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Comparative Embryology of the Carotid Body

Steven C. Hempleman[1] and **Stephen J. Warburton**
Department of Biology, Northern Arizona University, Flagstaff, AZ, 86011 USA

Abstract

Vertebrate carotid bodies and related structures (branchial arch oxygen chemoreceptors in fishes, carotid labyrinth in amphibians, chemoreceptors in the wall of the common carotid and its branches in birds) develop in embryos when neural crest cells, blood vessels, and nerve fibers from sympathetic and cranial nerve ganglia invade mesenchymal primordia in the wall of the 3rd branchial arch. This review focuses on literature published since the 1970's investigating similarities and differences in the embryological development of 3rd arch oxygen chemoreceptors, especially between mammals and birds, but also considering reptiles, amphibians and fishes.

Keywords

branchial arch; neural crest; carotid body

1. Introduction

Classical anatomical embryology of the carotid body (CB) has received considerable attention over the last century and produced many excellent research papers, monographs, and reviews, for example: Adams, 1958 (comparative morphology of the CB); Batten, 1960 (contribution of epidermal placodes to CB development), Murillo-Ferrol, 1967 (morphological development of the avian CB); Rogers, 1965 (uncertain embryonic origins of CB, especially glomus cells), and Smith, 1924 (morphological interactions of third arch mesoderm, blood supply, sympathetic trunk, glossopharyngeal, and vagal nerves in CB development). The present review includes main points from these earlier works, but focuses on developments from 1970 onward, when new methods such as the development of chick-quail chimeric embryos (Le Lièvre and LeDouarin, 1975), and histochemical, immunohistological, and transgenic animal studies added significantly to the knowledge of CB embryogenesis.

1.1 Evolutionary Background

The oxygen-sensitive CB glomus cells of higher vertebrates and similar glomus-like cells in the 3rd branchial arch derivatives of lower vertebrates are derived from the neural crest (reviewed by Milsom and Burleson, 2007). Gans and Northcutt in their paper "Neural crest and the origin of vertebrates: a new head" (1983) proposed that the extensive cephalic reorganization that first appeared in agnatha and gnathostomes (vertebrate ancestors of

[1]Corresponding Author: Dr. Steve Hempleman, Dept. of Biology, Northern Arizona University, PO Box 5640, Flagstaff, AZ. 86011, USA. steven.hempleman@nau.edu, Tel: +1(928)523-7220, Fax: +1(928)523-7500.

extant fishes, amphibians, reptiles, birds, and mammals) was a radical evolutionary specialization of the first vertebrates for life as active predators. These specializations included articulated jaws (except in agnatha) for prey capture and ingestion, enhanced respiratory and circulatory systems to support high metabolic rates, and a variety of paired sense organs (including carotid bodies, and lateral line organs) to support an active lifestyle and enable prey detection. According to Gans and Northcutt's theory, neural crest cells and associated epidermal placodes played key roles in this transformation.

Neural crest cells (NCC) are transitory features in vertebrate embryos formed along the mediodorsal lateral ridges of the neural tube during neurulation (reviewed by Dupin and Sommer, 2012; Le Douarin, 1986). During embryogenesis, multipotent NCC migrate according to their position along the border of the neural plate, and undergo transformation into other cell types that produce many characteristic structures of vertebrates, including the craniofacial skeleton, jaw, melanocytes, smooth muscle cells of the branchial arch arteries and their derivatives, and most of the peripheral nervous system including the sympathetic and parasympathetic autonomic ganglia, enteric ganglia, satellite glial cells in ganglia, dorsal root ganglia, Schwann cells, adrenal medullary chromaffin cells, and some paired cranial sense organs including the carotid bodies (Dupin and Sommer, 2012).

Epidermal placodes (EP) like NCC are ectodermal derivatives. They form adjacent to the neural plate, and migrate during embryogenesis, but unlike NCC, EP form only in the head region of the neural tube (Batten, 1960; Gans and Northcutt, 1983; Graham and Shimeld, 2012). Epidermal placodes help form ears, eyes, and nose, and sensory ganglia of many cranial nerves. The nodose ganglia of the vagi (X) and the petrosal ganglia of the glossopharyngeal nerves (IX) have a dual origin from EP and NCC (Le Douarin, 1986). Because the nodose and petrosal ganglia provide innervation to the avian and mammalian CB, respectively, NCC and EP derivatives are both required for CB embryogenesis.

1.2 Neural Crest Origin of the Carotid Bodies in Birds

Early attempts to study the migration and fate of NCC relied on detecting endogenous markers or applying exogenous markers to early stage NCC while they were aggregated near the neural plate, then observing the movement of marked NCC in the developing embryo (*reviewed by* Chai et al., 2000; Jiang et al., 2000; Le Douarin et al., 1980, 1986). Such techniques posed problems because the marked cells generally lost label intensity as cell division occurred, or cells ceased expressing an endogenous marker during differentiation. This problem was solved in 1972 when Le Douarin and coworkers (*reviewed by* Le Douarin, 1980, 1986; Le Lièvre and LeDouarin, 1975) developed a stable avian chimeric system for permanently marking and tracking the migration and fate of NCC. They exploited the fact that Japanese quail cells (*Coturnix coturnix japonica*) have a large concentration of heterochromatin in their interphase nucleoli, which appears clearly different from the even distribution of heterochromatin in chicken cells (*Gallus gallus*). This difference was visible with light or electron microscopy, and the grafted cells and their NCC derivatives retained distinctive heterochromatin even after differentiation. An "isotopic-isochronic" grafting procedure was developed involving the removal of donor neural crest primordium (segments of neural tube and neural fold, see Fig. 1 in Le Douarin, 1980) from quail embryos, and grafting this to recipient chicken embryos at the same developmental stage (isochronic), and at the same somatic position along the embryonic axis (isotopic). The recipient chick's own neural primordium was removed just prior to grafting the donor primordium. Quail-chick chimeras developed normally, and the transplanted NCC migrated and differentiated normally, a process that could be followed in serial sections by observing the characteristic quail cell heterochromatin. These chimeras were the first stable system for tracking the fate of NCC in developing embryos and were used to show the neural crest origin of avian CB glomus cells (Le Douarin, 1980, 1986; Le Lièvre and LeDouarin, 1975;

Pearse et al., 1973). For many years it was assumed that the same NCC origin of CB glomus cells was also likely true for other vertebrates.

1.3 Neural Crest Origin of CB in Mammals

Use of the avian technique of chimeric fate-mapping NCC wasn't feasible in mammals. However, equivalent information about the NCC origin of mammalian CB has been obtained with transgenic mice by exploiting the promoter *Wnt1* (a proto-oncogene uniquely expressed by migrating NCC) to control expression of a reporter gene for tracking NCC migration and fate (Chai et al., 2000; Jiang et al., 2000). Pardal et al (2007) analyzed CB development using a *Wnt1 Cre*/floxed *LacZ* transgenic mouse, in which NCC cells and their derivatives permanently expressed β-galactosidase as a reporter, and reacted positively to X-gal upon histological examination.

With the *Wnt1* Cre/floxed *LacZ* transgenic mouse model, Pardal et al. (2007) showed that neural-secretory glomus (type I) cells and glial-like sustentacular (type II) cells in the mammalian CB are both of cranial neural crest origin. Furthermore, they showed that exposure to chronic hypoxia would induce sustentacular cells in mature mice to transform into glomus cells, a stimulus long known to cause glomus cell hyperplasia in the CB. Since these experiments showed that some CB type II cells retain the stem cell-like multipotency of the NCC from which they derive, Pardal et al. (2010) proposed that the carotid body is a neurogenic niche in the peripheral nervous system. Retained multipotency of CB type II cells not only explains the physiological ability of the immature and adult CB to undergo hyperplasia in chronic hypoxia, it may also explain rarer tumorous growths (chemodectomas) of the CB, which interestingly occur more often in chronically hypoxic persons living at high altitude (Pardal et al., 2010). There is also evidence that autotransplantation of CB tissue into the substantia nigra of Parkinsonian animal models can relieve the motor symptoms associated with dopamine deficiency (a potential treatment for Parkinsonism). However, it is presently unclear whether these promising results are due to the transplanted CB stem cells transforming into glomus cells and producing dopamine, or due to the transplanted CB cells producing paracrine substances, such as glial derived neurotrophic factor, which could directly stimulate increased dopamine production from the existing nigro-striatal pathway (Pardal et al., 2010; Villadiego, 2005).

2. Development of the Mammalian and Avian CB: Overview

The first visible sign of CB development in both mammals and birds is a condensation of mesenchymal cells in the wall of the 3rd aortic arch artery (Rogers 1965; Kameda, 1994; Kondo, 1975; Murillo-Ferrol, 1967; Smith 1924). In mammals, thickening of the 3rd arch arterial wall occurs adjacent to the primordia of the superior cervical ganglion on the sympathetic trunk. In birds, the 3rd arch wall thickening occurs adjacent to the primordia of the vagal (nodose) ganglion and vagal recurrent nerve. At the conclusion of mammalian embryonic development, the bilateral carotid bodies lie in the upper neck at the carotid bifurcation and are innervated by the carotid sinus nerves carrying afferent fibers to the petrosal ganglia of the glossopharyngeal nerves (cranial nerve IX), and sympathetic (efferent) nerve fibers from the superior cervical ganglia. Conversely, the fully developed bilateral avian carotid bodies lie within the thoracic cavity next to the common carotid artery just cranial of the origin of the subclavian artery, and adjacent to the thyroid, parathyroid, and ultimobranchial glands. They are innervated by fine nerve branches arising from the adjacent vagus (cranial nerve X), vagal nodose ganglia, vagal recurrent nerves, and nerve fibers from the sympathetic trunk (arising at the level of the 14th cervical sympathetic ganglion in chicken, Kameda, 2002).

2.1 Human CB Embryology

Hervonen and Korkala (1972) studied the CB microanatomy of human midterm fetuses, gestational age 14–16 weeks (full term is 38 weeks), and described the carotid bodies as remarkably mature with structural characteristics like those of adult carotid bodies. Glomus (Type I) cells were plentiful and contained numerous dense-cored vesicles that exhibited characteristic monamine fluorescence after glutaraldehyde fixation. Sustentacular (Type II) cells lacking dense-cored vesicles surrounded the glomus cells. Axon terminals were seen in apparent synaptic contact with glomus cells, and axon bundles were in close contact with sustentacular cells. Blood capillaries arose directly from the carotid artery and formed an anastomosing network around which the other cell types were organized. The capillaries were also surrounded with adrenergic nerves.

Smith et al. (1993) studied the innervation pattern of CB in human fetuses and reported that one nerve bundle touched the CB primordia at 10 weeks, followed by another at 13 weeks, and both bundles then formed a plexus around the CB with some fibers entering the superficial region of the CB primordium. At 19 weeks nerve fibers had penetrated to the deepest part of the primordium and at 23 weeks synapses were seen between nerve fibers and glomus cells.

Korkala and Hervonen (1973) and Scraggs et al. (1992) evaluated the developmental time-course of carotid bodies from human fetuses at gestational ages ranging from 7–22 weeks and 13–19 weeks, respectively. At 7 weeks the primordial human CB was in close contact with the internal carotid artery and the sympathetic trunk anlage. Glutaraldehyde fixation (which induces monamine fluorescence) revealed mostly non-fluorescent fibroblasts and weakly fluorescent small cells. The sympathetic anlage had similar weakly fluorescing cells, and a cord of weakly fluorescing cells connected the sympathetic chain to the CB. Capillaries were present at the earliest appearance of fluorescent cells. By 9 weeks of gestation, the monamine fluorescent intensity of both the sympathetic trunk and the CB increased, but the cord of fluorescent cells linking the two structures was almost gone. At 11–16 weeks of gestation, the CB was clearly a separate organ from the carotid artery and sympathetic trunk. Fluorescent intensity was maximal and stayed relatively constant after 12 weeks, with cells in the center of the CB exhibiting the greatest fluorescent intensity. Sustentacular cells and glomus cells could first be distinguished from each other at about 13 weeks using paraffin-embedded sections stained with hematoxylin and eosin, and became easier to differentiate with increasing gestational age. By 17–22 weeks monamine fluorescence was uniformly distributed in the CB, the vascular system was well organized, and the organ had an identifiable hilus. Korkala and Hervonen (1973) suggested that the fluorescent cord of cells connecting the sympathetic chain with the CB at 7–9 weeks may indicate that glomus cell precursors populate the CB directly from the sympathetic anlage.

2.2 Rat and Rabbit CB Embryology

Kondo (1975) studied the developing anatomy of rat CB at embryonic days 13–18 (*normal rat gestation period is 21–23d*). In rat embryos aged 13d, the 3rd aortic arch was uniform with no evidence of localized wall thickening and no nerve fibers within or near the vessel wall. At 13.5d, a thickening of the arterial wall developed consisting of undifferentiated cells outside the *tunica media*. Unmyelinated nerve fibers ended close to the thickened area, and both clear and dense-cored vesicles were present in the nerve fibers, but no synapses were apparent. Small blood vessels from the external carotid artery penetrated the thickened wall. At 14d, the arterial wall aggregation had grown and four cell types were seen within the CB primordia: undifferentiated cells, endothelial cells, immature smooth muscle cells, and presumptive glomus cells containing dense-cored vesicles, especially at the periphery. Bundles of unmyelinated nerve fibers were common within the thickened area. At 14.5d the

CB anlage started to separate from the 3rd arch arterial wall. Presumptive glomus cells with dense vesicles were now more evenly distributed throughout the CB, undifferentiated cells lacking dense vesicles (possible sustentacular cell progenitors) enveloped the presumptive glomus cells, and unmyelinated nerve fibers were present but no synapses were seen. At 17d, the CB was separated from the artery wall and surrounded by a capsule, and asymmetric membrane specializations were present suggesting synapses between glomus cells and nerve fibers. At 17.5d, the CB was well developed structurally and similar to mature form. Kariya et al. (1990) reported similar developmental events (with somewhat slower time course) in a microscopic study of the embryonic rabbit CB.

2.3 Mouse CB Embryology

As in other mammals, the anlage of the superior cervical sympathetic ganglia in the mouse embryo lie adjacent to the 3rd aortic arch, and the CB primordia form where axonal processes and cells from the superior cervical (sympathetic) ganglion surround and then enter the wall of the 3rd aortic arch during development (Kameda et al., 2002). The sympathetic nervous system initially arises as a condensation of NCC along the dorsal aorta, which then migrate during embryogenesis under the control of various transcription and growth factors including Mash1, BDNF, FGF, Hoxa3, Hes1and Phox2b (Dupin and Sommer, 2012; Kameda 2005, Kameda et al., 2012).

Recently, transgenic mice have been important experimental models for investigating CB development and the roles of various genes expressing transcription factors and growth factors needed at critical times to guide development. For example, the *Mash-1* gene controls differentiation and survival of cells forming the central and peripheral nervous systems. Transgenic mice lacking *Mash-1* do not develop sympathetic ganglia, and therefore lack the superior cervical ganglion which is postulated to supply primordial neural crest-derived glomus cells to the CB during development. Kameda (2005) showed that although *Mash-1* null mutant mice do form a primordial CB in the wall of the 3rd arch, the CB never becomes populated by glomus cells—it consists only of mesenchymal cells, apparent sustentacular (type II) cells, and nerve fibers from the carotid sinus nerve. This supports the idea that the neural crest-derived glomus cells populate the CB by migration from the sympathetic nervous system. However, given the recent report from Pardal et al. (2007) that sustentacular cells are also of neural crest origin and are precursors to glomus cells, it is unclear how the CB of *Mash-1* null mice could have sustentacular cells but no glomus cells.

The transcription of *Mash-1* is transiently suppressed during embryogenesis by the expression of the *Hes1* gene, which helps orchestrate the normal development of the sympathetic ganglia and carotid body glomus cells. Kameda et al. (2012) found that *Hes1* homozygous null mutant mice developed with severe hypoplasia of the superior cervical ganglia and carotid body, suggesting that while *Mash-1* is required for sympathetic and CB development, the specific timing of *Mash-1* transcription, controlled in part by *Hes1,* is also very important.

The *Hoxa3* homeobox (HOX) gene is strongly expressed in the 3rd pharyngeal arch and pouch, and is necessary for development of the 3rd arch artery and its associated structures, including the carotid bodies (Chen et al., 2010). In *Hox3a* null mice, a short or non-existent common carotid artery is typical and therefore the embryological carotid bifurcation is much more central than in wild-type mice (Kameda et al., 2002; Kameda, 2006). The third arch begins to form but ultimately degenerates at embryonic age 11.5d-12.5d, consigning the third arch to the same lineage as the unrealized first and second arches in mammals. Interestingly, *Hoxa3* null mice develop normal, if somewhat hypertrophied, superior cervical ganglia, but they do not develop carotid bodies (Kameda et al., 2002), emphasizing the importance of the mesenchymal primordial cells (missing in the *Hoxa3* null mutants) in the

wall of the 3rd arch providing the target for neural crest cell migration and the framework for CB formation.

Fibroblast growth factor (FGF) is an important signaling molecule in the development of the nervous system, and FRS2α (FGF receptor substrate 2) is a key component for its transmembrane signal transduction. FRS2α mutants, where the tyrosine phosphatase Shp2-binding sites are targeted, result in a phenotype where the common carotid bifurcates normally, but no CB forms (Kameda et al., 2008). In this mutation, there is greatly reduced early investiture of the CB site by fibers from the carotid sinus nerve. There is little development of typical CB morphology and little to no presence of glomus cells at the carotid bifurcation. This suggests the fibers of the carotid sinus nerve are a necessary early step in CB development. Additionally, the superior cervical ganglion is more dorsal and caudad than in wild type animals.

Brain-derived neurotrophic factor (BDNF) is transiently expressed in the fetal cardiac outflow tract appearing to act as both target-derived survival factor and signaling molecule (Brady et al., 1999). Interestingly, BDNF is required for survival of neurons of specific organ systems, including the CB, rather than for specific modal types. Initially, BDNF originates in the target tissues at the onset of innervation and is vital for afferent survival. After innervation is underway, the afferents themselves begin to produce BDNF. Disruption of BDNF hinders survival of the afferent neurons innervating the CB (Erickson et al., 1996, Katz, 2005; Porzionato et al., 2008).

The HOX gene *Phox2b*, is also involved in CB development (Dauger et al., 2003). *Phox2b* controls: (1) development of sympathetic and parasympathetic ganglia, including the superior cervical ganglia, (2) development of the brainstem solitary tract nucleus (nTS), the first central synaptic relay nucleus for visceral afferents, and (3) development of afferent visceral pathways, including the mammalian CB afferents that receive synaptic input from glomus cells, have somata in the petrosal ganglia, and axons that project to the solitary tract nucleus *via* the glossopharyngeal nerve. In homozygous *Phox2b* null mutants, the CB degenerates during development, and the three epibranchial placode-derived visceral sensory ganglia (nodose, petrosal, and geniculate) are absent (Dauger et al., 2003). Although heterozygous *Phox2b* mutants may survive, they have a significantly altered response to hypoxia and hypercapnia, which provides an exciting model for research on congenital central hypoventilation syndrome, a dysautonomia characterized by impaired automatic control of breathing and chemosensitivty particularly apparent during sleep.

2.4 Avian CB Embryology

Most work on avian CB embryology has used chicken or quail as models, and the results are assumed representative of Aves in general. While mammalian carotid bodies are found in the neck near the angle of the jaw, avian carotid bodies lie within the thoracic cavity just lateral to the right and left common carotid arteries, between the origins of the subclavian and vertebral arteries (see fig. 1 in Kameda, 2002). Pharyngeal branchial derivatives including the thyroid, ultimobranchial, and parathyroid glands also lie alongside the common carotid arteries near the carotid bodies (Abdel-Magied and King, 1978; Kameda, 2002). In this thoracic location, the avian carotid bodies receive arterial blood from small branches of the common carotids, vagal innervation (including sensory afferents) from fine nerve fibers arising from the nodose ganglia, vagal recurrent nerve, and vagal trunk, and sympathetic (presumably efferent) innervation via nerve fibers from the nearby 14th cervical sympathetic ganglion (Abdiel Magied and King, 1978; Kameda, 2002). Kameda (1994) showed that peptidergic nerve fibers immunoreactive for calcitonin gene related product (CGRP), substance P, galanin, VIP, and somatostatin are densely distributed in CB parenchyma. Avian glomus cells have characteristic dense cored secretory vesicles showing

intense immunoreactivity for serotonin, and avian sustentacular (type II) cells are glial-like as in mammals.

In avian glomus cells, serotonin is the dominant monamine, and glomus cells are found not only in the carotid bodies, but also in the walls of the nearby common carotid and its branches between the subclavian and vertebral arteries (Kameda 1990, 1994, 2002). Kameda et al. (1994) used monoclonal antibodies against neuron-specific class III beta-tubulin isotype (TuJ1), anti-rat brain beta-tubulin, and anti-Leu-7 (HNK-1) to trace the migration pathways for cells populating the avian CB region, with the rationale that neurons would be labeled by TuJ1 and anti-rat brain beta tubulin, and NCC would be labeled by HNK-1. With this technique, the CB anlage were first discernible in chicken embryos aged 6–8d (*hatching occurs at 21d for chickens*) in the lateral portion of the 3rd branchial arch arteries. At this time, cells and nerve fibers positive for TuJ1 and HNK-1 were found in the distal vagal (nodose) ganglion and a stream of immunoreactive cells from the nodose ganglion projected to the area around (but not within) the CB anlage in the arterial wall. During embryonic days 9–12, immunoreactive cells streaming from the nodose ganglion entered the CB anlage, and became plentiful and widely dispersed within the wall of the common carotid and its branches. It was suggested that these neural crest-derived immunoreactive cells become glomus cells, and that they populate the avian CB and nearby wall of the common carotid by cell migration from the nodose (distal) ganglion of the vagus nerve (Kameda et al., 1994; Kameda, 2002). This pathway is different from the mammal CB, where the cervical sympathetic ganglion appears to be the proximal source of the NCC derived glomus cells (see above). This difference in cell migration during CB development may be related to the marked cervical elongation that occurs in birds above the level of the CB, producing a caudal shift the CB, carotid bifurcation, and pharyngeal glandular derivatives to the upper thorax in birds, whereas these structures are in the upper cervical region in mammals.

Although the dominant biogenic amine in avian glomus cells is serotonin, dopamine is also present but is less abundant than in mammalian carotid bodies. Kameda investigated the developmental patterns of glomus cells and peptidergic nerve fibers using immunohistochemistry for serotonin, neuropeptide Y (NpY), Substance P, met-enkephalin, CGRP, tyrosine hydroxylase (TH), and chromogranin A (Kameda, 1990, 2002). At embryonic day 9 Kameda found TH and chromogranin A in cells outside the CB primordium and wall of the carotid artery. On day 10 TH, chromogranin A, and serotonin immunoreactivity was inside the CB primordium and arterial wall, and by day 12 reactivity was intense and persisted through hatching. After hatching, immunofluorescence for TH persisted strongly in glomus cells within the CB, while glomus cells within the wall of the common carotid lost TH immunoreactivity. Similarly, NpY appeared in the glomus cells of the CB primordium and carotid arterial wall at embryonic day 10, and was intense by 14 days, persisting at a high level to hatching. However, NpY reactivity in glomus cells of the CB decreased after hatching (minimal at 20d post-hatch), but persisted to adulthood in the glomus cells of the arterial wall. These differences in post-hatch expression of TH and NpY may reflect functional differences in glomus cells that populate the CB and arterial wall.

Nerve fibers immunoreactive for Substance P and CGRP that are derived from the inferior and superior vagal ganglia penetrated the CB primordium and arterial wall at embryonic day 12 and increased to adult density by day 18 (Kameda, 2002). Only a few galanin and VIP immunoreactive nerve fibers were present during embryonic development of the avian CB, but they increased after hatching. In late embryos and after hatching---considerable numbers of met-enkephalin immunoreactive nerve fibers were found in connective tissue around CB.

In birds as in mammals, many neurotransmitter/secretory substances are synthesized, stored and released within the CB, but the function of this complicated system of messenger

molecules is not yet clear in either the Aves or Mammals, and remains an important area for study.

3. Branchial Arch Oxygen Chemoreceptors in Lower Vertebrates

In fishes, the 3rd branchial arch becomes the first gill arch, and the chemoreceptors in this location are innervated by cranial nerves IX and X (Nilsson, 1984). Jonz and Nurse (2006) noted the similarities between zebrafish (*Danio rerio*) gill arch neuroepithelial cells (NEC) and mammalian carotid body type I cells, including 5-HT production, cytoplasmic synaptic vesicles and innervation. It is interesting to note that, similar to mammalian and avian carotid bodies, zebrafish gill arches receive innervation prior to the production of specialized chemosensory cells, but the innervation of the cells themselves occurs only after the development of the cells is well underway (Jonz and Nurse, 2005, 2006, 2008). Whether or not zebrafish NEC are derived from neural crest cells is still unresolved, but given the origin of the CB of mammals and birds with NCC migration to mesenchymal primordia in the 3rd arch, innervation via cranial nerves IX and X, production of 5-HT by glomus cells (especially in Aves), and other morphological similarities to fishes, it appears likely that the CB of higher vertebrates is built from the same developmental program as branchial arch chemoreceptors in lower vertebrates.

The CB in mammals and some reptiles (e.g. lizards) is located at the internal/external carotid bifurcation (Adams, 1958), as is the homologous carotid labyrinth of amphibians (Kusakabe, 2002). Embryologically, this location corresponds to the 3rd pharyngeal arch. The carotid labyrinth (carotid gland in early literature) appears early in development at the third branchial arch (the first and second arches are evident only as developmental remnants). The carotid labyrinth remains at the third arch throughout amphibian metamorphosis as the dorsal aortic segment between the third and fourth branchial arches degenerates and the third arch becomes the common carotid artery, leaving the carotid labyrinth at the carotid bifurcation (Kusakabe, 2002). In birds and some reptiles (e.g. turtles), the CB is (apparently) divergently located in the thoracic cavity, more central than the CB of lizards and mammals. This is most likely due to the more central location of the third pharyngeal arch derivatives in birds and turtles. In these taxa, the true carotid bifurcation (the third arch) remains close to the heart, the external carotid artery atrophies early in development, and the internal carotid artery secondarily bifurcates in a more cephalad location, resulting in the so-called carotid bifurcation of the adult (*reviewed by* West et al., 1981). Thus in birds there is the emergence of a “secondary” carotid bifurcation rather than a translocation of the CB. The CB appears fixed at the location of the third embryological pharyngeal arch, regardless of the ultimate anatomical location in the adult species.

Innervation of the CB is via the IX cranial nerve, the X cranial nerve, or both, in all species studied to date. Presumably, the signal for creation of the CB in the vascular wall, i.e. induction by nerve fibers from the sympathetic and cranial nerves, is established by the location of the third branchial arch, and this location, in turn, is responsible for neural induction for supply of presumptive glomus cells. Thus, it appears that the developmental program for innervation of gill arch chemoreception via cranial nerves IX and X is highly maintained throughout the vertebrates as cranial nerves IX and X continue to innervate the carotid labyrinth and CB, located at the 3rd arch and continues to function in regulation of blood gas composition. For full discussion of CB evolution, see Milsom and Burleson, 2007.

4. Summary

Carotid bodies and related branchial arch O_2 chemoreceptors in vertebrates are derived from migration of NCC to mesenchymal primordia associated with the wall of the 3rd arch artery. Depending on vertebrate taxa, NCC populate the primordia from nearby ganglia on the

sympathetic paravertebral trunk or from ganglia of cranial nerves X or IX, and afferent and efferent innervation to the primordial CB extends from these same sympathetic and cranial nerve ganglia.

Carotid body embryology has clearly benefitted from the comparative approach. For example, the creation of embryonic quail-chick chimeras was critical for understanding the neural crest origin of the avian CB (Le Lièvre and LeDouarin, 1975; Pearse et al., 1973). Several decades later, advances in *Wnt1-Cre* transgenic mice permitted NCC fate-mapping in mammals as well, and confirmed and expanded earlier results from the pioneering work on *Aves* (Chai et al., 2000, Jiang et al., 2000, Pardal et al., 2007). Relevant to this comparative approach is the new science of "evo-devo" (evolutionary developmental biology), which seeks to understand how developmental plasticity among species orchestrates the evolution of anatomical and physiological characters (Carroll, 2006). In general, evo-devo approaches have shown that precise temporal and spatial expression patterns of regulatory genes in developing embryos produce the adult structures that are characteristic of the species. Recently, carotid body researchers have been using evo-devo approaches, as discussed above regarding phox2b, hoxa3, BDNF, mash1, hes1, and FRS2α. Continued discovery of the spatial and temporal expression patterns for genes regulating CB development in vertebrate embryos should be a fruitful area of research, and may explain many of the phylogenetic similarities and differences in CB structure and function. For example, it is interesting to note that the neotenic tiger salamander, *Ambystomatigrinum*, lacks carotid labyrinths (Kusakabe, 1990). Comparing the embryology of this animal to other amphibians may be a useful evo-devo approach for discerning growth factors, trophic factors and regulatory genes that control carotid labyrinth formation.

Promising lines for future inquiry include studies aimed at mechanistic understanding of how environmental factors affect the precisely orchestrated events of embryonic CB development (eg. chronic or intermittent hypoxia, nicotine, and other neuroendocrine disruptors), continued application of evo-devo approaches to investigate spatial and temporal patterning of gene expression in the embryonic CB, continued research on spatial and temporal patterning of growth and trophic factors regulating CB embryogenesis (Katz, 2005; and Porzionato et al., 2008), and continued use of transgenic approaches inCB embryology, including conditional knock-in and knock-out experiments to test the role of regulatory gene expression at various stages of development.

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