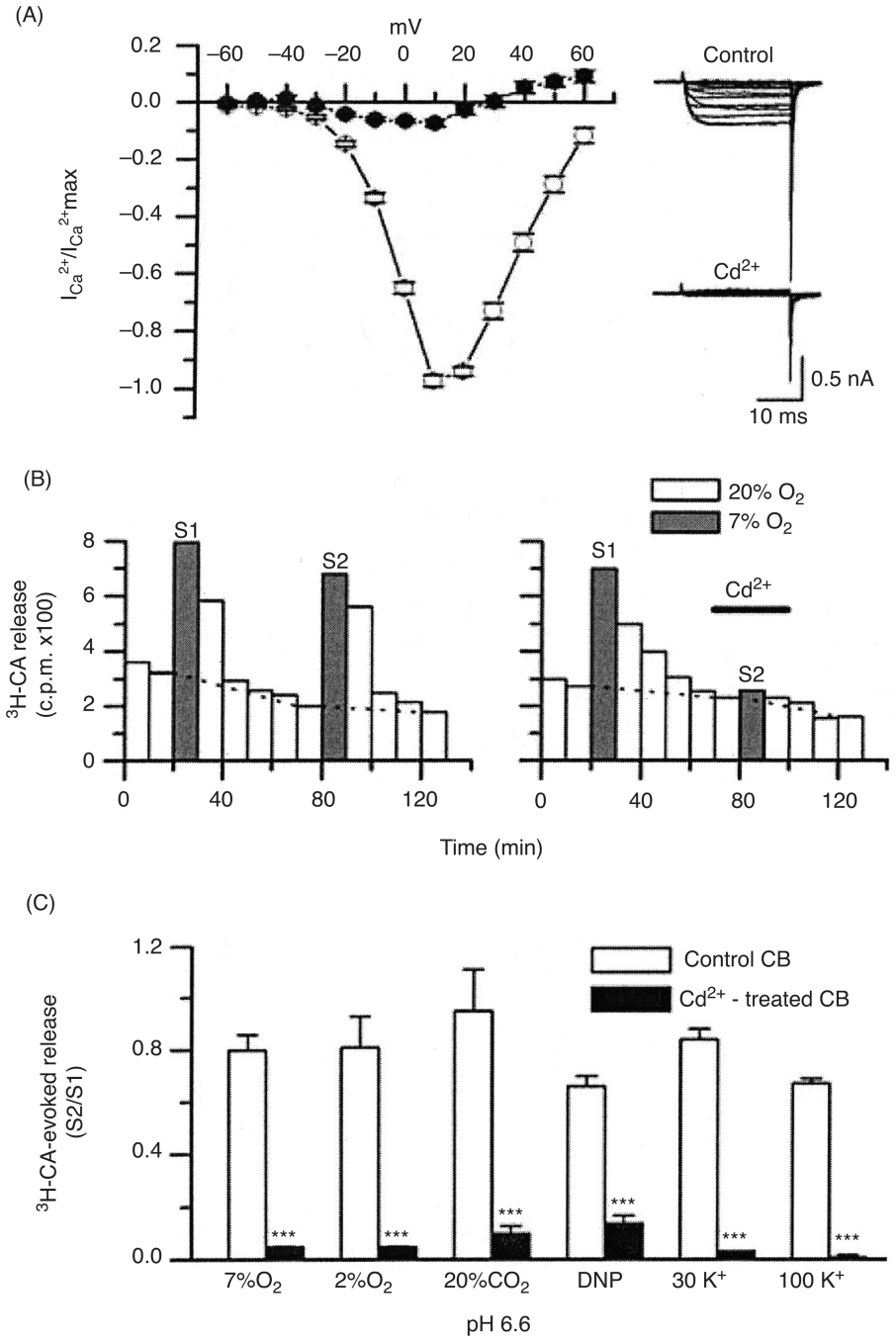

Figure 9.

Effect of cadmium on calcium currents and stimulus induced secretion of labeled catecholamine, [^{3}H]CA. (A) Normalized I–V relationships obtained in isolated chemoreceptor cells before (open circles) and during (filled circles) superfusion with 200 μM $CdCl_2$ (n = 4). From a holding potential of −80 mV, currents were elicited by 20 ms depolarizing pulses in 10 mV intervals. On the right, single currents obtained in a representative cell in both conditions. (B) General protocol used in the experiments of [^{3}H]CA release. The figure shows the actual release of [^{3}H]CA (c.p.m) from a control carotid body (CB; left panel) incubated in normoxia, a 20% O_2-, 5% CO_2-, and 75% N_2-

equilibrated solution (P_{O_2} *ca*. 150 mmHg; open bars), or hypoxia, a 7% O_2-, 5% CO_2-, and 88% N_2-equilibrated solution (P_{O_2} *ca*. 46 mmHg; gray bars). Hypoxia was applied twice (S1 and S2). Evoked release in every application of hypoxic stimuli corresponds to the sum of c.p.m. above dashed lines In the experimental CB (right panel), the protocol was identical except for the presence of 200 μM $CdCl_2$ during the time indicated by the horizontal line. (C) Effect of $CdCl_2$ (200 μM) on the evoked release induced by mild (7% O_2-equilibrated solution) and intense (2% O_2-equilibrated solution) hypoxia, hypercapnic acidosis (20% CO_2-equilibrated solution; pH 6.6), dinitrophenol (DNP; 100 μM) and 30 and 100 mM extracellular K^+_o. Experimental protocol as in B. For every type of stimulation, the figure shows averaged ratios of the evoked release in S2 to the evoked release in S1 (S2/S1), in control and cadmium-treated CB (n = 5–12 in every experimental condition, *** P<0.001). Adapted, with permission, from (746) John Wiley and Sons, Rocher et al.

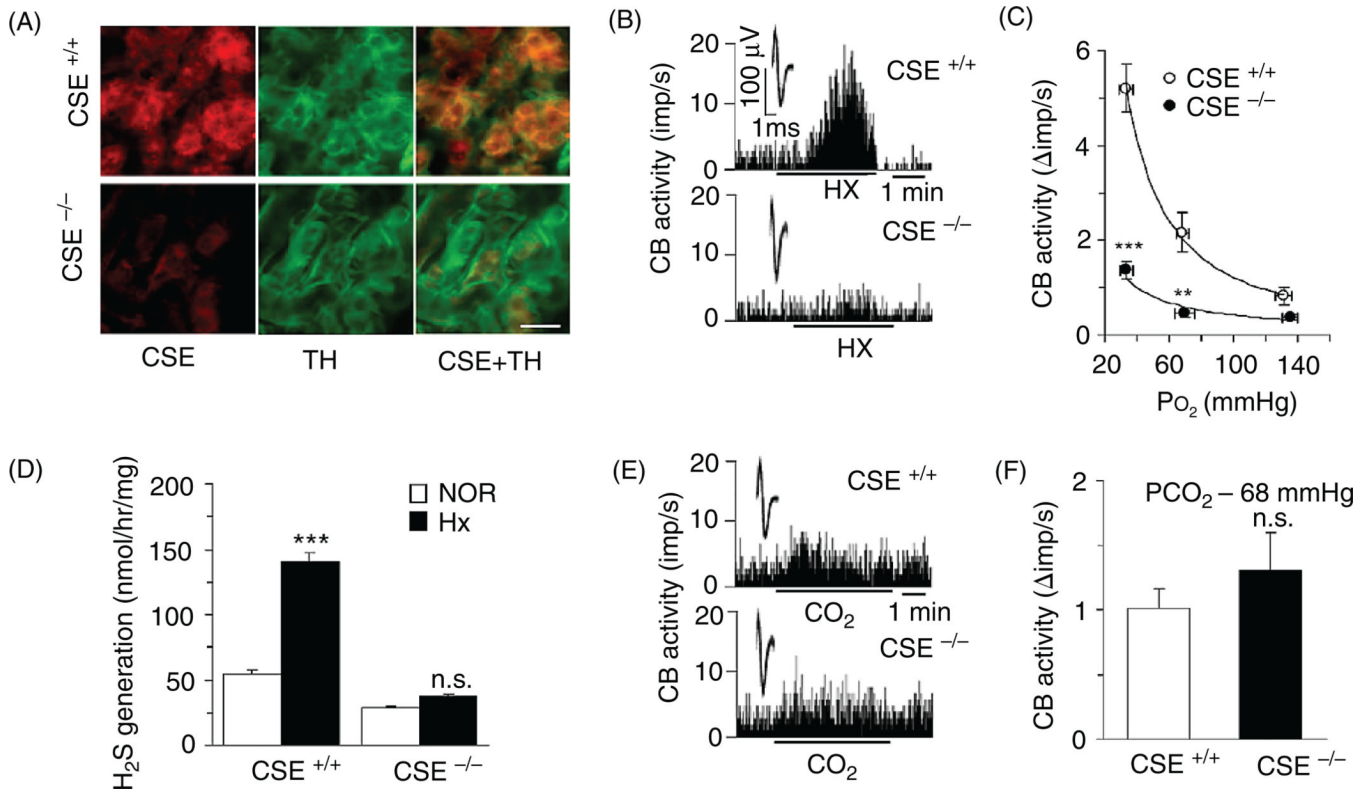

Figure 10.

Cystathionine γ-lyase (CSE) localization in the mouse carotid body and carotid body responses to hypoxia and hypercapnia in $CSE^{+/+}$ and $CSE^{-/-}$ mice. (A) CSE expression in carotid bodies from $CSE^{+/+}$ and $CSE^{-/-}$ mice. Carotid body sections were stained with antibodies specific for CSE or tyrosine hydroxylase (TH), a marker of type I cells. (Scale bar: 20 μm). (B) Sensory response of isolated carotid bodies to hypoxia (Hx) (P_{O_2} *ca*. 39 mmHg; at black bar) in $CSE^{+/+}$ and $CSE^{-/-}$ mice. Integrated carotid body sensory activity (CB activity) is presented as impulses per second (imp/s). Superimposed action potentials from the single fiber are presented in inset. (C) Carotid body responses to graded hypoxia from $CSE^{+/+}$ and $CSE^{-/-}$ mice, measured as the difference in response between baseline and hypoxia (D imp/s). Data are mean ±SEM of $n = 24$ ($CSE^{+/+}$) and $n = 23$ ($CSE^{-/-}$) fibers from eight mice each. (D) H_2S levels (mean± SEM) in carotid bodies from $CSE^{+/+}$ and $CSE^{-/-}$ mice under normoxia (NOR) and hypoxia (Hx) (P_{O_2} *ca*. 40 mm Hg) from four independent experiments. (E) Example illustrating carotid body responses to CO_2 (PCO_2 *ca*. 68 mmHg; at black bar) in $CSE^{+/+}$ and $CSE^{-/-}$ mice. (F) Average data mean (± SEM) of CO_2 response from $n = 24$ ($CSE^{+/+}$) and $n = 19$ ($CSE^{-/-}$) fibers from eight mice in each group. *** and **, $P<0.001$ and <0.01 respectively; n.s. (not significant), $P>0.05$. Adapted, with permission, from (684) Peng et al.

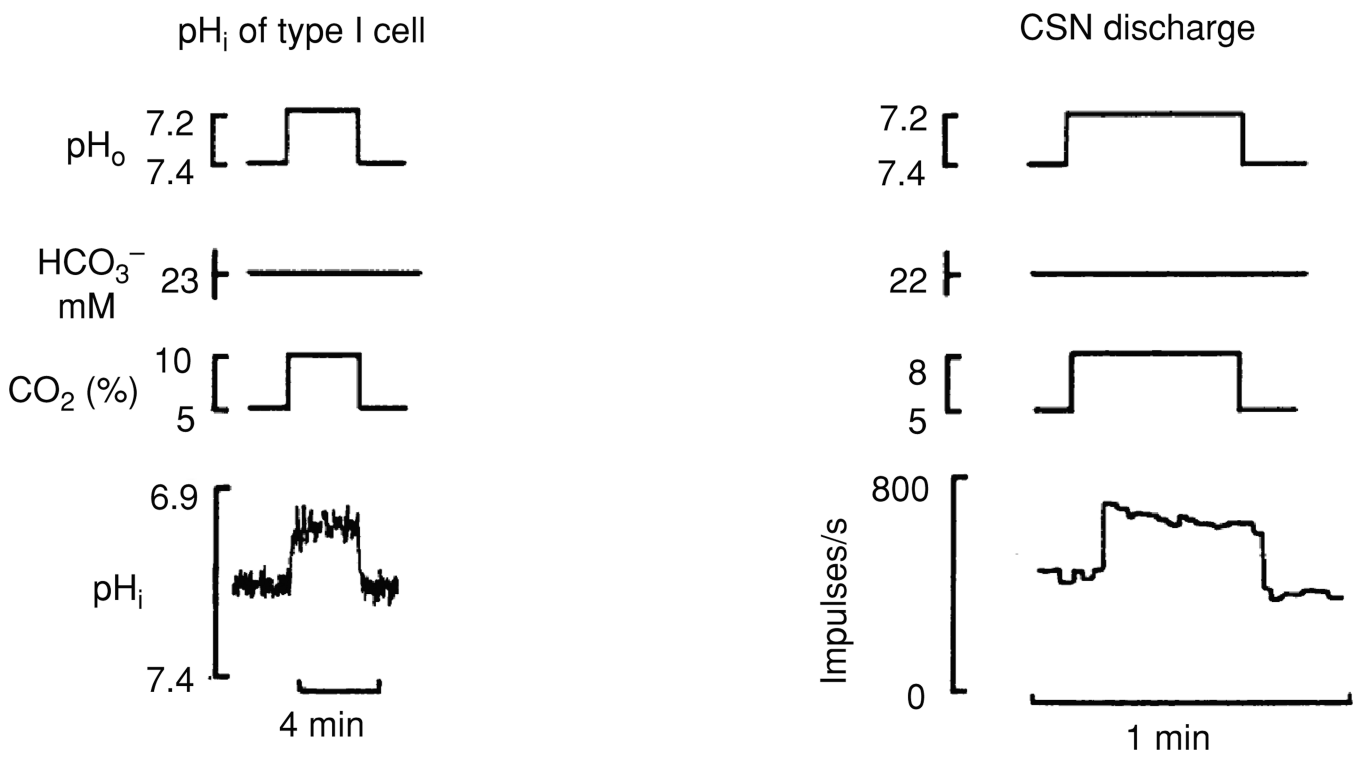

Figure 11.
Effect of respiratory acidosis upon pH_i and chemodischarge. A comparison of the effects on intracellular pH_i in the type I cell with carotid sinus nerve discharge rate (on the right: redrawn from Gray, 1968) of a simulated respiratory acidosis (i.e. increasing PCO_2 at constant $[HCO_3^-]_o$). Adapted, with permission, from (110) John Wiley and Sons, Buckler et al.

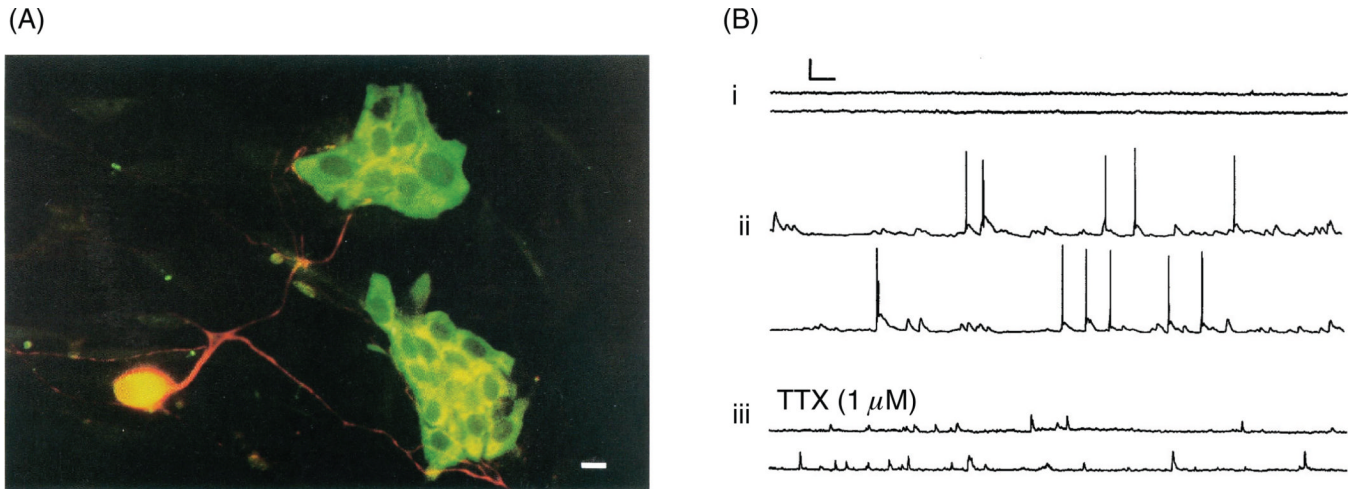

Figure 12.

Functional cocultures of rat type I cells and petrosal neurons. (A) Immunofluorescence staining of a coculture of dissociated rat petrosal neurons and carotid body type I cells. Culture was immunostained with tyrosine hydroxylase (TH) and neurofilament (NF, 68 kDa) antibodies and visualized with a fluorescein- and Texas Red-conjugated secondary antibody, respectively. The two type I cell clusters are TH positive (cytoplasmic green fluorescence), and are intimately associated with NF-positive petrosal processes (red fluorescence); nuclei of type I cells in the clusters appear dark. The single petrosal cell body is both TH and NF positive, accounting for the yellow-orange fluorescence; note an NF-positive pseudounipolar process leaves the petrosal cell body and appears to bifurcate into two main branches, each of which projects to a type I cell cluster. The petrosal neuron (PN) and type I cells were together for 8 days *in vitro*; scale bar represents 10 μm. (B) Effects of coculture on spontaneous membrane activity recorded with the perforated-patch technique from petrosal neurons. (i) Typical recording illustrating lack of spontaneous activity in a PN cultured without type I cells; membrane potential, indicated by the two displaced continuous traces remained relatively steady over time. Note in i–iii the right end of the top trace is continuous with the beginning (left end) of the lower trace. (ii) In coculture, atypical PN that was juxtaposed to a type I cell cluster showed spontaneous spikes and subthreshold potentials (two displaced traces), resembling excitatory post-synaptic potential (EPSPs) seem at chemical synapses. (iii) Perfusion of tetrodotoxin (TTX) (1 μM) to block action potentials, did not eliminate subthreshold potentials (SSPs) recorded in a different PN, juxtaposed to a type I cell cluster. Vertical scale bar (top left) represents 20 mV; horizontal bar represents 1 s in (i) and (ii) and 1.5 s in (iii). The resting membrane potential was −65 mV in (i), −60 mV in (ii), and −70 mV in (iii). Modified, with permission, from (951) John Wiley and Sons, Zhong et al.

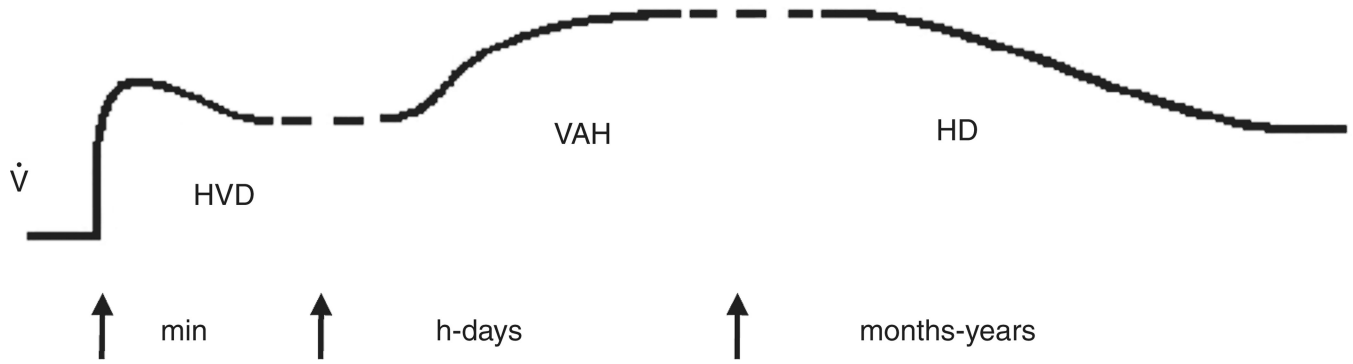

Figure 13.
Ventilatory response to chronic hypoxia. Ventilatory responses during and after prolonged hypoxic exposures include hypoxic ventilatory decline (HVD), ventilatory acclimatization to hypoxia (VAH), and hypoxic desensitization (HD). Modified, with permission, from, (714) Copyright Elsevier, Powell et al.

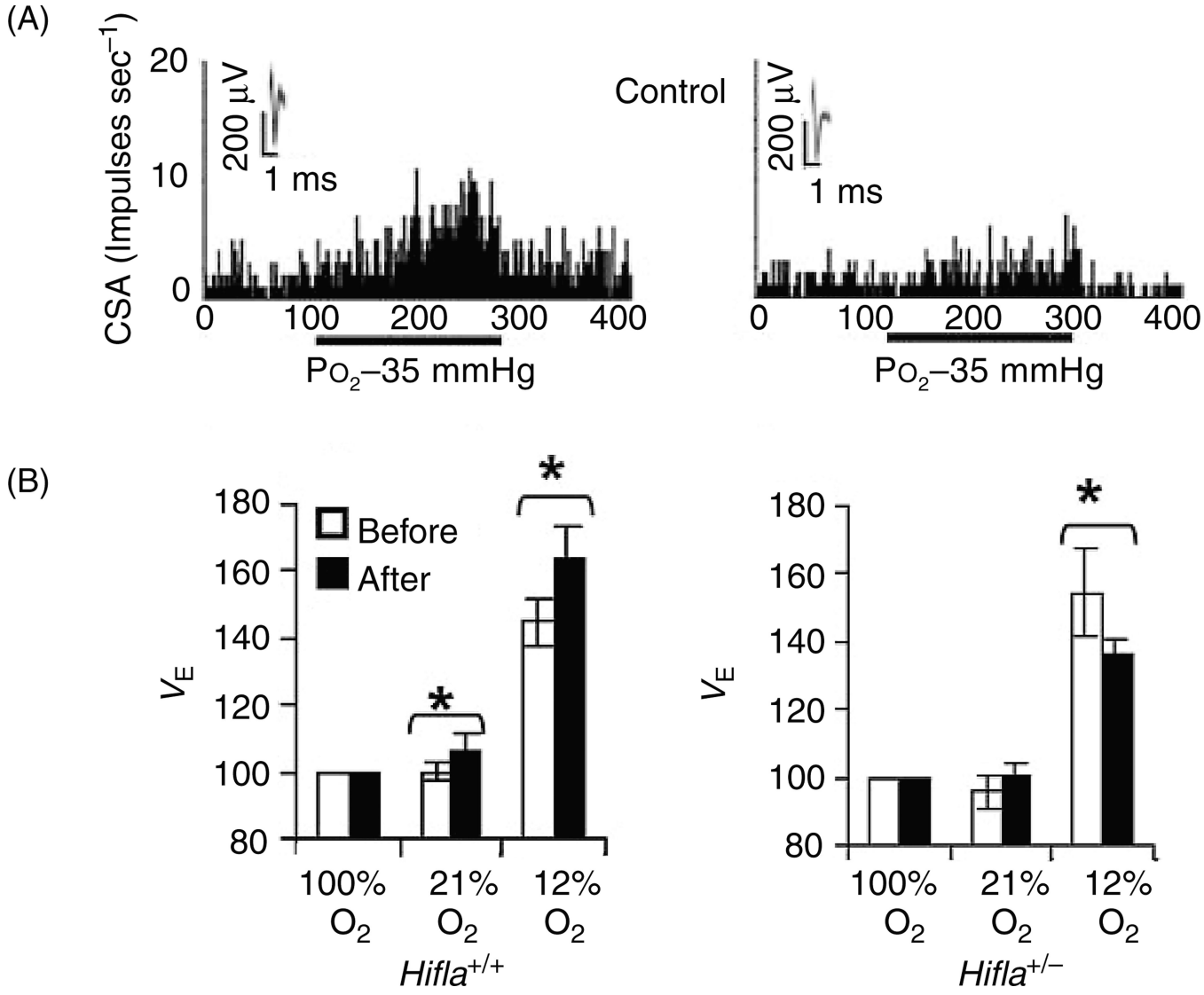

Figure 14.

Chemoafferent and ventilatory responses to hypoxia in HIF-1α deficient mice. (A) Sensory responses to hypoxia (black bars) in wild type (*Hif1a*$^{+/+}$; left panel) and HIF-1α deficient (*Hif1a*$^{+/-}$; right panel) mice. P_{O_2} = partial pressure of O2 in the perfusate. Superimposed action potential of "single" fiber from which the data were derived is shown (inset). Note the blunted carotid body response to acute hypoxia in HIF-1α deficient mice. Adapted, with permission, from (690) John Wiley and Sons, Peng et al. (B) Effect of chronic hypoxia on ventilatory response to acute hypoxia in wild type (*Hif1a*$^{+/+}$) (left panel) and HIF-1α deficient (*Hif1a*$^{+/-}$; right panel) mice. Changes in minute ventilation (VE) are expressed (mean ± SEM) relative to the values obtained while breathing 100% O2. Note the absence of ventilatory adaptation to hypoxia (VAH) in HIF-1α deficient mice. Modified, with permission, from (445) Kline et al.

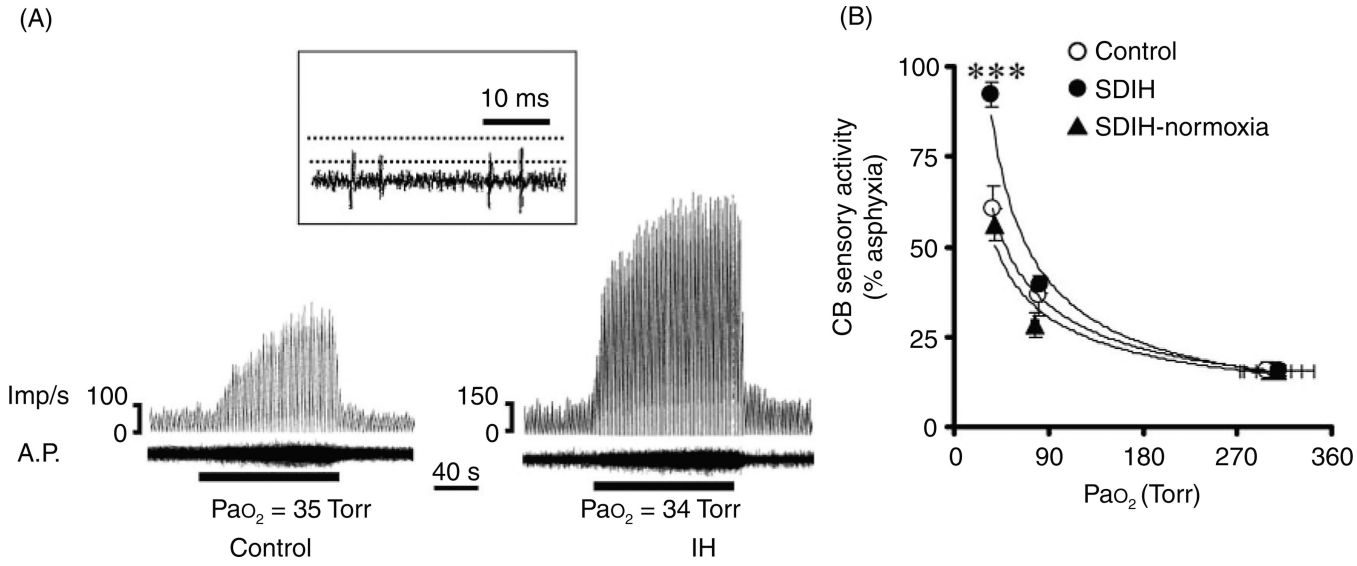

Figure 15.

Effect of intermittent hypoxia upon chemodischarge. (A) Representative tracings showing the changes in carotid body sensory discharges [impulses/second (imp/s)] in response to hypoxia in a control rat and a rat conditioned with 10 days of intermittent hypoxia (IH). AP, raw action potentials; time calibration = 40 s. Hypoxic challenges are marked under the tracings as solid bars, and arterial P_{O2} ($Pa_{O}2$) levels during hypoxic challenges are indicated under the bars. Inset: setting of the window discriminator for selection of action potentials above the baseline. (B) Average data showing the relationships of carotid body (CB) sensory activity (expressed as % of asphyxia response) against Pa_{O2} while arterial PCO_2 ($PaCO_2$) was maintained close to 35 Torr in control (n = 8,), IH-conditioned (n = 8,), and recovered rats (IH-normoxia, n = 7) that were conditioned with 10 days of IH followed by 10 days of normoxic exposure. *** $P < 0.001$, significantly different compared with control rats. Adapted, with permission, from (687) Peng and Prabhakar.

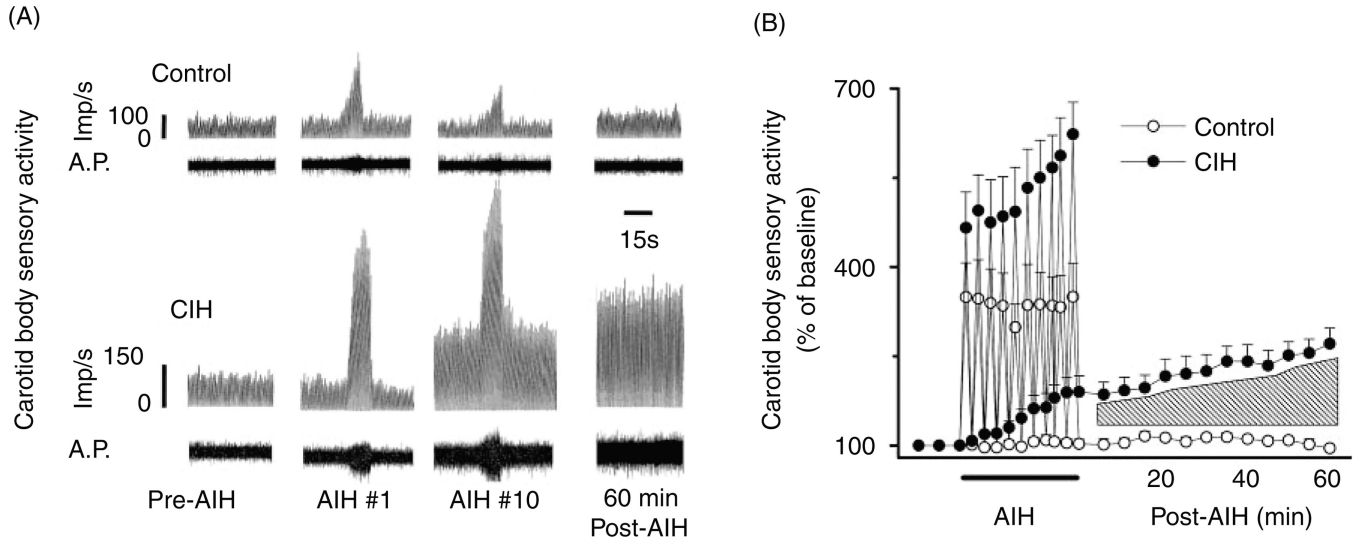

Figure 16.

Acute intermittent hypoxia (AIH) induces sensory long term facilitation (LTF) in the carotid body in chronic intermittent hypoxia (CIH) animals. (A) Carotid body sensory activity in a control (upper) and CIH-conditioned (lower) animal. Pre-AIH is baseline activity; AIH #1 and AIH #10 represent the first and 10th episodes of AIH; impulses (Imp) per s, integrated sensory discharge; A.P., action potentials. (B) Average changes in the sensory activity during AIH and during every 5 min of the post-AIH period. Average data represent mean ± SEM from control (n = 7) and 10 days CIH-conditioned (n = 7) animals. The shaded area represents the difference in baseline activity in CIH and control animals during the post-AIH period. Adapted, with permission, from (686) Peng et al.

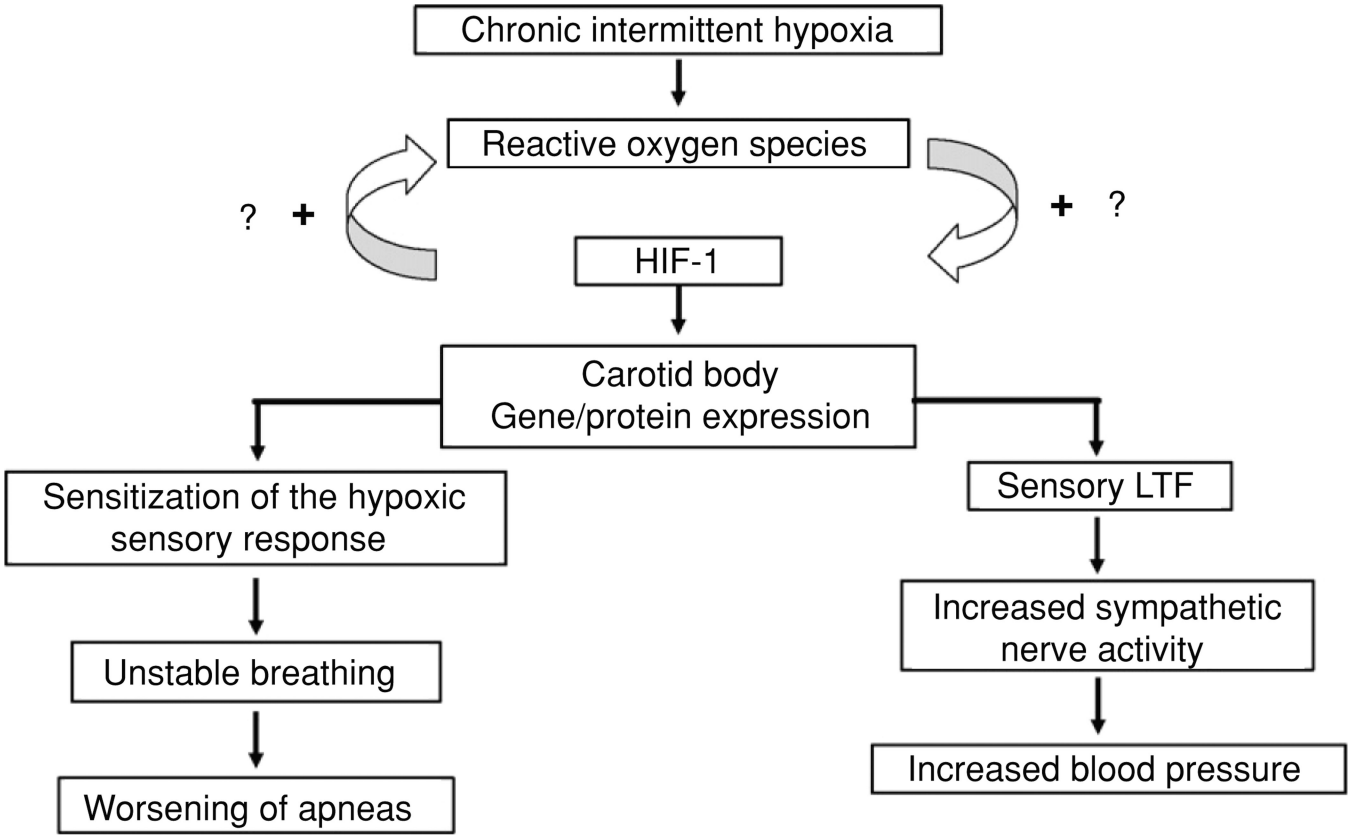

Figure 17.
Effect of CIH upon the cardiorespiratory system. Schematic illustration of the mechanisms and the consequences of chronic intermittent hypoxia (CIH)-induced changes in the carotid body on cardiorespiratory systems. Adapted, with permission, from (724), Copyright Elsevier, Prabhakar et al.

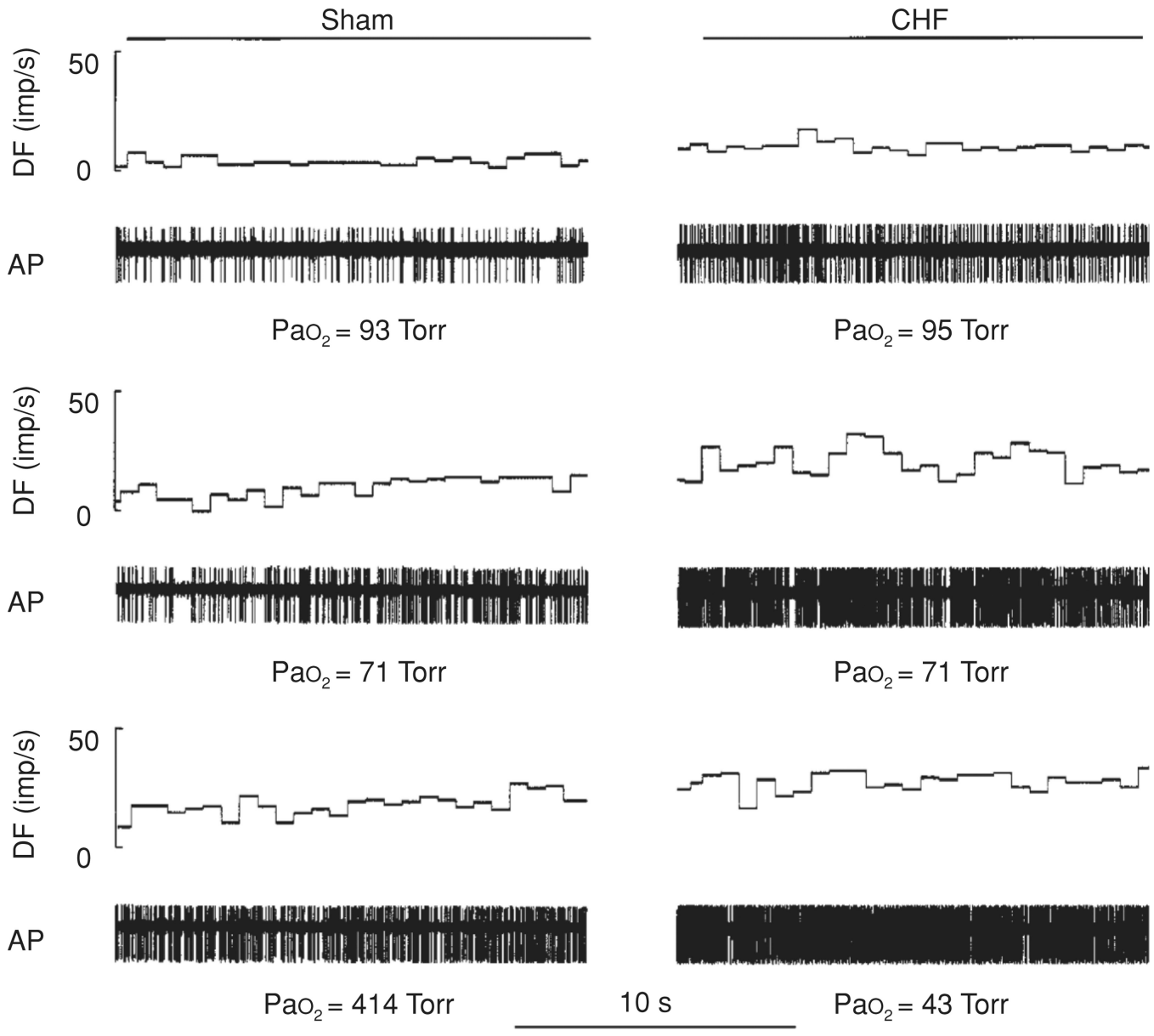

Figure 18.

Effect of chronic heart failure of chemodischarge. Representative recordings of afferent discharge of carotid body (CB) chemoreceptors during normoxia and two levels of isocapnic hypoxia from a sham (left) and a chronic heart failure (CHF) rabbit (right). Adapted, with permission, from, (812) Sun et al.