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The role of $P2Y_1$ receptor signaling in central respiratory control

V. Rajani[b,c], Y. Zhang[a,c], A.L. Revill[a,b,c], G.D. Funk[a,b,c,*]

[a] Department of Physiology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada
[b] Neuroscience and Mental Health Institute, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada
[c] Women and Children's Health Research Institute (WCHRI), Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada

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ABSTRACT

The profile of P2 receptor signaling in respiratory control has increased substantially since the first suggestions more than 15 years ago of roles in central chemoreception and modulating inspiratory motor outflow. Part of this reflects the paradigm shift that glia participate in information processing and that ATP is a major gliotransmitter. P2 receptors are a diverse family. Here, we review ATP signaling in respiratory control, highlighting G-protein coupled $P2Y_1$ receptors that have been a focus of recent work. Despite strong evidence of a role for glia and P2 receptor signaling in the central chemosensitivity mediated by the retotrapezoid nucleus, $P2Y_1$ receptors do not appear to be directly involved. Evidence that central P2 receptors and glia contribute to the hypoxic ventilatory response is compelling and $P2Y_1$ receptors are the strongest candidate. However, functional significance in vivo, details of the signaling pathways and involvement of other receptor subtypes remain important questions.

1. Introduction

Breathing, the essentially automatic, rhythmic movement of air in and out of the lungs, is a complex behavior requiring precisely coordinated activation of pump, airway and accessory respiratory muscles. Breathing is generated and controlled by a network in the brainstem. It includes a rhythm generator, which produces the basic oscillation and includes the preBötzinger Complex (preBötC) as a key component, and a pattern generator comprising premotoneurons and motoneurons that transform the basic oscillation into different patterns of activity in all the requisite muscles. Like most motor networks, breathing is modulated by mechanoreceptive feedback from multiple sources that keep the central network informed of the mechanical status of the lungs, chest wall and airways to maintain efficient breathing. Unlike other motor systems, however, respiratory network activity is sensitive to chemosensory feedback from oxygen (O_2) and carbon dioxide/pH (CO_2/H^+) sensors in the arteries and brain that match ventilation to metabolic demand and maintain blood gas homeostasis.

Purinergic, or ATP-gated, receptor signaling features prominently in these chemosensory reflexes. The purinergic P2 receptor family comprises 7 ionotropic $P2X_{1-7}$ and 8 metabotropic $P2Y_{1,2,4,6,11-14}$ receptor subtypes (Abbracchio et al., 2006). Fast ATP-mediated excitatory responses occur through P2X receptors, which are trimeric ligand-gated, non-selective cation channels with significant Ca^{2+} permeability. P2Y receptors couple to G-proteins and mediate slower responses to ATP, UTP, and ADP. $P2Y_{1,2,4,6}$ receptors signal primarily through $G\alpha_{q/11}$, $P2Y_{12,13}$ receptors through $G\alpha_i$, $P2Y_{14}$ receptors through $G\alpha_i/G\alpha_o$ and $P2Y_{12}$ receptors through $G\alpha_{q/11}$ and $G\alpha_s$ (Abbracchio et al., 2006). P2X and P2Y receptors are found throughout the CNS on astrocytes and neurons, with the exception of $P2Y_{11}$ receptors that are only found in neurons, and $P2X_7$ receptors, which appear limited to immune cells and glia (Burnstock, 2007). The pH sensitivity of the $P2X_2$ receptor and its potential as a pH sensor in central chemoreception put this subtype on center stage (Thomas et al., 1999). While the hypothesis that the $P2X_2$ subunit acts as a chemosensor was ultimately rejected (Rong et al., 2003; Lorier et al., 2004), interest in P2 receptor signaling did not diminish. Since then, the $P2Y_1$ receptor, which is distributed throughout the brainstem (Fong et al., 2002a), has received considerable attention (Lorier et al., 2007; Huxtable et al., 2009; Gourine et al., 2010; Huxtable et al., 2010). In part this was based on the finding that the preBötC rhythm in vitro is very sensitive to $P2Y_1$ receptor modulation (Lorier et al., 2007; Huxtable et al., 2009; Gourine et al., 2010; Huxtable et al., 2010), but also on the development of reasonably selective agonists/antagonists (MRS2365/MRS2179 and 2279). The objectives of this review are two-fold. First, we summarize current understanding of the role played by $P2Y_1$ receptors in modulating motoneuron excitability as

* Corresponding author at: University of Alberta, Department of Physiology, 3-020G Katz Group Centre, Edmonton T6G 2E1, Canada. Fax: +1 780 492 8915.
E-mail address: gf@ualberta.ca (G.D. Funk).

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well as their contribution to the homeostatic ventilatory responses evoked by hypoxia and hypercapnia. Second, we discuss the signaling cascades and ion channels through which $P2Y_1$ receptors might act to modulate respiratory neuron and network excitability, focusing on the preBötC where evidence of a role for $P2Y_1$ receptors is most compelling.

2. $P2Y_1$ receptor signaling in the central respiratory network

2.1. $P2Y_1$ receptor modulation of respiratory motoneuron behaviour

A role for P2 receptor signaling in modulating motor output from the mammalian CNS was first demonstrated in the in vitro respiratory network in which XII inspiratory motor output was potentiated by ATP (Funk et al., 1997). Hypoglossal (XII) motoneurons innervate muscles of the tongue, including the genioglossus muscle, which is the main tongue protruder important in maintaining airway patency. Loss of XII tone during sleep is strongly implicated in obstructive sleep apnea (Remmers et al., 1978; Dempsey et al., 2010). Inspiratory output from the phrenic nerve, which innervates the diaphragm, the main inspiratory pump muscle, is also potentiated by ATP (Miles et al., 2002; Alvares et al., 2014). ATP actions on motoneurons were originally attributed to P2X receptors, based on whole-cell recordings of current reversal potentials and membrane conductance changes (Funk et al., 1997; Miles et al., 2002; Ireland et al., 2004). However, $P2Y_1$ receptors also appear to contribute, at least at XII motoneurons. $P2Y_1$ receptor responses in C4 nerve in vitro are only evoked at concentrations that question physiological relevance (Alvares et al., 2014). XII motoneurons express $P2Y_1$ receptors and $P2Y_1$ agonists potentiate XII nerve burst amplitude by ~60% (Fong et al., 2002a; Alvares et al., 2014). $P2Y_1$ receptor activation increases XII MN excitability, at least in part, by potentiating $I_{CAN,}$ a non-inactivating mixed cation current that requires elevated intracellular Ca^{2+} and membrane depolarization to activate (see Section 3.3, I_{CAN}). Whether other P2Y receptors modulate XII MN excitability is not known. Key questions include the physiological or pathophysiological conditions that evoke endogenous ATP release in the XII nucleus and the source of ATP. One possible source is via co-release with noradrenaline from locus subcoeruleus, which occurs at some central synapses (Poelchen et al., 2001). XII motoneurons receive strong noradrenergic innervation (Chan et al., 2006), and this may provide a pathway through which ATP contributes to the state-dependent modulation of XII MN excitability and airway tone.

2.2. $P2Y_1$ receptors in the hypoxic ventilatory response

The ventilatory response to acute hypoxia is biphasic, comprising an initial rapid increase in ventilation, followed by a secondary depression (Moss, 2000). While there are several hypoxia sensitive regions in the CNS that could contribute to the initial increase, including the caudal hypothalamus, the nucleus tractus solitarius (NTS), C1 and the preBötC (Solomon, 2000; Neubauer and Sunderram, 2004) it is primarily attributed to the peripheral carotid body chemoreceptors. The multiple roles of carotid body P2 receptors, including $P2Y_1$ receptors, in mediating the initial increase are reviewed elsewhere (Tse et al., 2012). The mechanisms underlying the secondary depression are still under debate but central mechanisms are strongly implicated (Teppema and Dahan, 2010). A role for P2 receptor signaling in the hypoxic ventilatory response first emerged in 2005 with the use of biosensors that detected hypoxia-induced ATP release at the ventral surface of the medulla in anesthetized rats. The peak ATP signal followed the initial increase in ventilation, while application of the P2 receptor antagonist, PPADS (pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid, 100 μM; i.e., concentrations that could affect glutamatergic transmission) over the ventral medullary surface caused a greater respiratory depression without affecting the initial increase in ventilation. These data suggested that the ATP is released during hypoxia and that its excitatory actions attenuate the secondary hypoxic depression (Gourine et al., 2005b). Several questions remained. First, concerns over selectivity of the biosensors and specificity of PPADS raised doubts about the involvement of ATP. Additional questions included: (i) the site and source (neuronal or glial) of ATP; (ii) the mechanism of hypoxia sensing; (iii) the mechanism of ATP release; and (iv) the mechanism by which ATP increases frequency. Substantial progress has been made in addressing these questions.

2.2.1. Site of ATP signaling

Application of biosensors to hypoxia-exposed horizontal medullary slices revealed, at least in vitro, that hypoxia evokes ATP release from the ventral respiratory column, including the preBötC (Gourine et al., 2005b). The exact site at which ATP is released to increase breathing during hypoxia in vivo is unknown. However, the preBötC, a critical site for inspiratory rhythm generation is a strong candidate. Expression of TMPAP (transmembrane prostatic acid phosphatase) on neurons and glia of the preBötC and surrounding regions (via injection of a lentiviral vector expressing TMPAP and enhanced green fluorescent protein under the control of elongation factor 1α promoter), which degrades ATP to adenosine to minimize ATP actions, increased the secondary hypoxic respiratory depression similar to P2 receptor antagonists (Angelova et al., 2015). In addition, mapping of the ventrolateral medulla in rhythmic medullary slices using local injections of P2 receptor agonists and antagonists indicates that ATP acts predominantly via $P2Y_1$ receptors in the preBötC to increase inspiratory frequency (Lorier et al., 2007; Zwicker et al., 2011).

2.2.2. Source of ATP

Glia were first implicated in the response to ATP via in vitro experiments in the neonatal rhythmic slice showing that the frequency increase evoked by ATP, but not Substance P, in the preBötC was markedly reduced following incubation of the slice with glial toxins (Huxtable et al., 2010). In addition, glial cells cultured specifically from the preBötC express $P2Y_1$ receptors and show $P2Y_1$ receptor-dependent, ATP-evoked increases in intracellular Ca^{2+}. These data led to the hypothesis that hypoxia evokes ATP release from unknown sources, and that the ATP acts in part on glial cells via ATP-evoked ATP (or glutamate) release to increase frequency (Huxtable et al., 2010). More recent data from primary dissociated glial cultures from multiple brain regions, suggest that astrocytes are the source of ATP in hypoxia. Total internal reflection fluorescence microscopy (TIRF) imaging revealed loss of ATP-containing vesicles (marked with fluorescent quinacrine or tagged vesicular nucleotide transporter) upon exposure to hypoxia (Angelova et al., 2015). However, autocrine, ATP-evoked ATP release did not contribute to the glial response to hypoxia; the rate of hypoxia-induced vesicle exocytosis was not affected by the $P2Y_1$ receptor antagonist, MRS2179, or apyrase (Angelova et al., 2015). Thus, the ATP-evoked ATP release proposed based on data from rhythmic slices does not occur in cultured astrocytes. Whether this mechanism contributes to the hypoxic ventilatory response remains to be determined.

2.2.3. Hypoxia sensing and ATP release

Hypoxia triggers increases in intracellular Ca^{2+} in astrocytes in organotypic brain slice cultures, acute slices, primary dissociated glial cultures and in cortical astrocytes in vivo, suggesting that astrocytes are ubiquitously capable of sensing hypoxia (Angelova