Published in final edited form as:
Respir Physiol Neurobiol. 2007 July 1; 157(1): 123–129.

Purines, the Carotid Body and Respiration

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Abstract

The carotid body is essential to detecting levels of oxygen in the blood and initiating the compensatory response. Increasing evidence suggests that the purines ATP and adenosine make a key contribution to this signaling by the carotid body. The glomus cells release ATP in response to hypoxia. This released ATP can stimulate P2X receptors on the carotid body to elevate intracellular Ca^{2+} and to produce an excitatory response. This released ATP can also be dephosphorylated to adenosine by a series of extracellular enzymes, which in turn can stimulate A_1, A_{2A} and A_{2B} adenosine receptors. Levels of extracellular adenosine can also be altered by membrane transporters. Endogenous adenosine stimulates these receptors to increase the ventilation rate and may modulate the catecholamine release from the carotid sinus nerve. Prolonged hypoxic challenge can alter the expression of purinergic receptors, suggesting a role in the adaptation. This review discusses evidence for a key role of ATP and adenosine in the hypoxic response of the carotid body, and emphasizes areas of new contributions likely to be important in the future.

Keywords

carotid body; glomus cells; hypoxia; purines; ATP release; neurotransmitter; ion channels, oxygen sensing; P1 receptors; P2 receptors

1. Introduction

The carotid body has historically been known as the first oxygen sensor, and the elucidation of this role by Corneille Jean Francois Heymans was deemed worthy of the Nobel prize (Fitzgerald & Lahiri, 1996). Our understanding of the complexities involved in detecting oxygen and processing this information by the carotid body has increased a great deal since then, and while it is recognized as the primary site of oxygen sensing, responding to hypoxia without the need for new protein synthesis, a critical input from multiple signaling systems is now recognized. Considerable evidence now suggests that extracellular signaling by the purines ATP and adenosine make a key contribution to the process. Numerous reports detailing purinergic signaling in the carotid body will be reviewed below, with emphasis on the role of purines in the dynamic regulation of oxygen sensing and the processing of this information by the carotid body.

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2. Oxygen Sensing and the Carotid Body

The carotid bodies are present bilaterally in the bifurcation of the carotid arteries. These are relatively minute organs, 1-2 mg in small animals, and can first sense oxygen levels at 100 Torr, far above the levels that can be detected by other cells in the body. This sensitivity reflects contributions from both the sensors themselves and the associated signaling mechanisms. There is good evidence that mitochondrial oxidative phosphorylation, through cytochrome c oxidase, is an important oxygen sensor for regulation of carotid chemoreceptor activity, cardiac blood flow and many other related functions (Nuutinen *et al.*, 1982). It should be noted, however, that mitochondrial cytochrome c oxidase is not the only oxygen sensitive component in the cells, and there are likely multiple sensors, acting at multiple levels.

The glomus cells (GC) of the carotid bodies are central in the response to changes in oxygen and carbon dioxide. Cumulative knowledge tells us that glomus cells are clustered together in groups of 5-12 cells. These clusters are innervated by one or more fibers from pertrosal ganglion neurones (PGN), the cell bodies of which reside in the petrosal ganglia and the axons of which travel to the brainstem neurons. These neurons are the basis of reflexive respiratory function. A decrease in arterial oxygen pressure elicits a reflexive increase in respiration; the stimulus leads to the suppression of current through K^+ channels, subsequent membrane depolarization, and the opening of voltage-gated Ca^{2+} channels. The influx of Ca^{2+} through these channels raises intracellular Ca^{2+} sufficiently to release neurotransmitter, which in turn activates afferent nerves and the reflex response. It is interesting to note that the PGNs are not directly affected by hypoxia or acidosis (Alcayaga *et al.*, 2003) but depend upon transmitters released from GCs.

The sensitivity of the carotid body to oxygen is subject to modulation. It is known, for example, that the carotid body of adult animals is much more sensitive to oxygen than that of newborn, and this sensitivity changes continuously during the maturation process (Carroll, 2003). Even within adult animals, the oxygen sensitivity of the carotid body is much greater than that for aortic body (Lahiri *et al.*, 1981). This indicates that the oxygen sensor and the downstream signaling mechanisms are being modulated by the metabolic environment and transmission mechanisms. The oxygen sensitivity can be seen at several levels, including oxygen delivery to the tissue, the set-point for cellular energy metabolism, oxygen/carbon dioxide interaction, etc. It may be the mechanisms downstream from the sensor itself that determine the difference in oxygen sensitivity observed between the carotid body and other sensing tissues such as the aortic bodies (Lahiri *et al.*, 1981).

3. Adenosine and the Carotid Body

While extracellular ATP and adenosine both modulate carotid body function, the effects of adenosine are known in more detail. There are four subtypes of receptor stimulated by adenosine; A_1, A_{2A}, A_{2B} and A_3. While all four are coupled to G-proteins, they differ in their affinity for adenosine and their downstream connectivity. The human A_1 receptor is stimulated by adenosine with a potency of 300-3000 nM (Fredholm *et al.*, 2001). Although the A_1 receptor has traditionally been associated with the inhibition of adenylate cyclase through pertusis toxin sensitive G-proteins (van Calker *et al.*, 1979), it also activates phospholipase C (PLC) with corresponding increases in inositol triphosphate (IP_3), diacyl glycerol (DAG) and Ca^{2+} (Iredale *et al.*, 1994;Megson *et al.*, 1995;Rogel *et al.*, 2005). The A_1 receptor is detected in rabbit (Rocher *et al.*, 1999) but not in rat glomus cells (Gauda, 2000;Kobayashi *et al.*, 2000a). The A_{2A} receptor shows a sensitivity similar to the A_1 receptor, with adenosine acting at a potency of 700 nM (Fredholm *et al.*, 2001). Stimulation of the A_{2A} receptor activates adenylate cyclase and leads to an increase in cAMP; coupling is primarily via G_s, at least in the periphery, but involves G_{olf} in the striatum (Kull *et al.*, 2000). A_{2A} receptor stimulation can also lead to

transmitter release through activation of protein kinase C (PKC) independent of protein kinase A (PKA; Cunha *et al.*, 2000), and stimulation of PKC indirectly through PKA has also been reported (Huang *et al.*, 2001). The A_{2B} receptor is the least sensitive to adenosine, with a potency of 24,000 nM at the human receptor (Fredholm *et al*., 2001). This receptor stimulates adenylate cyclase and PLC, through actions at G_s and G_q proteins respectively (Peakman & Hill, 1994;Gao *et al.*, 1999;Linden *et al.*, 1999). The A_{2A} and A_{2B} receptors are the predominant types in tyrosine hydroxylase-expressing (glomus) cells (Weaver, 1993;Gauda, 2000;Gauda & Lawson, 2000;Gauda *et al*., 2000;Kobayashi *et al*., 2000a;Conde & Monteiro, 2006). Expression of the A_3 adenosine receptor was not detected in carotid body chemoreceptor cells using the polymerase chain reaction (Kobayashi *et al.*, 2000a).

The effects of extracellular adenosine on the carotid body are diverse. Adenosine increases ventilation in several species (Watt & Routledge, 1985;Monteiro & Ribeiro, 1987;Reid *et al.*, 1987;Watt *et al.*, 1987a;Watt *et al.*, 1987b;Monteiro & Ribeiro, 1989,1991;Reid *et al.*, 1991). In primates, an intravenous injection of adenosine produces hyperventilation and dyspnoea resulting from a direct activation of the carotid body (Conde & Monteiro, 2006). This activation can be antagonized by the non-specific adenosine antagonist caffeine in premature infants (Aranda & Turmen, 1979;Bairam *et al.*, 1987) and rhesus monkeys (Howell & Landrum, 1995). Adenosine increases the ventilation depth and the minute ventilation rate in human clinical assays, with some secondary effects including heat sensation, flushed face, dyspnoea and chest discomfort (Uematsu *et al.*, 2000). The increased ventilation rate is dependent on the dose and the proximity of the infusion site to the carotid body (Watt & Routledge, 1985;Watt *et al.*, 1987b). Adenosine also enhances the ventilatory response to hypoxia but not to hypercapnia (Griffiths *et al.*, 1997). This argues against a major contribution from the central chemosensory centers, where adenosine increases the sensitivity to hypercapnia, consistent with the inability of adenosine to readily cross the blood-brain barrier (Berne & Rubio, 1974). Instead, these data suggest a role for peripheral sensors in the ventilatory response to adenosine (Maxwell *et al.*, 1986;Maxwell *et al.*, 1987).

Intravenous infusion of adenosine also enhances ventilation in rats (Monteiro & Ribeiro, 1987) and cats (McQueen & Ribeiro, 1983,1986). Adenosine and the agonists 2'-chloroadenosine (CADO), N6-methyladenosine, 5'-N-ethylcarboxamidoadenodine (NECA), L-N6-phenylisopropiladenosine (L-PIA) and D-N6-phenylisopropyladenosine (D-PIA) increase tidal volume, respiratory frequency and minute volume parameters in rats (Monteiro & Ribeiro, 1987). The effects of adenosine are antagonized by the general adenosine receptor antagonist theophylline and by cutting the carotid sinus nerve. Levels of endogenous adenosine can be elevated by either erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA), an adenosine deaminase inhibitor or dipyridamole, an adenosine uptake blocker; EHNA and dipyridamole both emulate the excitatory effects of adenosine in rats (Monteiro & Ribeiro, 1989). This enhancement is abolished by carotid sinus nerve section and antagonized by the poorly selective adenosine receptor antagonist 1,3-dipropyl-8(p-sulfophenyl) xanthine (DPSPX). Stimulation of the A_{2A} receptor was shown to increase ventilation in rats at low concentrations (Sebastiao & Ribeiro, 1996). A similar process occurs in cat; adenosine increases the discharge of the carotid sinus nerve; this increase is mimicked by NECA, CADO, L-PIA and D-PIA and inhibited by theophylline, suggesting A_2 receptor involvement (McQueen & Ribeiro, 1983, 1986). This increased discharge from the carotid sinus nerve was also detected in response to application of adenosine to cat cells in vitro (Runold *et al.*, 1990a,b).

The mechanisms underlying the increase in catecholamine discharge from the carotid sinus nerve in response to adenosine have been elucidated by multiple investigators and involve A_{2A} receptor stimulation, increased cAMP and PKA, block of K^+ channels, membrane depolarization, opening of voltage-gated Ca^{2+} channels, Ca^{2+} influx and transmitter release from glomus cells (Buckler & Vaughan-Jones, 1994;Gonzalez *et al.*, 1994;Lopez-Lopez *et*

al. 1997;Vandier *et al.*, 1999; Xu et al., 2006). The Ca^{2+} increase in glomus cells triggered by adenosine is emulated by A_{2A} agonist CGS21680 and by increasing cAMP with forskolin, but inhibited by specific A_{2A} receptor antagonist ZM241385 and by PKA inhibitor H89 (Xu et al., 2006). The increased cAMP decreases the amplitude of 4-AP sensitive and voltage-independent K^+ outward currents in isolated rabbit (Lopez-Lopez *et al.*, 1993) and rat glomus cells (Lopez-Lopez *et al.*, 1997;Vandier *et al.*, 1999). This block of K^+ currents by adenosine is largely Ca^{2+}-independent (Lopez-Barneo *et al.*, 1988), although a small but significant contribution involves the inhibition of Ca^{2+}-dependent K^+ channels (Peers, 1990). The influx of Ca^{2+} through L-type channels (Buckler & Vaughan-Jones, 1994) leads to Ca^{2+} dependent transmitter release (Gonzalez *et al.*, 1994).

In rabbit glomus cells, adenosine acts at A_1 receptors to produce the opposite effect; it inhibits the release of catecholamines by inhibiting L-type Ca^{2+} channels (Rocher *et al.*, 1999). Antagonists of the A_1 receptor including 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and 8-cyclopentyl-1,3-dimethylxanthine (8-CPT) prevent the inhibition, as does the L-type Ca^{2+} channel blocker nisoldipine. These inhibitory actions of adenosine are mimicked by the A_1 receptor agonists R-PIA and 2-chloroadenosine, and by the adenosine uptake blocker dipyridamole. The absence of the A_1 receptor in rat cells eliminates this contribution.

The actions of adenosine on the carotid body are of interest primarily as they may increase in response to hypoxia (Gonzalez *et al.*, 1994). Although the final contributions of adenosine to the cellular responses in hypoxia remain to be clarified, hypoxia induces production of adenosine through the release of ATP and the dephosphorylation of nucleotides, as discussed below (Conde & Monteiro, 2004). The increase in adenosine concentration during the hypoxic challenge induces the increase in intracellular cAMP via stimulation of A_{2A} receptors (Chen *et al.*, 1997). This response is mimicked by CGS21680, blocked by the A_2 receptor antagonist 3,7- dimethyl-L-propargyl-xanthine (DMPX) and potentiated by dipyridamole. Stimulation of A_{2A} receptors occurs after hypoxia, while the modulating function of adenosine is prevented by A_{2A} antagonist ZM241385 (Kobayashi *et al.*, 2000b).

4. ATP and the Carotid Body

While the effects of adenosine on the carotid body are now understood in some detail, our appreciation of how extracellular ATP modulates the detection of oxygen levels is expanding rapidly. Extracellular ATP mediates its actions by stimulating the ionotropic P2X receptors and the metabotropic P2Y receptors. There are seven P2X subunits termed $P2X_{1\text{-}7}$, with three subunits in each receptor forming both homomeric and heteromeric combinations (North, 2002). The G-protein coupled P2Y receptors are classified into two main families based on the particular G-protein affiliation (von Kügelgen, 2006). The actions of extracellular ATP described to date have primarily involved P2X receptors, with PGNs expressing $P2X_2$, $P2X_3$ $P2X_5$ and $P2X_7$ receptors (Campanucci *et al.*, 2006). However, inhibitory effects mediated through P2Y receptors may also contribute to the response from glomus cells (Xu *et al.*, 2005).

The excitatory effects of ATP on the carotid body were known long before specific P2 receptors had been identified, with numerous studies demonstrating a physiological response to extracellular ATP (Jarisch *et al.*, 1952;Spergel & Lahiri, 1993). Barbe *et al.* (2002) have reported that the carotid chemoreceptor discharge initiated by CO was completely suppressed by the ATP receptor antagonist PPADS, while ATP increases cytosolic calcium in the glomus cells (Mokashi *et al.*, 2003). Additional support is provided in Figure 1; intracellular Ca^{2+} levels in a co-culture of PC-12 cells and rat PGNs rapidly increased after hypoxic stimulation with a PO_2 of 10 Torr. However, over 60% of this response was blocked by the P2 inhibitor suramin (100 μM), while the remainder was abolished by application 50 μM hexomethonium,

consistent with involvement of both ATP and ACh in the initial Ca^{2+} elevation triggered by hypoxia. Although the relative non-selectivity of suramin and PPADS precludes assignment of these effects to any particular P2 receptor, these findings provide additional support for the importance of ATP signaling in the hypoxic response. Other transmitters may of course also be involved; a recent study found in anesthetized but spontaneously breathing cats that both the carotid body and ventilatory response to hypoxia persisted after combined cholinergic and purinergic blockade (Reyes *et al.*, 2007). It is also possible that expression of receptors could be affected by the choice of model.

The $P2X_2$ and $P2X_3$ receptors likely make a considerable contribution to signaling in the carotid body, although their precise input is proving more complex than originally predicted. Using co-cultures of rat carotid body and petrosal ganglion cells, receptors on the PGNs forming functional chemosensory units with the carotid body cells were investigated (Zhang *et al.*, 2000;Prasad *et al.*, 2001). Molecular and immunohistochemical techniques were used to identify both $P2X_2$ and $P2X_3$ receptors on the afferent PGNs that could be stimulated by both hypoxia and CO_2. As agonist potency at the $P2X_2$ receptor increases with acidity (King *et al.*, 1996;King *et al.*, 1997) and as the potentiation of the $P2X_2$ receptor response to ATP by noradrenaline, nicotine and serotonin are all dependent upon the acidification of the bath (Wildman *et al.*, 1997), it was thought that these transmitters could alter purinergic contribution to chemosensory signaling in the carotid body. However, the ventilatory response in either $P2X_2$ or $P2X_3$ knockout mice exposed to hypercapnia was the same as wildtype (Rong *et al.*, 2003). In contrast, the increase in ventilation during hypoxia was markedly reduced in the $P2X_2$ -/- mice. These mice showed a profound depression of ventilation when exposed to 2.5% oxygen, similar to the effect found in CSN denervated cats (Lahiri *et al.*, 1993). The response to hypoxia in $P2X_3$ knockout mice was normal. These observations indicate that the $P2X_2$ receptor subunits are essential for the development of normal ventilatory response to hypoxia, although they cast doubt on the contribution of the $P2X_3$ receptors. They also suggest that either these transgenic mice effectively compensate for the loss of $P2X_2$ and $P2X_3$ receptors subtype, or other subtypes of P2 receptors are involved in central CO_2/H^+ chemotransduction. The recent identification of A- 317491, a relatively specific antagonist for the $P2X_3$ and $P2X_{2/3}$ heteromeric receptor may help unravel the contributions (McGaraughty & Jarvis, 2005), although the presence of $P2X_5$ and $P2X_7$ receptors in PGNs suggests additional complications (Campanucci *et al.*, 2006).

5. Regulation of Extracellular Purines in the Carotid Body

The complex responses of the carotid body to extracellular purines are dependent upon the levels of purine available to stimulate receptors in addition to the particular distribution of these receptors. It is now well accepted that the endogenous agonists used in most purinergic signaling are not derived from the pathological outpouring of ATP following cell lysis but instead result from the controlled release of ATP (Lazarowski *et al.*, 2003). Adenosine is typically produced by the subsequent dephosphorylation to adenosine or by transport of adenosine directly out of cells (Thorn & Jarvis, 1996;Robson *et al.*, 2006). The work of Colin Nurse and colleagues using co-cultures of rat glomus cells and pertrosal ganglion neurones suggested that the co-release of ATP and acetylcholine (ACh) was central for hypoxic chemotransduction (Zhang *et al.*, 2000;Buttigieg & Nurse, 2004). The sensitivity of the afferent postsynaptic responses to the P2 purinergic blocker suramin suggested an important role for ATP release in carotid body function (Prasad *et al.*, 2001;Buttigieg & Nurse, 2004;Zhang & Nurse, 2004;Nurse, 2005). Nurse's studies also tested the effect of carbon dioxide on the co-release of ATP and ACh in the co-cultures (Prasad *et al.*, 2001). The PGNs depolarized, with spike frequencies indicating an interaction between carbon dioxide and hypoxia that was reminiscent of that found previously in the chemosensory discharge from the cat carotid body (Lahiri & DeLaney, 1975). However, release in vivo may differ from this co-culture model.

For example, it has not been directly demonstrated that ACh is released from glomus cells in the intact animal. Immunohistochemical and in situ hybridization experiments demonstrate that rate-limiting enzymes for ACh synthesis and transport are not expressed in glomus cells, but are detected in the autonomic microganglion cells within the carotid body (Schutz et al., 2001;Gauda et al., 2004). Whether model-dependent discrepancies also exist for ATP release is currently unclear. It is also possible that other neurotransmitters are co-released with ATP, as found in chromaffin cells (Hollins & Ikeda, 1997). Readers are referred to a recent review on the release of ATP from the carotid body for more detail (Zapata, 2007).

Extracellular levels of adenosine surrounding the CB are determined by dephosphorylation of nucleotides, adenosine transport and other mechanisms yet to be determined. Ecto-5' nucleotidase (CD73) catalyzes the extracellular dephorphorylation of 5'AMP into adenosine (Zimmermann, 1992;Reigada *et al.*, 2006); inhibition of ecto- 5'nucleotidase by α,β -mADP (AOPCP) inhibits 40% of the extracellular adenosine released under both normoxic and hypoxic conditions and unmasks a contribution of the equilibrative adenosine transporter (Conde & Monteiro, 2004). Inhibition of both to ecto-5'nucleotidase and equilibrative adenosine transporter reduces extracellular adenosine levels by only 50-75%, suggesting an additional source of adenosine may contribute. As the adenosine release that accompanies activation of nicotinic receptors in the carotid body was inhibited 72% by α,β -mADP (Conde & Monteiro, 2006), the relative contribution of extracellular ATP may vary with stimuli.

While this review is primarily concerned with peripheral oxygen sensing, it is worth noting that Gourine et al. found that 10% O_2 hypoxia was followed by ATP release in the ventral surface of the medulla in peripherally chemo-denervated animals (Gourine *et al.*, 2005). Blockade of ATP receptors in the ventrolateral medulla by PPADS augmented the hypoxia induced secondary slowing of the respiratory rhythm. This indicates that ATP-initiated purinergic signaling in the ventrolateral medulla may also contribute to respiratory activity.

6. Involvement of Purines in Plasticity

The usefulness of the carotid body in maintaining homeostasis is magnified on its plasticity. Responses are not fixed at birth but rather adapt with maturation and experience to environmental stresses. Much of this plasticity arises from changes in gene expression. The full complement of genes of the chemoreceptors are not all active simultaneously but are brought into play as needed. Our understanding of the processes involved in the enhancement and suppression of the genes involved in the purinergic neurotransmission is just beginning, but promises to accelerate as new technologies are applied to the problem.

It is well known that subacute hypoxia continues to increase ventilation above that due to acute hypoxia despite an increase in arterial oxygen pressure and decrease in carbon dioxide pressure (Lahiri *et al.*, 2002) This is due, at least in part, to a continued increase of peripheral chemoreceptor activity (Barnard *et al.*, 1987). If ATP plays a key role in the mediation of arterial chemoreceptor activity, it follows that either sensitivity of the chemoreceptors to ATP is increased, extracellular ATP concentration is increased or both (Lahiri, 2006). He et al. have reported that continuous hypoxia for several days diminishes the effectiveness of acetylcholine as a synaptic transmitter in the carotid body while the response to ATP is maintained (He *et al.*, 2006), suggesting the relative contribution from P2 receptors may increase with time. The contribution of purines may also be developmentally regulated. The A_{2A} adenosine and D_2 dopamergic receptors are differentially expressed in glomus cells during development, with greater relative expression of mRNA message for the A_{2A} receptor found in earlier stages and of the D_2 receptors in the adult animal (Gauda & Lawson, 2000;Gauda *et al.*, 2000). The dynamic modulation of the purinergic contribution in both the aging animal and as a form of adaptation is a key area for future research.

Improved technology has greatly accelerated our ability to identify genes whose expression is modulated. Recent studies have used oligonucleotide microarray techniques to analyze changes in genomic expression in the whole carotid body under physiologic levels of hypoxia. Ganfornina et al. (2005) studied carotid bodies obtained from mice exposed to 21 or 10 percent oxygen for 24 hours. Transcriptional profiles obtained from the carotid body were subtracted from profiles obtained from the adrenal medulla of the same mice. Hypoxia led to a mild downregulation of the A_{2B} receptor, suggesting the regulation of P1 receptors may contribute to the adaptive processed in hypoxia. It should be noted, however, that changes in mRNA may not directly correspond to changes in protein expression or receptor impact.

Several investigators have used model systems rather than the carotid body itself to study gene expression. For example, a clonal line of pheochromocytoma cells, PC12 cells, have been used to trace the pathways employed during hypoxia by transcription factors that are critical in hypoxic adaptation. Many hypoxia inducible genes have been shown to have promoter and enhancer elements for multiple transcription factors. Studies by Seta and Millhorn (2004) using PC-12 cells exposed to continuous hypoxia have reported that genes for adenosine receptors, particularly the A_{2A} receptors, increase in expression. Chronic hypoxia was also shown decrease the expression of adenosine kinase, adenosine deaminase and the adenosine transporter, while increasing the expression of ecto-5' nucleotidase (Kobayashi *et al.*, 2000b). These results all suggest that levels of extracellular adenosine and of P1 receptors are dynamically regulated, and emphasize that these genetic tools are well suited to examine how changes in purinergic signaling may contribute to the plasticity of oxygen sensing.

7. Summary

Our knowledge of the signaling mechanisms that contribute to oxygen sensing has been expanding ever since the properties of the carotid body were identified by Heymans in the 1930s. A large number of studies have stressed that purines play a key role in the process. The complex effects of both ATP and adenosine enable the hypoxic signal to be transduced from chemoreceptors to afferent neurons, with multiple receptors allowing for a nuanced transmission unavailable to single-action transmitters. As ATP and adenosine can have contrasting effects on neurons (Zhang *et al.*, 2006), the integrated response to all purines in vivo needs to be understood as a whole, and not just as the discrete effect induced by specific agonists. The increasing variety of pharmaceutical tools specific for purinergic receptors and regulatory enzymes, combined with new genomic and proteomic approaches, promise the next decade will reveal even more exciting information about the role of purines in oxygen sensing by the carotid body.

Acknowledgements

Supported by NIH grants HL43413 (SL), HL50180 (SL), EY013434 (CHM) and EY015537 (CHM). The authors would like to thank HuiLing Hu for assistance.

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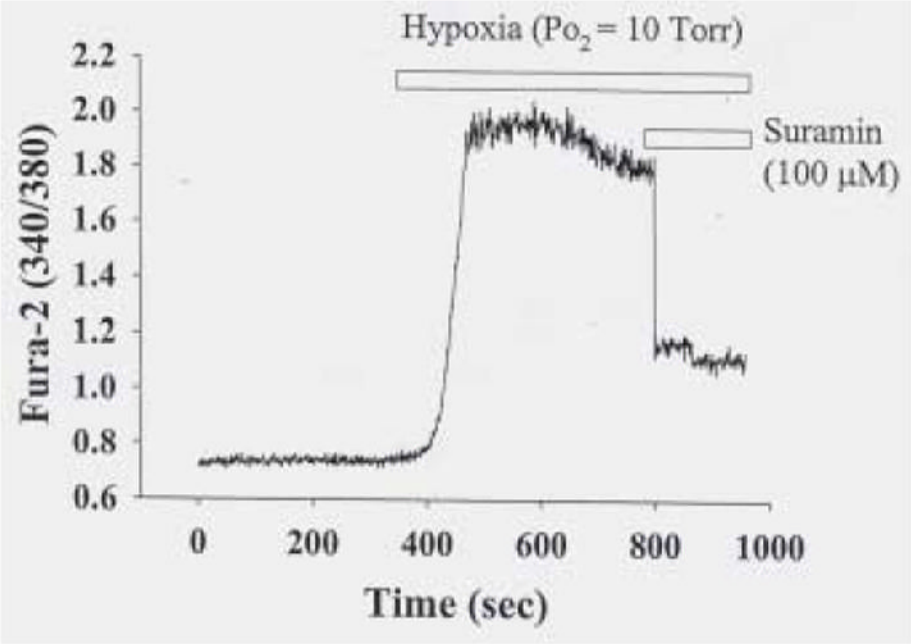

Figure 1.

The purinergic contribution to the hypoxic response is substantial. Lowering PO_2 to 10 Torr led to a substantial and sustained increase in the intracellular Ca^{2+} levels of a PGNs co-cultured with PC-12 cells, as determined from the ration of light excited at 340 nm to 380 nm in cells loaded with the dye fura-2. The P2 receptor antagonist suramin (100 μM) decreased the sustained Ca^{2+} elevation by 60%. Subsequent addition of 50 μM hexomethonium abolished the remainder of the response (not shown). Unpublished observation (Baby SM, Roy A and Lahiri S) based on previously published approaches (Mokashi *et al*., 2003).

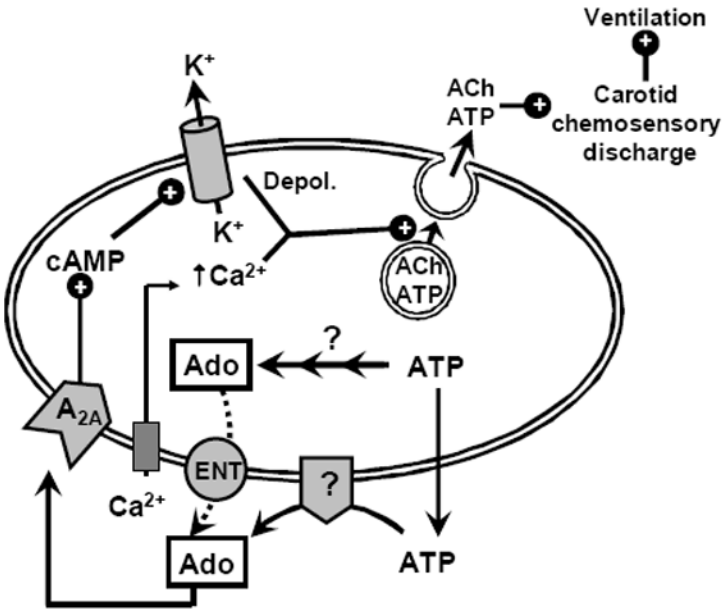

Figure 2.
Schematic diagram showing co-release of ATP and acetyl choline and primary consequences of stimulating P1 receptors. Alternate release patterns are also possible (see text for details).