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Hypoxia Modulates the Adenosinergic Neural Network

Susana P. Gaytán and Rosario Pasaro

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<http://dx.doi.org/10.5772/65242>

Abstract

The aim of this study was to review the latest findings about the neural plasticity on the adenosinergic neural network after the exposition to hypoxia. Identification of the neuromorphology that supports the physiological adaptations underlying the response of organisms to environmental factors including injurious exposures (specifically hypoxia) has been one of the major research challenges in biomedicine. To know these responses would connect the metabolic needs and the vegetative neuronal networks in an integrated way. Hypoxia refers to a state in which oxygen supply is insufficient and several neural cardiorespiratory structures are responsible for correcting and preventing its effects. Although hypoxia is often a pathological condition, variations in arterial oxygen concentrations can be part of the normal physiological responses, for example, during hypoventilation training or strenuous physical exercise. Also, hypoxia is a serious consequence of preterm birth in the neonate. Neural plasticity is a persistent change in the morphology and/or function based on prior experiences, and it is crucial for understanding its effects. Plasticity is well evident when the triggering experience occurs early in life; but in the case of respiratory control plasticity, could also be present in adult life. The regulation of adenosinergic neural network maturation, especially in central cardiorespiratory areas, could provide new perspectives in respiratory new-born distress symptoms.

Keywords: hypoxia, purinergic network, adenosine, central respiratory control, neuronal plasticity

1. Introduction

One of the main functions of the cardiorespiratory system is to guarantee that all tissues are adequately oxygenated at all time, maintaining the normal mitochondrial oxidative process and ATP production. The most common electron acceptor is molecular oxygen (O_2), and when O_2 is

present, the mitochondria will undergo aerobic respiration. To maintain O_2 levels, in healthy animals, ventilation is tightly controlled by a system that must maintain the precise constancy of alveolar and arterial blood gases and acid-base status, as well as minimizing the work and metabolic cost of breathing. Deviations from these normal values lead to hypoxic tissue environments [1–6].

In mammals, a lack of O_2 (hypoxia) induces acute reflexes including increasing ventilation and sympathetic tone in order to almost immediately improve the uptake and distribution of O_2 to all tissues of the organism. When the conditions of hypoxia were prolonged, during hours or days, as a defence mechanism it would induce the expression of different genes that, consequently, would modify the ATP metabolism. Indeed, all the homeostatic control of the animal would be affected and other changes would be induced such as increased ventilation, erythropoiesis and angiogenesis, all of that would result in improved O_2 tissue levels. In order to produce the complete response to hypoxia, a group of specialized cells are key to mediate these fast reflex responses. These cells are crucial because they are capable of sensing small variations of O_2 , and this information is crucial to maintain O_2 homeostasis. Among the organs that respond acutely to hypoxia, the carotid body (CB) is currently attracting renewed medical interest, as its over-activation seems to be involved in the autonomous dysfunction that accompanies numerous highly prevalent disorders such as sleep apnoea, diabetes, hypertension and chronic heart failure [1–6].

The aim of this study was to review the latest findings about the neural plasticity of the adenosinergic neural network implicated in the central regulation of breathing after the exposition to hypoxia. Four types of hypoxia are currently known: first, the hypoxemic type, in which the blood O_2 levels fall down and they could not saturate the molecules of haemoglobin. Secondly, in the anaemic type, low concentrations of functional haemoglobin avoid the erythrocytes that could make an effective transportation of O_2 . Thirdly, the stagnant type, in which hemodynamic is altered and the velocity and volume of the blood flow is diminished, as that occurs in shock or syncope. Finally, the histotoxic hypoxia was referred to a reduction due to a deficiency in the utilization of O_2 by the cells. To compensate for hypoxia, cardiovascular and respiratory functions are implemented increasing the cardiac rhythm, causing hypertension, modifying the ventilatory rhythm and increasing the activity of the accessory breathing muscles of neck and upper chest. As the hypoxia continues to worsen, these compensatory mechanisms would begin to fail [1–6].

All of these systemic responses are controlled by specific brain areas that integrate the information about the hypoxic conditions and conduct the changes during the hypoxic insult to initiate an adaptive process. As in other systems, the neural plasticity is a central cue for the cardiovascular and respiratory responses to the hypoxia functional adaptation. Neural plasticity implies a persistent change in the morphology and/or function based on prior experiences and it is crucial for understanding the changes in the central control of cardiorespiratory functions. Plasticity is well evident when the triggering experience occurs early in life; but in the case of respiratory central control, plasticity could also be present in adult life [3, 5, 7–11]. Since ATP production is compromised during exposure to hypoxia, it is interesting to try to discuss the possible role of the purinergic signalling, in general, and the neuronal

adenosinergic network, among other neural structures, responsible of the defence response against hypoxia. This interplay could confer emergent properties to the central respiratory control system. Understanding these mechanisms and their interactions may enable us to optimize hypoxia-induced plasticity as a way to improve treatments for patients that suffer from different ventilatory impairments or other related pathologies [1–6]. On the other hand, the hypoxic hypometabolism differs in adults or young animals. Indeed, it would have a more evident effect in mammals when the levels of O₂ consumption are higher (i.e. in small or young animals when they are exposed to cold). It is clear that a good strategical adaptation to low O₂ levels (hypoxia) requires coordinated down-regulation of metabolic demand, as well as tissue supply, in order to prevent a mismatch in ATP utilization and production that might end in a bioenergetic collapse. In this way, substantial experimental evidence suggests that common integrative structures are probably involved in the metabolic and ventilatory responses to hypoxia [12–15]. The synthesis of adenosine is related to the cellular ratio AMP/ATP (**Figure 1**) and, obviously, to the energy metabolism of cells. In addition, in the central nervous system (CNS), an increase in neuronal activity needs a higher expense of energy and, for this reason, the extracellular levels of adenosine would be modified. The adenosinergic system acts, in CNS, to bind adenosine to one of the different adenosine receptors (A-Rs). Usually, the increased high levels of extracellular adenosine would induce a decrease in neuronal activity. Because the cells reduce its activity, its need for energy falls down too. This nucleoside usually acts via receptor-dependent mechanisms, and could also use receptor-independent mechanisms. Anyway, its complex and wide range of actions imply that adenosine could have a significant role in the defence against cell damage in areas of increased energy requirements, in tissues as well as in recovering the normal/physiological state from a pathologic one [6, 12–22].

Furthermore, the above-mentioned hypometabolism is mediated by an activation of the chemoreceptors by depletion in the arterial O₂ partial pressure (Pa_{O₂}) among other factors. The sensing of the Pa_{O₂} is the principal afferent pathway to modify the alveolar ventilation, which assure the O₂ supply. Thus, arterial chemoreceptors (aortic bodies and CBs) serve an important role in the control of alveolar ventilation, but they also exert a powerful influence on cardiovascular function. Aortic bodies sense likewise the levels of arterial carbon dioxide partial pressure (Pa_{CO₂}) to regulate the depth and rhythm of breathing, but not changes in the blood H⁺ concentration ([H⁺]). To detect this last factor it is necessary to understand the role of the CB that detects all the previously described arterial variables, and, as its major quality, they do not desensitize. Finally, central chemoreceptors located on the ventrolateral surface of medulla oblongata detect changes in cerebrospinal fluid [H⁺] (**Figure 1**) [5–7, 9–11].

It is obvious then that the hypoxic response is a complex effect that must be studied at different levels, including the central areas where the respiratory rhythm and pattern is generated, as well as newly described functions of the CB, the integrative nature of central chemoreceptors and the interaction between peripheral and central chemoreception. Furthermore, it must be also taken into account the metabolic signalling influence of purinergic control, in general, and, in particular, the adenosinergic influence [1, 13, 17, 18, 23, 24].

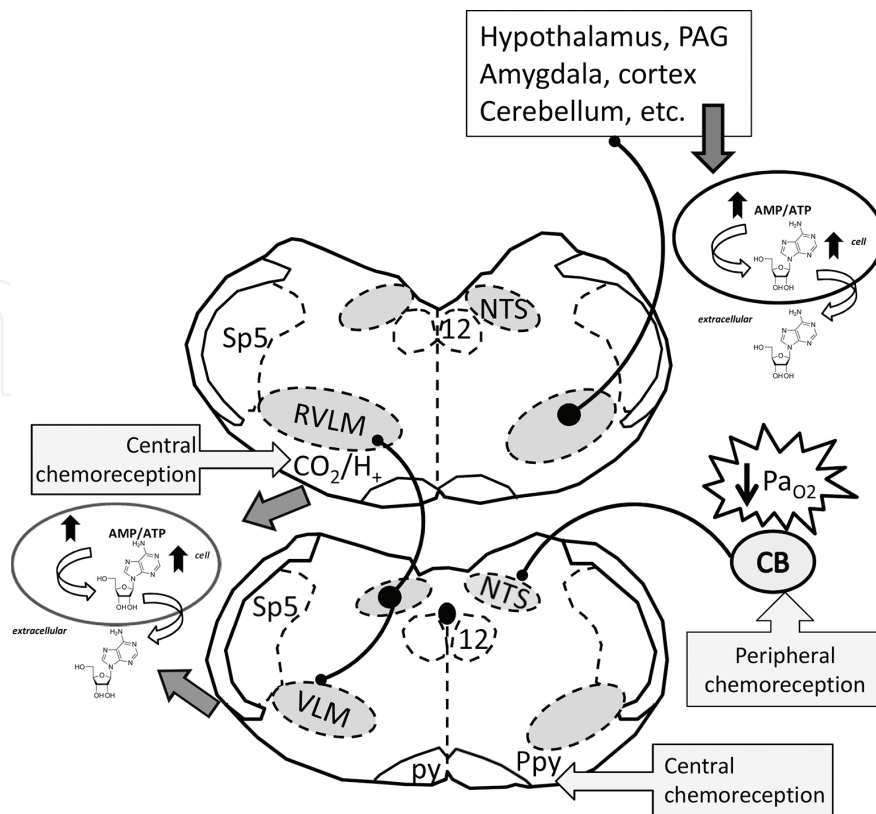


Figure 1. Schematic diagram of the hypoxic response generated at the ventrolateral medulla after the integration of peripheral and central chemoreceptors, including the role of metabolic signalling within the central neuronal network. Abbreviations: 12, hypoglossal nucleus; py, pyramidal tract; Sp5, spinal trigeminal nucleus.

2. Brain, hypoxia and pathophysiology

Normal breathing must be continuously adjusted to maintain homeostasis of arterial blood gases by means of feedback, feedforward and adaptive control strategies that depend of the brainstem respiratory network. How this process is centrally controlled is still under discussion, despite the advances (especially thanks to the development of *in vitro* preparations) that have been recently made [2, 3, 6, 7]. The precise mechanisms (cellular, synaptic and molecular) that underlie the generation and modulation of respiratory rhythm/pattern still remain largely unknown. This lack of fundamental knowledge in the field of neural control of respiration, and its relationship with other neurovegetative controls, is likely due to the complexity of the mammalian brain where synaptic connectivity between central cardiorespiratory neurons, motoneurons and their peripheral counterparts, to the present day, cannot be reliably mapped [2, 3, 7, 8].

Adaptive responses have evolved in different animal species to guarantee a sufficient supply of O_2 to tissues and to facilitate the survival of cells under transient or sustained conditions of limited O_2 availability. Although hypoxia is often related to a pathological condition, it is of great importance to recognize that variations in Pa_{O_2} can be part of normal situations that

require strenuous physiological responses, for example, during hypoventilation training or intense physical exercise [4, 5, 16, 21, 25–28]. It has to be also taken into account the condition of hypercapnia that results from an excess of Pa_{CO_2} , which results in acidification of blood and tissues. The respiratory central medullary rhythm/pattern generator must respond to these chemosensory cues to maintain O_2 and carbon dioxide (CO_2) homeostasis in the blood and tissues. To do so, sensorial cells located in the periphery and CNS monitors the Pa_{O_2} and Pa_{CO_2} and initiates respiratory and autonomic reflex adjustments during hypoxic and hypercapnic. Activation of either the hypoxic or hypercapnic chemoreflex elicits both hyperventilation and sympathetic activation [4, 5, 16, 25–28]. However, the hypoxic insult is a fundamental drive to increase respiratory rate.

Traditionally, physiological research has been focused on the effect of a chronic sustained hypoxia (CH), but relatively few works were directed to the effect of periods of intermittent hypoxia that is maintained chronically. However, the different protocols resemble several pathological states that occur when patients suffer discontinuous expositions to hypoxia by malfunction of the ventilatory system. Nevertheless, these chronic intermittent hypoxia (CIH) laboratory protocols vary greatly between researches in lifespan of hypoxic exposure periods, numbers of hypoxic episodes *per day* and the total number of days of exposure. In any case, and in spite of the lack of a uniform definition, most of the recent data suggest that animals exposed to CIH would present multiple long-term pathophysiological consequences that are similar to those observed in clinic and, for that, it would be a good animal model to study different respiratory pathologies [4, 5, 16, 21, 25–28].

2.1. The role of carotid body as chemoreceptor

O_2 sensing is necessary for the activation of cardiorespiratory reflexes that permit the survival of individuals under hypoxic environments, like high altitude or pathological conditions (with reduced capacity for gas exchange between the lung alveoli and the blood). Changes are detected by the arterial chemoreceptors, in particular CB, to facilitate rapid adaptations to hypoxia including hyperventilation and sympathetic activation. The CB is located at the carotid bifurcation although its precise location varies between mammalian species. The CB is composed of functional units named glomeruli, which are clusters of cells separated by a profuse network of small capillaries and connective tissue. Each glomerulus (in close contact with blood vessels and nerve fibres) contains neuron-like glomus (or type I) cells, which can be easily identified because they are strongly dopaminergic. Glomus cells are surrounded by processes of sustentacular (type II) cells that are positive for antibodies against glial fibrillary acidic protein and other glial markers. It has been shown that type II cells, or a subpopulation of them, are quiescent stem cells that are activated under hypoxia to proliferate and differentiate into glomus and other cell types [5, 9–11]. Glomus neuron-like cells contain O_2 -sensitive K^+ channels, which are inhibited by hypoxia acting through several mechanisms, including release of gaseous transmitters (NO , CO , H_2S), AMP-activated protein kinases and/or reactive oxygen species. Finally, it has been demonstrated that CBs are polymodal receptors that would respond not only to modifications in Pa_{O_2} , Pa_{CO_2} and H^+ , but also to stimuli as K^+ , several

neurotransmitters (i.e. norepinephrine), changes in temperature and osmolarity, as well as variations in the levels of glucose or insulin. Furthermore, reductions in CB blood flow (in addition to a decrease in Pa_{O_2}) also provide powerful CB stimulation and remodelling over time [5, 9–11].

The feedback from the CB is sent to the cardiorespiratory centres in the medulla oblongata via the afferent branches of the glossopharyngeal nerve. The afferent neurons to CB have their somas in the petrosal ganglion. This ganglion is anatomically distinct in several species of mammals like cat and rabbit, but in others (i.e. rat) it is part of a structure that includes the jugular and nodose ganglia. Their afferent fibres project to the commissural or medial subnuclei of the nucleus tractus solitarius (NTS) (**Figure 1**) that convey sensory information regarding cardiorespiratory homeostasis in the form of graded action potential frequencies in fibres of the carotid sinus branch of the ninth cranial (glossopharyngeal) nerve. The efferent innervation arises primarily from the sympathetic fibres originating from the superior cervical ganglion constituting the ganglio-glomerular nerve. Efferent innervation may best be considered as a modulating influence affecting the CB chemosensitivity largely, but not solely, via a modulation of CB blood flow [5, 7–11, 29, 30].

2.2. Central integrative chemoreception process

The brainstem is the central structure that operates the integrative process of the different chemoreceptors and baroreceptors inputs and which also generates the respiratory rhythm/pattern. From these structures it should be outlined that the NTS is composed of a series of clusters of neuronal cell bodies forming a vertical column of grey matter embedded in the dorsal medulla oblongata. The NTS projects to, among other regions, the reticular formation, parasympathetic preganglionic neurons, hypothalamus and thalamus, conforming circuits that contribute to autonomic regulation (**Figure 1**). Anatomical and physiological experiments have shown that the dorsomedial part of the NTS is the primary termination site of glossopharyngeal and vagal baroreceptors, integrating the baroreceptor afferents, while the midline area, caudal to the *calamus scriptorius*, has been identified as a primary central termination site for CB afferents. The NTS neurons are stimulated by hypoxia or hypercapnia, and most profoundly by a combination of both. Under normal or pathological conditions, CB information reaches the respiratory pattern generator neuronal network via NTS glutamatergic neurons, which also target the rostral ventrolateral medulla oblongata (RVLM) presympathetic neurons, thereby raising sympathetic nerve activity (**Figure 1**). For that, NTS second-order neurons could induce chemoreceptor reflex responses that include hyperpnoea, bradycardia and a sympathetically mediated vasoconstriction for a long-term acclimatization to hypoxia [5, 7, 9–11, 29, 30].

Other group of neurons to be highlighted is the RVLM, containing several functionally distinct types of neurons, which control and orchestrate cardiovascular and respiratory responses to hypoxia and hypercapnia (**Figure 1**) [3, 7, 8, 29, 30]. At this level, chemoreceptors regulate presympathetic neurons and cardiovagal preganglionic neurons indirectly via inputs from the neurons related to the respiratory pattern generator. Secondary effects of chemoreceptors on

the autonomic outflows result from changes in lung stretch afferent and baroreceptor activity [3, 7, 8, 29, 30].

On the other hand, central respiratory chemosensitivity is caused by direct effects of cerebrospinal $[H^+]$ on neurons and indirect effects of CO_2 via astrocytes. Central respiratory chemoreceptors are not definitively identified but several brainstem areas have been demonstrated to have a role as chemoreceptor. First, the retrotrapezoid nucleus (RTN), located at the rostral end of RVLM, is a particularly strong candidate (**Figure 1**). Indeed, the absence of RTN likely causes severe central apnoeas in congenital central hypoventilation syndrome. The RTN chemoreceptor neurons provide a CO_2/H^+ -dependent drive to breathe and serve as an integrator centre of convergence of chemosensory information from other central and peripheral sites, including the CBs. Finally, the RTN chemosensitive neurons also appear to serve as important sites of integration of several stimuli, as these neurons are significantly modulated by inputs from vagal-mediated pulmonary stretch receptors and from the hypothalamus [29, 30].

Another cluster of RVLM cells (constituted by a population of C1 catecholaminergic neurons) controls sympathetic vasomotor tone in resting and in hypoxic and hypercapnic conditions, including the peripheral chemoreflex [29, 30]. The increased sympathetic outflow elicited by peripheral chemoreceptors is mediated primarily by activation of the presympathetic neurons of the RVLM, the majority of which are C1 neurons. In fact, the cardiorespiratory effects of peripheral chemoreceptors are mediated in part by the direct glutamatergic inputs from the NTS to C1 neurons (**Figure 1**) [2, 3, 7, 8, 29, 30].

Recently, the description of the structures related to the respiratory rhythmogenesis has improved with the advent of the *in vitro* neonatal rodent brainstem preparation [31]. This recording technique has allowed for precise identification of specific medullary sites for separate but coupled rhythm generation or “oscillators”. These neurons reside in the pre-Bötzinger complex and in the parafacial respiratory group (pFRG) located in the RVLM [29, 30]. The most exciting result so far was the finding that some inspiratory neurons in RVLM act as inspiratory pacemakers; they continue to produce rhythmic bursts of potentials even when the synaptic connections are blocked [2]. Although the inspiratory pacemaker neurons do not constitute a well-defined group within the medulla, this group of neurons named pre-Bötzinger complex certainly play an important role in the generation and/or modulation of the breathing rhythm [2]. Of the several models proposed for generating respiratory rhythm, the most promising appears to be a hybrid model, which combines emergent properties of networks of synaptic connections and intrinsic membrane properties of individual neurons together with independent pacemaker-type neurons [1–3, 7, 8, 23, 29, 30].

Furthermore, several facts support that the pFRG/RTN complex is likely to be the major site of central CO_2 chemo-responsiveness. First, pFRG/RTN is characterized by glutamatergic interneurons that strongly express Phox2b (that codes for the homeodomain transcription factor expressed exclusively in the nervous system, in most neurons that control the viscera, like cardiovascular, digestive and respiratory systems). Besides, the Phox2b neurons are part of an uninterrupted chain of neurons in a circuit that includes the CBs and their afferents as well as the NTS projections to the RTN. The functional consequences of this linkage are that

stimulation of the peripheral chemoreceptors enhances the slope of the central CO₂ ventilatory response, and conversely, inhibition of the CBs reduces the slope of the central CO₂ response [1–3, 7, 8, 23, 29, 30].

Another interesting central chemosensitive area is the caudal parapyramidal (Ppy), located near the ventral surface of the medulla, at the level of the pyramidal decussation and may function as well as the pFRG/RTN complex (**Figure 1**). Furthermore, medullary neurons activated in response to hypercapnia were only found in the Ppy area. Nevertheless, neurons in both regions, RTN and PPy, could belong to the same cell population based on their histochemical and physiological properties and their location, near the medullary surface that facilitates the sensing of the arterial composition [1, 23].

In any case, the brainstem cardiorespiratory control areas are connected with other areas such as periaqueductal gray (PAG), hypothalamus, amygdala, cortex and cerebellum (**Figure 1**). These areas also exert influences over the respiratory rhythm/pattern generator. In this way, it has been found, from data obtained by clinical evidences in patients submitted to deep brain stimulation (by means of stimulating electrodes that recorded field potentials during neurosurgical procedures), that the PAG and the subthalamic nucleus have a key role in activating the central command of cardiorespiratory responses to stress. The PAG is an integrative structure that maintains a wide network of connectivity with different neural systems, such as prefrontal cortex, hypothalamus and nociceptive pathways. Moreover, the PAG efferent projections also addressed to the medullary cardiorespiratory control areas. Finally, anatomical evidences support the connectivity to amygdala and cortex from RVLM and neurons of the respiratory pattern generator that supports, among others effects, the vegetative correlate of emotions or learning (**Figure 1**) [3, 7, 8, 30, 31].

All of the above described structures are part of an extended neuronal network that participates in the regulation and integration of cardiovascular and respiratory functions. From all of the neurotransmitters shared by this complex neuronal network, the purinergic network is one of the choices to regulate the physiologic responses to hypoxia. Recent evidence suggests that ATP-mediated purinergic signalling at the level of the RVLM coordinates cardiorespiratory responses triggered by hypoxia and hypercapnia by activating RTN and C1 neurons, respectively. For all of that, the role of ATP-mediated signalling in the RVLM must be critical for cardiovascular and respiratory activities (**Figure 1**) [3, 7, 8, 29, 30].

2.3. Pathophysiological responses to hypoxia

Since the condition in which the whole body (or a region) was exposed to variations in arterial O₂ concentrations can be part of the normal physiology, a mild and non-damaging intermittent hypoxia (IH) is used intentionally, for example, during altitude training to develop an athletic performance adaptation at both the systemic and cellular level [4, 5, 16, 21, 26–28]. However, hypoxia is a deprivation of adequate O₂ supply at the tissue level and, often, a pathological condition with very serious consequences of preterm birth in the neonate, for example. The main cause for this pathology is that the lungs of the human foetus are among the last organs to develop during pregnancy. The perinatal hypoxic-ischemic cerebral injuries found in the clinic are a main problem of paediatrics because of its severe consequences for the posterior

development of the infants, such as the appearances of cerebral paralysis. Accumulating evidence points to an evolving process of brain injury after intrapartum hypoxia-ischemia, initiated *in utero* and extending into a recovery period. This process in the neonate originates numerous functional deficits, such as impaired resting ventilation and ventilatory response to hypoxia [17, 28, 31, 32].

On the other hand, abnormalities or mutations of the medullary neuronal breathing rhythm/pattern networks may also have a great impact on the progress of human diseases in children or adults. Failures in the breathing pattern with severe consequences are well-documented. These problems, often cause CO₂ retention in awake, and in particular, in sleeping subjects, that could be associated to neurodegenerative diseases such as Parkinson's disease, amyotrophic lateral sclerosis or post-polio syndrome. It is also been proved that these breathing alterations are often associated to medullary and multiple system atrophy of patients. These syndromes have been linked to deficits in neurons related with the respiratory control in the pre-Bötzinger complex, pontine raphe and adjacent areas. Obviously, an understanding of how the response to hypoxia is organized and when or why the system become maladapted and could induce cell damage is extremely important for knowing how to fight against the diseases in the future [4, 5, 16, 21, 26–28].

It is well known that disruption of the drive to breathe is thought to contribute to the mortality of certain pathologies, including stroke or epilepsy, and it is the cause of sudden infant death syndrome (SIDS) [1–6]. In the case of SIDS, it has generally been accepted that, in the absence of trauma, children death occurs to either respiratory or circulatory failure. The events appear to be a sequential process, first hypoxia occurs, and then there must be a failure to recover from hypoxia. The failure to recover could occur when the infant does not arouse from sleep and/or self-resuscitation mechanisms fail. For that, it has been proposed that there should be three necessary components for development of SIDS: congenital or acquired vulnerability, a critical “time-window” during maturational development and an acute stressor. The arousal response is essential for avoiding the hypoxic conditions due to certain microenvironments that could cause the loss of consciousness or the risk of dying. A failure of the neural system that would induce the arousal response from sleep, in the hypoxic condition, could be related to the progress or, in certain cases, the fatal result in diagnosed SIDS. However, these kinds of malfunctions do not explain all the process that should appear in SIDS. In fact, it seems clear that an initial respiratory failure and hypoxemia ignites the sequence of responses that, dramatically, may cause the death. Respiratory chemoreceptor studies on infants at risk for SIDS have suggested that a decreased sensitivity to CO₂ could play a causal role in these deaths [1–6, 33].

Concerning CB function, there is a significant increase in sensitivity of the peripheral chemoreceptors during the first few weeks of life and it has been frequently shown that CB denervation in animal models is followed by hypoventilation and sudden death later on. Therefore, these denervated animals for the most part are markedly symptomatic prior to death. The principal problem in translating these results to humans is that SIDS infants do not appear to have any symptoms before death. This fact implies that it could be a problem related to the central integration of CB information in SIDS, but its role is still under discussion. However,

CB must be taken into account in the pathogeny of SIDS, because a partial decrease in the sensitivity to hypercapnia or hypoxemia would be a causal role in this syndrome [2, 33].

Several studies indicate that changes in the strength and/or pattern of respiratory-sympathetic coupling may have pathological implications in the control of arterial pressure levels. Such dysfunctions can be observed in the experimental condition of CIH, and also is commonly observed in patients suffering from obstructive sleep apnoea (OSA). OSA consists of a repetitive obstruction of the upper airways during sleep. Each obstruction causes an episode of hypoxia leading to a picture of CIH causing a fall in the Pa_{O_2} and arterial haemoglobin saturation. OSA is characterized by repetitive collapse or near collapse of the upper airway during sleep, and these repetitive events impose substantial adverse effects on multiple organ systems. As a result of these mechanical changes in the airway, hypoxemia and hypercapnia develop, which further stimulate respiratory effort. Without airway opening the increased drive is ineffective at increasing ventilation. Hypoxic episodes stimulate the CB, triggering an increased motor muscles towards the inspiratory output and an arousal reaction, which together solve the obstruction [1–6]. Following even very brief periods of IH interspersed with normoxia, hyperventilation and increased sympathetic activity are sustained over an hour or more (i.e. the so-called long-term facilitation). Central adaptive responses occur following CIH in the persistent elevation of tonic hyperactivity of neurons at the level of the hypothalamus and other structures [4, 16, 21, 26–28]. As OSA progresses, it frequently generates a syndrome with associated pathologies at different systems: cardiovascular (hypertension and augmented acute vascular accidents), hepato-metabolic (insulin resistance, glucose intolerance, fatty liver disease) and neuropsychiatric (anxiety, depression and cognitive-executive deficits). Clinical and experimental studies indicate that CIH is an important event in the occurrence of OSA-associated pathologies because it causes CB sensitization [1–6]. The process probably includes increasing CB chemoreceptor input to the brainstem leading to an exaggerated sympathetic tone, which generates hypertension and subsequent cardiovascular and metabolic pathologies. In OSA patients, the repetitive respiratory events lead to IH and CO_2 retention, both of which can augment sympathetic nerve activity via stimulation of central and peripheral chemoreceptors. Conditions of hypoxia, both chronic and intermittent lack of O_2 , seem to induce CNS plasticity of respiratory and sympathetic functions neuronal networks and metabolic changes that could also lead to pathological states [4, 5, 27, 28].

3. Purinergic neuronal networks and hypoxia

ATP is released in an activity-dependent manner from different cell types in the brain, fulfilling different roles as a neurotransmitter, neuromodulator, in astrocyte-to-neuron communication, propagating astrocytic responses and modulating microglia responses. So, purinergic signaling has been found to contribute at all levels of the nervous system, including enteric, autonomic and central [34–38]. The term purinergic receptor was classically introduced to name specific classes of membrane receptors that mediate the release of ATP (P2 receptors) or adenosine (P1 receptors). The group of adenosine P1 receptors (A1-R, A2a-R, A2b-R, A3-R) are

expressed on presynaptic and postsynaptic neurons, on astrocytes, microglia and mature and precursor oligodendrocytes. The mechanisms of ATP signalling are equally diverse, acting by means of P2 receptors, including ionotropic (P2X-R) and metabotropic (P2Y-R) subtypes, as well as varying methods of transmission, including vesicular, volume-regulated anion channel and gap junction hemichannel release of ATP from neuronal and non-neuronal cells [34–38].

ATP is involved in central respiratory control and may mediate changes in the activity of medullary respiratory neurons during hypercapnia. The P2 receptor family comprises seven ionotropic P2X-R subunits (P2X1-7), forming both homomeric or heteromeric receptors and eight metabotropic P2Y-R subtypes (P2Y1, 2, 4, 6, 11, 12, 13, 14). The brain displays a robust mRNA expression, an intense binding, and immunoreactivity for both P2X-R and P2Y-R in neuronal and non-neuronal elements, although the role of central P2-R remains ill defined. The ATP-mediated signalling in respiratory control and central chemoreception is associated to the profile of the P2X2-R subunit. This subunit is expressed, by physiologically identified respiratory neurons, in areas of the ventral medulla, the pontine locus coeruleus, the NTS, and the raphe nuclei. There are several evidences that sustain the hypothesis that purinergic signalling could play a central role in the mechanisms underlying the chemosensitivity of RVLM (**Figure 1**). It has been demonstrated the responses evoked by ATP in neurons expressing P2X-Rs to changes in extracellular $[H^+]$. In that way, this evidence supports the putative mechanism of chemosensitivity of RVLM cells, and it would be necessary the tonic release of ATP. This may be the case, when P2-R blockade reduces the baseline firing of RVLM respiratory neurones. The modulation of P2X2-R function, evoked by acidification of the extracellular environment during hypercapnia, contributes to the changes in activity of the RVLM respiratory neurones that express these receptors [34–38].

Furthermore, it has been shown that several medullary areas may have chemosensitive responses mediated by ATP. In this way, experiments made in brain slices using cell-attached recordings of membrane potentials have shown that CO_2/H^+ -receptive NTS neurons are activated by focal ATP applications. However, it has been evidenced that purinergic P2-R blockade did not affect their CO_2/H^+ responsiveness [38]. On the other hand, CO_2/H^+ -sensitive raphe neurons were unaffected by ATP or P2-R blockade [34, 38]. When the experiments were realized *in vivo*, ATP injection into the NTS increased cardiorespiratory activity; however, injections of a P2-R antagonist into this area did not change the baseline breathing or the CO_2/H^+ responsiveness [34, 38]. Indeed, a significant proportion of respiratory neurones located in the vicinity of the Böttinger and pre-Böttinger areas express the P2X2-R subunit and respond with an increase in discharge during ATP application. This fact could mean that purinergic signalling plays an additional role in the generation and shaping of central respiratory output, as well as premotoneurons that are responsible for transmitting this rhythm to the spinal motoneurons controlling the diaphragm and intercostal muscles [34–38].

Finally, as above stated, RVLM contributes to peripheral chemoreceptor modulation of breathing and blood pressure, by chemosensitive RTN neurons and presympathetic C1 neurons, respectively, and these neurons are activated by purinergic agonists. In contrast, the blockade of P2-Rs in the RVLM blunted cardiorespiratory responses to peripheral chemoreceptor activation in anesthetized rats [34–38]. RTN neuronal activity was found to be

independent of temperature and stimulus strength and was wholly retained when synaptic activity was blocked using high-Mg⁺⁺, low-Ca⁺⁺ solution. In the RTN, mechanisms of chemoreception involved direct H⁺-mediated activation of chemosensitive neurons and indirect modulation by purinergic signalling. This modulation implies a CO₂/H⁺-evoked ATP release by RTN astrocytes, contributing to respiratory drive. ATP injection into the RTN increased breathing and blood pressure by a P2-R dependent mechanism, at the cellular and systems level [38]. However, because the results using antagonists of P2-R and focal injections did not elucidate the cells that were responsible, it is necessary more experimental evidence to determine the putative chemoreceptors and, if it is the case, the above observed effects could be indirect ones. Nevertheless, purinergic signalling also modulates the activity of CO₂/H⁺-sensitive neurons at least in two other brainstem regions thought to contribute to central chemoreception (i.e. the caudal NTS and medullary raphe). In any case, these evidences suggest that purinergic signalling is a unique feature of RTN chemoreception and point out to a unique CO₂/H⁺ sensing mechanism in the RTN [34–38].

4. Adenosine receptors, brain development and pathophysiological hypoxic response

The role of adenosine, as an extracellular signalling molecule, was defined after the observations of the ability of purines to control the functioning of the heart. Adenosine modulates the activity of the nervous system at cellular level both presynaptically by inhibiting or facilitating transmitter release, and postsynaptically by hyperpolarising or depolarising neurons, as well as exerting non-synaptic effects (i.e. on glial cells). It is usually assumed that adenosinergic signalling provides a neuroprotective role. However, several researches have shown that, under determined circumstances, changes in the levels of adenosine could have the opposite effects, contributing to neuronal damage and cell death [6, 12–15, 19–22]. These two ways of actions could be determined by the union of adenosine to different subtypes of A-Rs. Furthermore, changes in the levels of expression of the different subtypes, interactions between these receptors, differential actions on neuronal and glial cells and several “time-windows” (that are critical during development) could also provide different actions at different events, as well as adenosinergic agonist and antagonist compounds administration. Moreover, adenosine do not work isolated, and, in spite of this, it is still unclear if the role of A-R subtypes (A1-R and A2-R) in the control of neuroprotection is mostly due to the control of glutamatergic transmission. Another possible role of adenosine is that its protection is mediated by one of the homeostatic roles of its receptors, such as control of metabolism, neuroglial communication, inflammatory response, neurogenesis or mechanism of action of growth factors [6, 12–15, 19–22].

Adenosine acts in parallel as a neuromodulator and as a homeostatic modulator in the CNS [6, 12–22]. The adenosine role as a neuromodulator is especially important around the time of birth and is involved in the suppression of foetal and neonatal breathing, particularly during hypoxia when extracellular levels of this nucleoside rapidly increase. Apnoea of prematurity, defined as cessation of breathing lasting longer than 15 s and accompanied by bradycardia or

hypoxia, is common occurring in 85% of infants born less than 34-week gestation. Preterm birth constitutes approximately 6–12% of all births in industrialized countries and accounts for 70% of neonatal mortality and 75% of neonatal morbidity [6, 12–21]. Depending on gestational age and birth weight, preterm infants present a wide range of abnormal physiological responses due to their immature organ systems [17, 28, 31, 32]. During development A1-Rs are especially important, being the earliest receptors expressed in the embryonic brain and heart. A1-R activation potently inhibits the development of axons and can lead to leukomalacia [18, 32].

The most common method of treatment of the apnoeas of prematurity is continuous positive airway pressure and administration of a methylxanthine. The family of methylxanthines includes caffeine (1, 3, 7-trimethylxanthine), one of the most popular human stimulants, and all of them derivate from xanthine, that is a purine present in human and other organism's tissues and fluids. This group of alkaloids has therapeutically been used for their effects stimulating respiratory function by means of its excitatory effects on the CNS, because of its capacity to suppress respiratory depression, reduce periodic breathing and enhance diaphragmatic activity. Caffeine also increases ventilatory drive and improves sensitivity and/or responsiveness to changes in the level of Pa_{O₂} [6, 12–22]. The

discovery that methylxanthines acted as antagonists of adenosine receptors represented a crucial step to establish the idea that adenosine indeed acted as an extracellular signalling molecule operating on selective receptors. Caffeine, at high doses, can also inhibit phosphodiesterases, block GABA_A receptors or cause a release of intracellular Ca⁺⁺. Furthermore, caffeine acts on the respiratory cycle by antagonizing the actions of endogenous A1-R, A2a-R or A2b-R [6, 12–22]. Studies on A1-R have demonstrated that these receptors are found at high density in the brainstem and hypothalamus while A2a-Rs are widely distributed in the medulla [14, 17, 18]. Animal studies have shown that caffeine treatment alters A-R expression and distribution, cause transient motor impairments and could also be neurotoxic to the newborn. In rats, limited exposure to therapeutic doses of caffeine during early life (postnatal days 3–6, P3–P6) changes the distribution, density and sensitivity of A1-Rs in several regions of the CNS; these changes could persist until adulthood. Caffeine treatment at P2–P6 mimics the clinical use of caffeine in human neonates. Since the relative level of maturation of the CNS in newborn rats in the first week of life is similar to that of a premature newborn human between 20 and 40 weeks postconception, newborn rats could serve as a suitable animal model to test the potential impact of perinatal caffeine treatment on the adenosinergic system. The oral administration of caffeine in critical periods of newborn rat and immunohistological experiments showed an increase of A1-R labelling in restricted cardiorespiratory related areas. These labelled structures were the anterior hypothalamic area, ventromedial hypothalamic nucleus, parabrachial complex and ventrolateral medulla of the caffeine-treated group at P6. For the subtype A2a-R, it was found a moderate increase of immunolabelling in pontomedullary and other hypothalamic areas also related to vegetative functions. Indeed, increased A1-R and A2a-R gene expression was observed in both the brainstem and hypothalamus at P5. These results showed an up-regulation of adenosinergic maturation

in central cardiorespiratory areas when the animals were caffeine treated in the neonatal period and could explain the pharmacological effects observed in caffeine treated premature infants, and it would also imply that caffeine mediated a modification of the post-natal development of the adenosinergic system during a critical period or “time-window” [6, 12–22]. To date, human data show that such caffeine treatment has no major side effects on neurodevelopmental outcome in children in the 38–42 weeks following birth and up to 2 years after the treatment. However, further research is required to determine the long-term pathologic and functional effects of caffeine and the combination of caffeine and other substances on the developing immature brain [6, 12–22].

Anyway, adenosine, is not only crucial in development, it also mediates multifactorial forms of ventilatory responses. The reduced hypoxic ventilatory response could be attributed to depressed adenosinergic peripheral excitatory mechanisms and to enhanced adenosinergic central depression mechanisms, both of which contribute to the blunted ventilatory response in different metabolic states (**Figure 1**). Several important groups of clinical studies, in which the adenosinergic network role has been demonstrated, are related to OSA, asthma and interstitial lung disease such as idiopathic pulmonary fibrosis (IPF) [1–6]. Levels of adenosine receptors are altered in the lungs of asthmatics and OSA patients and a recent study has shown that the A2b-R is increased in remodelled airway epithelial cells of rapidly progressing IPF patients [6, 12–22]. Furthermore, CIH (as an experimental OSA model) elicits phrenic long-term facilitation by an adenosine-dependent mechanism [2, 6, 12–22]. All of the above are interesting evidences about the mechanisms that support and induce inflammatory and tissue remodelling processes in these pathological states; however, it is necessary to do more research on the pathways that provoke their progressive and chronic evolution. For example, there are already implemented several models of deregulated or overactive wound healing pathways to explain how these processes contribute to an excessive remodelling response such as seen in chronic lung disease [2, 6, 12–22]. Consistent with this, adenosine levels are elevated in the lungs of patients with chronic lung disease, where it is hypothesized that adenosine regulates the balance between tissue repair and excessive airway remodelling. Furthermore, it has been demonstrated that exogenous adenosine treatment can elicit acute bronchoconstriction in patients with asthma or OSA [1–6]. In contrast, the administration of adenosine to healthy subject did not affect them, suggesting a fundamental difference with respect to adenosinergic signalling in the treated patients. The differential response could be mediated by the activation of A-Rs that would modify the activity of different cell types that play a central role in chronic lung disease. These groups of possible targets include mast cells, eosinophils, macrophages, airway epithelial cells, pulmonary fibroblasts and airway smooth muscle cells. Indeed, recent studies directly demonstrate that adenosine is involved in the regulation of pulmonary fibrosis. Lastly, there are correlations between the degree of inflammation and damage and adenosine accumulations in adenosine deaminase-deficient individuals. Furthermore, purinergic metabolism and signalling components are altered in a manner that promotes adenosine production in tissue samples from patients with OSA and IPF. These modifications were related to the very important changes found in the expression of the promoter molecules of inflammatory process that could be induced by A2b-R signalling. Finally, it was interesting to point out that it has been demonstrated that activation of A2b-Rs can

influence the production of inflammatory and fibrotic mediators from macrophages isolated from these patients [6, 12–22].

All of the above findings suggest that adenosine-based therapeutics may be beneficial in the treatment of chronic lung diseases such as OSA and IPF. On the other hand, it is known that inflammation-induced release of prostaglandin E₂ changes breathing patterns and the response to CO₂ levels. This bioactive eicosanoid regulates many biologically important processes as a potent activator of several signalling pathways, through four distinct G-protein-coupled receptors. All of this alters neural network activity in the pre-Bötzinger rhythm-generating complex and in the chemosensitive brainstem respiratory regions, thereby increasing sigh frequency and the depth of inspiration with implications for inspiration and sighs throughout life, and the ability to autoresuscitate when breathing fails [2, 6, 7, 12–15, 19–22, 29, 30].

5. Conclusion

Identification of the neurophysiological mechanisms underlying the response of organisms to environmental factors, in particular, to injurious exposures like hypoxia, represents one of the most important research problems in biomedicine. Neural plasticity, as a persistent change in the morphology and/or function based on prior experiences, is crucial for understanding the effects of O₂ supply changes over neuronal networks. Plasticity is well evident when the triggering experience occurs early in life; but in the case of respiratory control plasticity, could also be present in adult life. The regulation of adenosinergic neural network maturation, especially in central cardiorespiratory areas, could provide new perspectives in respiratory newborn distress symptoms. Adenosine acts as an extracellular signalling molecule operating on selective receptors. Regulation of adenosinergic maturation in central cardiorespiratory areas in caffeine-treated neonatal mammals could explain the pharmacological effects of caffeine observed in premature infants. Anyhow, the neuroplasticity observed in the cardiorespiratory network is fundamental to maintain life in many adverse conditions.

The central and peripheral chemical drive to breathe is associated with several widespread autonomic disorders. Deficits in central chemical drive are associated with central sleep apnoea, a debilitating disease with few therapies besides constant positive airway pressure. In addition, disruption of the drive to breathe is thought to contribute to mortality of certain pathologies, including SIDS, stroke and epilepsy. Finally, in OSA, certain forms of hypertension and heart failure, it has been observed sensitization of peripheral chemoreceptor drive, particularly the sympathetic component and this over-activity is thought to contribute to the pathology.

Purinergic signalling has been proposed to be an excellent system to target for therapies of numerous pathologies, mainly due to novel pharmacological agents being developed. As more detailed understanding of the purinergic mechanisms involved in the chemical drive to breathe are uncovered, these would allow to possible pharmacological treatments of the aforementioned pathologies with the newly developed purinergic agents.

Author details

Susana P. Gaytán* and Rosario Pasaro

*Address all correspondence to: sgaytan@us.es

Department of Physiology, University of Seville, Sevilla, Spain

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