

Synaptic and paracrine mechanisms at carotid body arterial chemoreceptors

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Abstract Mammalian carotid bodies are the main peripheral arterial chemoreceptors, strategically located at the bifurcation of the common carotid artery. When stimulated these receptors initiate compensatory respiratory and cardiovascular reflexes to maintain homeostasis. Thus, in response to low oxygen (hypoxia) or increased CO_2/H^+ (acid hypercapnia), chemoreceptor type I cells depolarize and release excitatory neurotransmitters, such as ATP, which stimulate postsynaptic $\text{P2X}_2/3$ receptors on afferent nerve terminals. The afferent discharge is shaped by autocrine and paracrine mechanisms involving both excitatory and inhibitory neuromodulators such as adenosine, serotonin (5-HT), GABA and dopamine. Recent evidence suggests that paracrine activation of P2Y_2 receptors on adjacent glia-like type II cells may help boost the ATP signal via the opening of pannexin-1 channels. The presence of an inhibitory efferent innervation, mediated by release of nitric oxide, provides additional control of the afferent discharge. The broad array of neuromodulators and their receptors appears to endow the carotid body with a remarkable plasticity, most apparent during natural and pathophysiological conditions associated with chronic sustained and intermittent hypoxia.

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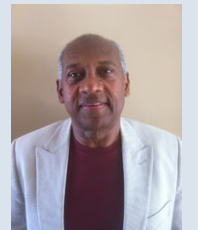
Abbreviations ACh, acetylcholine; Ado, adenosine; NO, nitric oxide; nNOS, nitric oxide synthase; PKC, protein kinase C; VAChT, vesicular acetylcholine transporter.

Introduction

The maintenance of homeostasis in the respiratory and cardiovascular systems of mammals depends on signal processing within receptor organs located in the periphery. The carotid bodies, and the less well-studied aortic bodies, represent a class of peripheral chemoreceptor organs involved in the sensing of chemicals in arterial

blood such as low O_2 (hypoxia), high CO_2/H^+ (acid hypercapnia) and low glucose (hypoglycaemia). The location of the bilaterally paired carotid bodies at the bifurcation of the common carotid arteries is strategic, as the blood composition is sampled just before it reaches the brain, an organ that is very sensitive to oxygen and glucose deprivation. Thus, in response to hypoxaemia, chemoreceptor cells in the carotid body (CB) depolarize and

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release neurotransmitters that excite afferent terminals of the carotid sinus nerve. Afferent signals are then relayed to the central pattern generator in the brainstem, leading to reflex hyperventilation and restoration of blood O₂ homeostasis (Gonzalez *et al.* 1994). While central chemoreceptors play a key role in sensing elevated CO₂, the relative impermeability of the blood–brain barrier to protons means that pH sensing during metabolic acidosis is dependent mainly on peripheral CB chemoreceptors. The CB is richly vascularized and reputed to have the highest blood flow per unit weight of any tissue in the body (McDonald, 1981).

The main cellular components of the CB are the chemoreceptor cells, also known as glomus or type I cells, and ensheathing glial-like type II cells in a ratio of approximately 4:1 (McDonald, 1981). Type I cells communicate by both chemical and electrical synapses, and are innervated by afferent nerve terminals whose cell bodies reside in the petrosal ganglia. There is morphological evidence for reciprocal chemical synapses (McDonald, 1981), and functional evidence for electrical coupling (Eyzaguirre, 2007), between type I cells and afferent terminals as well as between neighbouring

type I cells. In addition, the CB receives an efferent inhibitory innervation via the carotid sinus nerve. A schematic representation of the cellular organization and innervation of the carotid body chemoreceptor complex is shown in Fig. 1. The type I cells synthesize a variety of neurotransmitters and neuromodulators, both excitatory and inhibitory, and express a range of corresponding ionotropic and metabotropic receptors. This organization suggests the presence of widespread autocrine and paracrine mechanisms that may help fine tune the afferent discharge during chemoexcitation. There is now compelling evidence that the type I cells are the actual transducers of CB chemostimuli. These cells depolarize in response to hypoxia and acid hypercapnia, primarily as a result of inhibition of K⁺ channels. The reader is referred to other excellent reviews for a discussion of the transduction mechanisms in type I cells, as this continues to be an active area of investigation (Buckler, 2007; Peers *et al.* 2010; Kumar & Prabhakar, 2012). In this mini-review I will highlight some of the neurotransmitter and autocrine/paracrine mechanisms involved in signalling processing in the CB, as well as recent data suggesting that type II cells may participate in these events

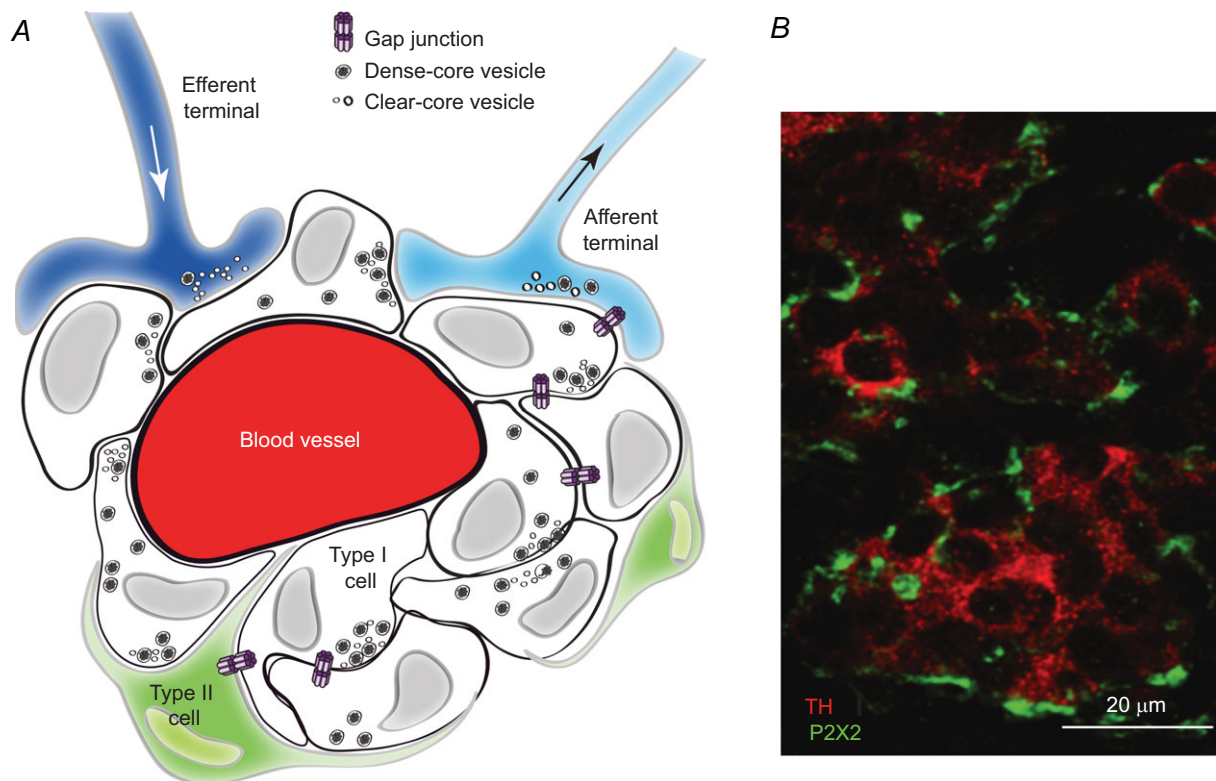


Figure 1. Cellular organization and innervation of the rat carotid body

A, schematic illustration of type I cell clusters in intimate association with glial-like type II cells. Type I cells contain dense-core granules and receive afferent innervation from the petrosal ganglia; inhibitory efferent terminals from autonomic neurones terminate near type I cells. Adapted from Piskuric and Nurse (2013). *B*, section of rat carotid body showing clusters of type I cells immunostained for tyrosine hydroxylase (red); type I cells are innervated by afferent terminals, immunostained for purinergic P2X2 subunit (green).

via 'gliotransmission' mediated by pannexin 1 channels. Although the focus here is on small molecules, various neuropeptides are known to play important modulatory roles in CB function, as described elsewhere (Prabhakar, 2006; Kumar & Prabhakar, 2012).

A brief historical perspective on signal processing in the CB

The first description of the CB appeared around the mid 18th century and is attributable to the studies of von Haller (reviewed by Kumar & Prabhakar, 2012). However, it was the seminal histological studies of De Castro in the 1920s that led to the idea that the CB was a sensory organ, designed to detect chemicals in arterial blood. Subsequently, the physiological role of the CB in controlling ventilation in response to changes in blood levels of O₂, CO₂ and H⁺ ions was demonstrated in the laboratory of Corneille Heymans, who later received the Nobel Prize in Physiology and Medicine (Heymans *et al.* 1930). Early studies on signalling mechanisms focused on acetylcholine (ACh) as a major excitatory neurotransmitter in the CB (Hollinshead & Sawyer, 1945). However, this view was later challenged in the mid 1950s (Douglas, 1954), only to be resurrected in more recent times (Eyzaguirre & Zapata, 1984; Gonzalez *et al.* 1994; Nurse & Zhang, 1999; Fitzgerald, 2000; Nurse, 2010). Other transmitter candidates including dopamine and substance P were also considered as excitatory mediators in the CB (Gonzalez *et al.* 1994; Iturriaga & Alcayaga, 2004; Prabhakar, 2006). Many studies, based on extracellular nerve recording during exogenous application of transmitter candidates to the whole CB, identified several agents, including ACh, that were capable of exciting the afferent carotid sinus nerve (Gonzalez *et al.* 1994; Iturriaga & Alcayaga, 2004). Indeed ATP, now considered a key CB excitatory neurotransmitter (Nurse, 2010; Piskuric & Nurse, 2013), has been known for some time to cause an increase sinus nerve discharge during intra-carotid injections (Jarish *et al.* 1952). Additionally, exogenous administration of the ATP breakdown product, adenosine, increased ventilation *in vivo*, as well the afferent sensory discharge *in vivo* and *in vitro* (reviewed by Conde *et al.* 2009). Other small molecules, including dopamine, serotonin (5-HT) and histamine, may act as direct chemoexcitants, or modulators of chemosensory activity (Gonzalez *et al.* 1994; Iturriaga & Alcayaga, 2004; Jacono *et al.* 2005; Kumar & Prabhakar, 2012; Nurse & Piskuric, 2013), and in many cases, receptors for these ligands have been localized on chemoreceptor cells and/or afferent nerve terminals. However, dopamine still remains the best studied CB neurotransmitter and there is abundant evidence that it is released from type I cells during chemoexcitation (Gonzalez *et al.* 1994; Lopez-Barneo, 2003). However, with the exception of in the rabbit, its role

appears to be that of an inhibitory neuromodulator in most species, as described in detail elsewhere (Gonzalez *et al.* 1994; Iturriaga & Alcayaga, 2004; Iturriaga *et al.* 2009; Nurse, 2010; Kumar & Prabhakar 2012).

Synaptic transmission between chemoreceptors and afferent nerve terminals

To identify the main excitatory neurotransmitters that cause the increase in afferent discharge during chemoexcitation, several laboratories have relied on the isolated CB–sinus nerve preparation *in vitro* (Gonzalez *et al.* 1994; Iturriaga & Alcayaga, 2004; Kumar & Bin-Jaliah, 2007; Donnelly, 2009; Kumar and Prabhakar, 2012). CB output as reflected by sinus nerve discharge frequency is directly recorded in these preparations, which have the advantage that secondary cardiovascular variables are eliminated. The development of a functional co-culture model consisting of isolated rat CB chemoreceptor cell clusters and juxtaposed petrosal neurones has contributed significantly to our understanding of synaptic mechanisms (Nurse & Zhang, 1999; Nurse, 2010). More recently, this model has been used to record simultaneously responses in both chemoreceptor type I cell(s) and the adjacent postsynaptic neurone using double patch-clamp recording or ratiometric Ca²⁺ imaging (Zhang *et al.* 2009; Nurse & Piskuric, 2013). In this co-culture model, ATP, acting via postsynaptic P2X2/3 receptors, was identified as a key excitatory neurotransmitter during hypoxia and hypercapnia (Zhang *et al.* 2000; Zhang & Nurse, 2004). In concert with these findings, P2X2 receptor subunit expression has been detected by immunohistochemistry on afferent nerve terminals apposed to tyrosine hydroxylase-positive type I cells in the rat carotid body *in situ* (Fig. 1B). A critical role of P2X2 receptor subunits in the sinus nerve chemosensory discharge, and in the whole animal hypoxic ventilatory response, was validated in a transgenic mouse model deficient in the P2X2 subunit (Rong *et al.* 2003). An excitatory role of co-released ACh was also identified in the co-culture model, although controversy still surrounds its importance in sensory transmission (Fitzgerald, 2000; Donnelly, 2009; Nurse, 2010; Kumar & Prabhakar, 2012; Piskuric & Nurse, 2013). The co-culture model also provided evidence that GABA, released from chemoreceptor cells, may act postsynaptically on ligand-gated GABA_A receptors to inhibit the sensory discharge by a shunting mechanism (Zhang *et al.* 2009).

Paracrine and autocrine mechanisms in the CB: interactions among type I and type II cells

Several endogenous neuromodulators, acting via G-protein coupled receptors, appear to play important roles in regulating CB output via paracrine and autocrine

mechanisms. Some of them play predominantly inhibitory roles such as dopamine acting via D2 receptors (Iturriaga *et al.* 2009), and GABA acting via GABA_B receptors (Fearon *et al.* 2003), on type I cells. Here, I briefly consider the roles of predominantly excitatory neuromodulators, i.e. adenosine, 5-HT and ATP, acting at type I or type II cells.

Adenosine. Adenosine (Ado), generated via the breakdown of extracellular ATP by ecto-5'-nucleotidase, or released from type I cells via an equilibrative nucleoside transporter, appears to play important auto-crine/paracrine roles during acute hypoxia (Conde *et al.* 2012). There is evidence that Ado may act on high affinity A2a receptors present on type I cells to enhance type I cell depolarization via protein kinase A-dependent inhibition of TASK-like background K⁺ channels (Tse *et al.* 2012). Also, selective blockers of A2a receptors appear to inhibit the hypoxia-induced receptor potential in type I cells at nanomolar concentrations (Nurse & Piskuric, 2013). Low affinity A2b receptors are also present on type I cells, allowing for further modulation of their secretory functions (Conde *et al.* 2009, 2012; Livermore & Nurse,

2013). Indeed, exposure to chronic hypoxia appears to alter Ado signalling in the CB via mechanisms that probably involve regulation of presynaptic A2 receptors in type I cells. For example, it was recently shown that exposure of rat CB cultures to chronic hypoxia (2% O₂; 24 h) led to a marked increase in Ado-evoked intracellular Ca²⁺ transients and catecholamine secretion in type I cells, mediated via A2b receptors (Livermore & Nurse, 2013). This pathway could potentially play a role during ventilatory acclimatization to hypoxia, as occurs during sojourns to high altitude, when CB sensitivity to acute hypoxia is augmented (Conde *et al.* 2009; Teppema & Dahan, 2010). Ado may also act postsynaptically via A2a receptors to enhance the firing pattern in the afferent nerve (Conde *et al.* 2009, 2012; Piskuric & Nurse, 2013).

Serotonin (5-HT). Using immunocytochemistry, 5-HT and 5-HT_{2a} receptors have been localized to type I cells in tissue sections of the rodent CB (Zhang *et al.* 2003). In recent studies, the 5-HT biosynthetic enzyme tryptophan hydroxylase and the serotonin transporter have also been localized to type I cells *in situ* (Yokoyama *et al.* 2013). Moreover, detection of 5-HT release from whole rodent

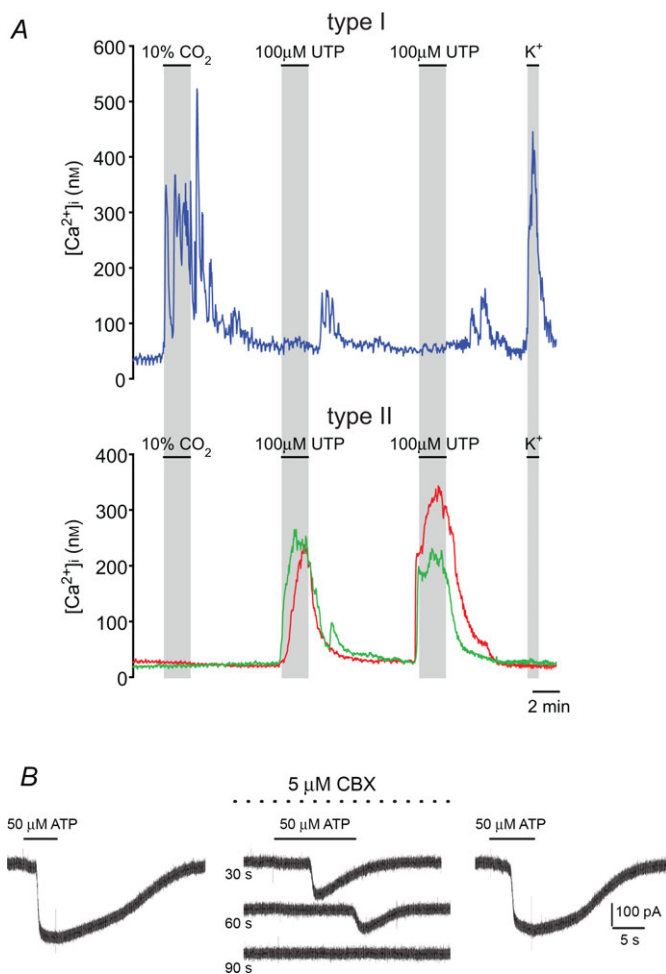


Figure 2. Effects of P2 receptor agonists on type I and type II cells

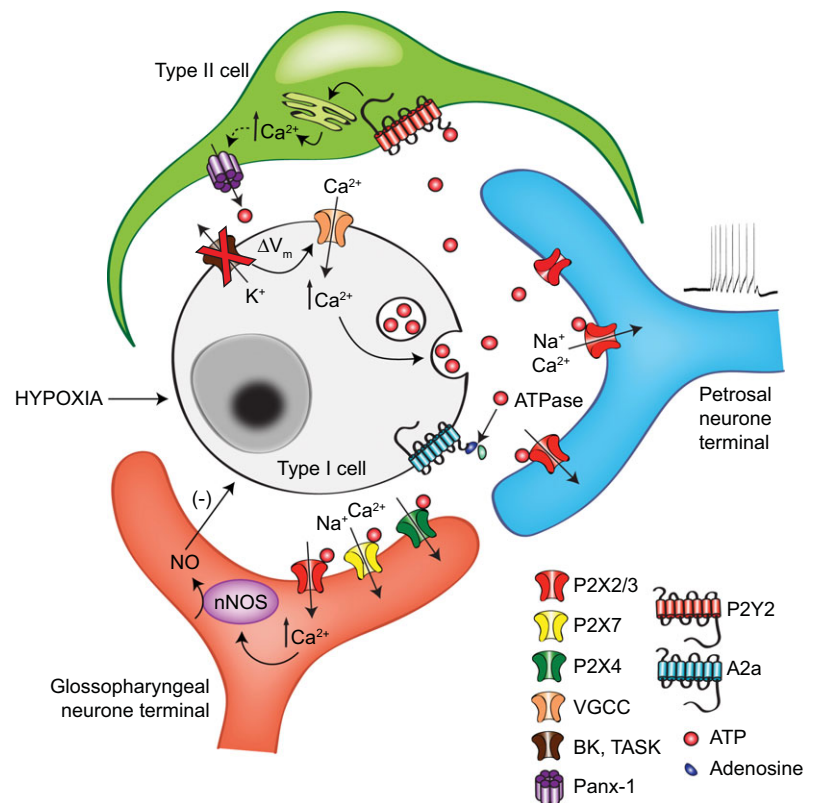
A, intracellular calcium transients elicited by different stimuli in type I versus type II cells. In the upper trace, a type I cell responds with an increase in [Ca²⁺]_i when exposed to hypercapnia (10% CO₂) and high K⁺, but not to UTP or ATP; delayed off responses of unknown origin were sometimes seen on removal of UTP and ATP. In lower traces, two different type II cells failed to respond to hypercapnia and high K⁺, but elicited robust increases in [Ca²⁺]_i when exposed to UTP and ATP, consistent with activation of P2Y2 receptors. **B**, in a type II cell, ATP activated an inward current that was reversibly inhibited by 5 μM carbenoxolone (CBX; middle traces at time intervals indicated), a selective blocker of pannexin-1 channels; holding potential was -60 mV. Data for both **A** and **B** were reproduced from Zhang *et al.* (2012).

CB by high-performance liquid chromatography has been observed under certain conditions (Jacono *et al.* 2005; Peng *et al.* 2009). In the co-culture model of the CB, the hypoxia-induced receptor potential in type I cells was reversibly inhibited by 5-HT_{2a} receptor blockers, consistent with a paracrine positive feedback role for endogenously released 5-HT (Zhang *et al.* 2003). In some type I cells, exogenous 5-HT induced membrane depolarization via a protein kinase C (PKC)-dependent inhibition of a resting K⁺ conductance. 5-HT also appears to play an important modulatory role in CB plasticity. For example, exposure of rats to chronic intermittent hypoxia leads to sensory long-term facilitation where the CB sinus nerve discharge in response to acute hypoxia is markedly potentiated (Peng *et al.* 2009). These authors showed the mechanism involved 5-HT release and activation of 5HT_{2a} receptors coupled to PKC. Taken together, these studies support a neuromodulatory role for 5-HT via paracrine interactions that facilitate the chemoexcitatory response of the type I cell.

ATP. In addition to its major role as a fast-acting excitatory neurotransmitter in the CB, there is increasing evidence that ATP may have paracrine functions involving glial-like type II cells (Tse *et al.* 2012; Piskuric & Nurse, 2013; Piskuric & Nurse, 2012). Earlier studies showed that ATP, acting via G-protein coupled P₂Y₂ receptors, caused a rise

in intracellular Ca²⁺ in isolated rat type II cells (Xu *et al.* 2003; Tse *et al.* 2012). More recently, activation of these P₂Y₂ receptors was shown to lead not only to a rise in intracellular Ca²⁺ but also to the opening of large-pore, pannexin-1 channels in type II cells (Piskuric & Nurse, 2012; Zhang *et al.* 2012), as illustrated in Fig. 2. In fact, these open pannexin-1 channels acted as conduits for the further release of ATP, and could be reversibly blocked by the selective blocker carbenoxolone (5 μM; Fig. 2). This ‘ATP-induced ATP release’ appears to be a potential paracrine mechanism for boosting the excitatory ATP signal at the chemosensory synapse. Preliminary evidence suggests that other CB neuromodulators can also regulate intracellular Ca²⁺ levels in type II cells (Tse *et al.* 2012), as well as the opening of pannexin-1 channels (S. Murali, M. Zhang and C. A. Nurse, unpublished observations). ATP itself may also help control its own extracellular levels within the sensory synapse. For example, at high concentrations ATP can act in a negative feedback manner to inhibit pannexin-1 channels in type II cells (Nurse & Piskuric, 2013), and to inhibit the hypoxia-induced receptor potential in type I cells via P₂Y₁ receptors (Tse *et al.* 2012). In another chemosensory organ, i.e. the taste bud, high extracellular levels of the excitatory neurotransmitter ATP can lead to severely diminished taste responses, attributable to desensitization of the rapidly adapting P₂X₃-containing receptors on the afferent nerve (Kinnamon & Finger, 2013). Similarly, in the CB, the

Figure 3. Proposed model for purinergic paracrine signalling in the rat carotid body
 Type I cells release the excitatory neurotransmitter ATP following hypoxia-induced membrane depolarization. ATP excites afferent terminals via postsynaptic P₂X_{2/3} receptors. ATP may also activate P₂Y₂ receptors on neighbouring type II cells, leading to a rise in intracellular Ca²⁺ and opening of pannexin-1 channels. When open, pannexin-1 channels cause further release of ATP, thereby boosting the excitatory signal. The inhibitory efferent pathway may also be activated by ATP acting on a variety of P₂X receptors on nNOS-positive, autonomic terminals; stimulation of these P₂X receptors leads to synthesis and release of NO, which in turn hyperpolarizes type I cells. Breakdown of extracellular ATP by ecto-5'-nucleotidase generates adenosine, which may also be transported directly from type I cells. Adenosine can further enhance excitatory pathways in the CB via presynaptic A_{2a} and A_{2b} receptors on type I cells, and postsynaptic A_{2a} receptors on afferent terminals. Some of these receptors, as well as other paracrine pathways, have been omitted for clarity. Adapted from Piskuric & Nurse (2013).



necessity to prevent the accumulation of high levels of ATP via these (and other, see below) negative feedback inhibitory pathways may have evolved in part to limit desensitization of P2X2–P2X3 heteromeric receptors on petrosal chemoafferent terminals (Prasad *et al.* 2001).

Role of nitric oxide in efferent inhibition

Evidence for an inhibitory efferent innervation of the CB via autonomic (parasympathetic) neurones has been reviewed elsewhere (Prabhakar, 1999; Campanucci & Nurse, 2007; Moya *et al.* 2012). These efferent neurones are immunopositive for vesicular acetylcholine transporter (VAcHT) and neuronal nitric oxide synthase (nNOS) and are distributed in small groups along the glossopharyngeal and carotid sinus nerves. Inhibition of CB function during hypoxia is mediated by nitric oxide (NO) released from these efferent fibres situated near chemoreceptor type I cells (Fung *et al.* 2001). The nNOS-positive efferent neurones express a variety of P2X purinergic and nicotinic ACh receptors (Campanucci *et al.* 2006, 2012; Campanucci & Nurse, 2007). Stimulation of these receptors with ATP and ACh leads to an increase in intracellular Ca^{2+} , activation of nNOS and synthesis of NO that can be detected by diaminofluorescein (DAF) fluorescence (Campanucci *et al.* 2012; Lowe *et al.* 2013). Moreover, in co-cultures containing CB type I cell clusters and juxtaposed efferent neurones, application of ATP led to type I cell hyperpolarization that could be prevented by the NO scavenger carboxy PTIO (Campanucci *et al.* 2006). These data suggest that release of the excitatory neurotransmitter ATP from chemoreceptor type I cells during hypoxia may have an additional, paracrine, negative feedback role, i.e. the trigger for NO synthesis and release from efferent fibres, leading to inhibition of the receptor potential in type I cells. Given that nicotinic ACh receptor stimulation can also lead to NO synthesis in efferent neurones (Campanucci *et al.* 2012), it is plausible that local release of ACh from type I cells, or from VAcHT-positive efferent neurones themselves, could lead to a similar NO-mediated chemoreceptor inhibition.

Summary and concluding remarks

This short review has highlighted several features of information processing in the chemosensory CB with a focus on only few selected ligands. The presence within this tiny organ of a plethora of neurotransmitters and neuromodulators, as well as an even broader spectrum of their receptors, emphasizes the complexity of information processing. Nevertheless, compared to the challenges posed in understanding comparable mechanisms in the brain, the CB offers an attractive alternative. Indeed, it provides a unique opportunity for studying fast chemical synaptic transmission, autocrine–paracrine

signalling, electrical coupling, efferent inhibition and even ‘gliotransmission’ within a fairly localized region (Figs. 1, 3). Moreover, the sensitivity of the CB to chemostimuli can be modified by different patterns of environmental challenges (e.g. hypoxia), providing an opportunity for studies on synaptic plasticity (Kumar & Prabhakar, 2012; Teppema & Dahan, 2010). Understanding these mechanisms can have benefits to human health. For example, enhanced CB activity occurs during exposure to chronic intermittent hypoxia (e.g. sleep apnoea), thereby increasing the risk for hypertension (Peng *et al.* 2009; Kumar & Prabhakar 2012). Furthermore, increased CB activity and disordered breathing patterns occur in congestive heart failure, serving only to exacerbate the condition and increase the risk of mortality (Schultz *et al.* 2013).

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Additional information

Competing interests

The author declares no conflict of interest.

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