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Signalling mechanisms of long term facilitation of breathing with intermittent hypoxia

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Abstract

Intermittent hypoxia causes long-term facilitation (LTF) of respiratory motor nerve activity and ventilation, which manifests as a persistent increase over the normoxic baseline for an hour or more after the acute hypoxic ventilatory response. LTF is likely involved in sleep apnea, but its exact role is uncertain. Previously, LTF was defined as a serotonergic mechanism, but new evidence shows that multiple signaling pathways can elicit LTF. This raises new questions about the interactions between signaling pathways in different time domains of the hypoxic ventilatory response, which can no longer be defined simply in terms of neurochemical mechanisms.

Introduction

During periods of systemic hypoxia (e.g. from lung disease or high altitude), the body's first line of defence is the hypoxic ventilatory response, a reflex increase in ventilation mediated by arterial chemoreceptors, primarily in the carotid bodies [1]. The hypoxic ventilatory response is a complex interplay between several distinct mechanisms whose net effect varies depending on the pattern and intensity of hypoxic exposure. Depending on the pattern of hypoxic stimulation, the hypoxic ventilatory response may change as a result of short-term effects that temporarily alter synaptic activity (e.g. increased neurotransmitter release) or long-term effects that alter the strength of chemical synapses of ventilatory control circuits (e.g. receptor modification or new protein synthesis). These changes result in either facilitation or depression of ventilation that lasts from seconds to years [2]. Since such mechanisms alter future ventilatory responses, they are examples of neuroplasticity in the ventilatory control system [3]. For example, a train of brief episodes of intermittent hypoxia results in LTF of ventilation, which manifests primarily as an increase in tidal volume that lasts for up to 90 minutes after the final stimulus [2,4,5]. Alternatively, chronic sustained hypoxia results in ventilatory acclimatization to hypoxia, which is an increase in ventilation (mainly breathing frequency) that lasts for days to weeks following removal of the hypoxic stimulus [2]. Different time domains of the hypoxic ventilatory response may be involved in different diseases with hypoxemia, e.g. LTF in sleep apnea with intermittent hypoxia and ventilatory acclimatization to hypoxia in chronic obstructive pulmonary disease with chronic hypoxemia.

In 1998 [2], the different time domains of the hypoxic ventilatory response were defined and distinguished on the basis of the following: (1) the pattern and intensity of hypoxic exposure; (2) the time course of the response (seconds to years); (3) the effects of this stimuli on the various physiological components of the hypoxic ventilatory response (e.g. breathing frequency and tidal volume); (4) whether these effects result in an increase or decrease in ventilation; and (5) the neurochemicals necessary for the manifestation of these responses [2]. Recently, considerable progress has been made in the study of LTF in particular, and it has become clear that multiple signaling pathways can cause the same change in ventilation. It can be expected that specific mechanisms will be activated

and extinguished at different times depending on species, experimental preparations and individuals. Thus, defining a given time domain of a ventilatory response in terms of a neurochemical or signaling pathway can be ambiguous when trying to compare results between different studies. To resolve this dilemma, we now propose to define the different time domains of the hypoxic ventilatory response as physiological responses to a given hypoxic stimulus, which may have multiple underlying molecular and cellular mechanisms. A corollary is that a specific mechanism should not be assumed for each different time domain of the hypoxic ventilatory response, and it is critical to specify a given mechanism if it is important for designing an experiment or interpreting results about the hypoxic ventilatory response. Here, we highlight recent work on the study of LTF to illustrate how multiple signaling pathways can induce the same physiological hypoxic ventilatory response.

LTF – historically a serotonin-dependent pathway

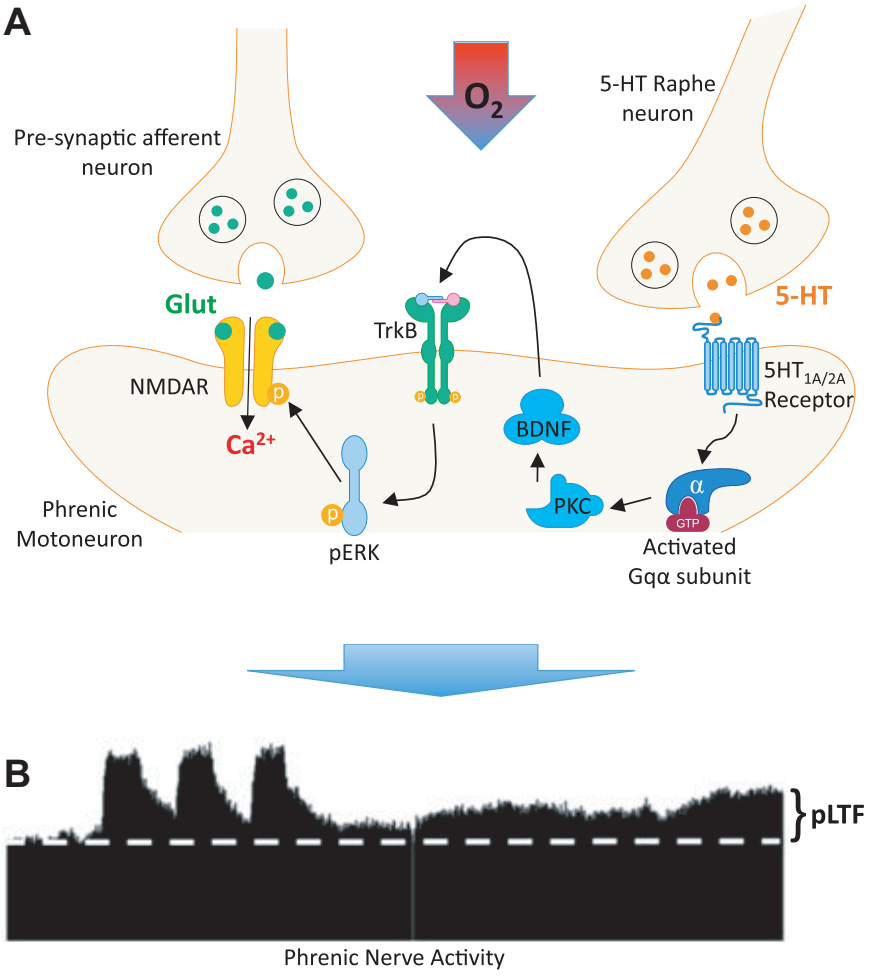
LTF has been observed in a wide variety of animals, both as increased ventilation (ventilatory LTF) or enhanced phrenic nerve activity (phrenic LTF) in awake or anesthetized animals, respectively [2,6-9]. Ventilatory LTF is more difficult to study experimentally and appears to depend on sleep-wakefulness state, species, and the hypoxic induction protocol; this topic has been expertly reviewed recently [10,11]. Recent studies show that ventilatory LTF may be the sum of plasticity in genioglossal, hypoglossal, and intercostal motor responses, in addition to phrenic responses [2,10,12,13]. Most of the experimental work defining neurochemical mechanisms of LTF has been done in anesthetized animal preparations and focuses on phrenic LTF. Probably the first description of LTF in the literature was the report of serotonin-dependent "afterdischarge" in phrenic activity in anesthetized cats in response to repeated bouts of carotid sinus nerve stimulation [14,15]. The hypoxic stimulus for LTF must be intermittent as it is not induced by continuous hypoxia of the same duration as the sum of the intermittent episodes [16].

Until recently, serotonin type 2 receptor ($5\text{-}HT_2R$) activation during, but not after, intermittent hypoxia was thought to be the primary signaling mechanism for ventilatory LTF and phrenic LTF [17-19]. Experimental evidence for this includes the observations that phrenic LTF induced by intermittent hypoxia or carotid sinus nerve stimulation is prevented by $5\text{-}HT_2R$ blockade with the general 5-HTR antagonist methysergide [20], or ketanserin, a specific $5\text{-}HT_2R$ antagonist [21-23]. The working model for the $5\text{-}HT_2$ mechanism of LTF has been as follows (Fig. 1). First, episodic hypoxia activates serotonergic Raphe neurons in the medulla, which results in the release of the neuromodulator 5-HT near phrenic motor neurons; such 5-HT release has been measured when the carotid sinus nerve is electrically stimulated [24], and there is strong evidence for it occurring with hypoxia as well [25-29]. 5-HT then activates a variety of downstream signals that activate protein kinases to initiate new protein synthesis and enhance glutamatergic neurotransmission [22,30,31]. Presumably, this involves inserting glutamate receptors into the post-synaptic membrane and/or phosphorylating them to enhance sensitivity to pre-synaptic inputs, as described for other glutamatergic systems [32-34]. However, it has not been conclusively demonstrated that the mechanisms of LTF are explicit to synapses on respiratory motor neurons, and potential roles for changes in cellular excitability, interneurons, or glia have not been ruled out. Episodic activation of $5\text{-}HT_2Rs$ leads to synthesis of brain-derived neurotrophic factor (BDNF) in the spinal cord near the phrenic motorneurons. Evidence supporting this includes observations that a single intrathecal BDNF administration induces LTF without a hypoxic stimulus, and that blocking BDNF translation and protein synthesis with RNAi approaches abolishes hypoxia-induced LTF [30]. BDNF subsequently activates high-affinity receptor tyrosine kinases (TrkB), which in turn activate extracellular signal-regulated kinases 1 and 2 (ERK1/2) [30,35,36]. ERK1/2 regulate glutamatergic receptor phosphorylation and/or density at the postsynaptic membrane in other systems [37] and presumably this results in phrenic LTF [22,23].

It is interesting to note that although intermittent, but not chronic, hypoxic exposures are required to induce LTF, a single bolus injection of BDNF is sufficient to activate LTF. This raises interesting questions about the activation of LTF and how the ventilatory control system differentiates between patterns of hypoxic exposure. Presumably, the increase in BDNF with the first bout of intermittent hypoxia or the start of sustained hypoxia is not sufficient to induce LTF. However, it is not known how multiple short bouts of hypoxia increase BDNF differently to cause LTF.

Chronic intermittent hypoxia, studied by exposing animals to several hours of intermittent hypoxia per day for between 4 days to 5 weeks, increases phrenic LTF [23,38]. Increased phrenic LTF with chronic intermittent hypoxia involves both elevated carotid body chemoreceptor responses to a given hypoxic stimulus (sensory LTF [39]) and increased central nervous system (CNS) gain of the hypoxic ventilatory response, which is demonstrated by a potentiated phrenic nerve response to electrical stimulation of the carotid sinus nerve [23]. This effect has been reported in animals treated with intermittent hypoxia using hypoxic bouts between 15 seconds (plus 68-85 seconds of graded

Figure 1. Classic model of signaling for phrenic long-term facilitation



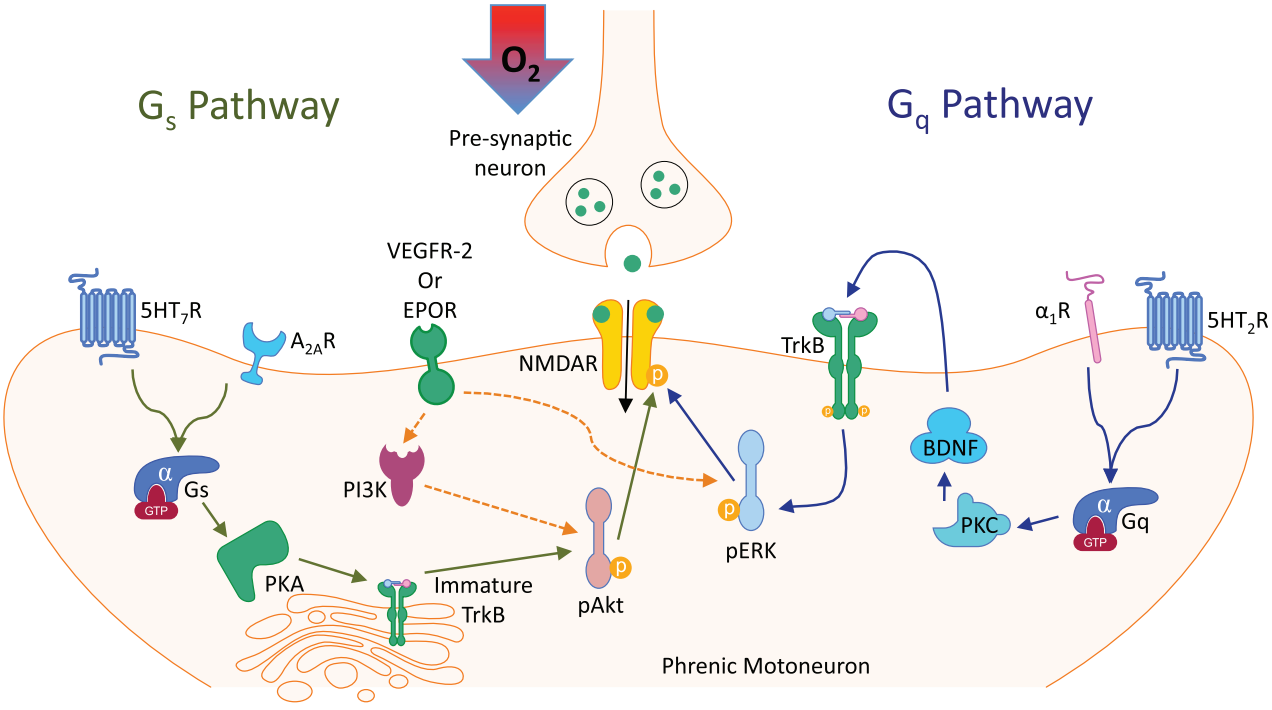
Intermittent hypoxia increases ventilatory drive during acute hypoxia and normoxic (baseline) ventilation remains elevated for over an hour after intermittent hypoxia. **(A)** Carotid body stimulation by IH releases serotonin (5-HT) from neuromodulatory Raphe neurons, which binds to 5-HT type 1A and 2A receptors on phrenic motorneurons. 5-HT activates G_q protein signaling cascades to activate protein kinase C (PKC) and induce the synthesis of brain-derived neurotrophic factor (BDNF). BDNF binds to tyrosine kinase receptors (TrkB) that activate phospho-extracellular signal regulated kinase (pERK). In other systems, pERK has been shown to phosphorylate glutamatergic N-methyl-D-aspartate receptors (NMDARs) in post-synaptic neurons and increase sensitivity to pre-synaptic glutamate release. **(B)** Physiologically, this increased sensitivity manifests as enhanced phrenic nerve activity and increased ventilation (primarily increased tidal volume). Figure 1 is modified from [44,53].

hypoxia during the change from normoxia to the target level of hypoxia) and 5 minutes long for several days, but it does not occur in response to chronic sustained hypoxia [10,39]. Chronic intermittent hypoxia induces new synthesis of the proteins that mediate the LTF pathway [40] and increases phrenic LTF [23,39]. Interestingly, phrenic LTF after chronic intermittent hypoxia still depends on 5-HT_2Rs, but the increment in phrenic LTF with chronic versus acute intermittent hypoxia involves central (versus carotid body) effects of a different subtype of 5-HTR, which is sensitive to methysergide [23]. This finding provided early evidence that LTF could be regulated by multiple mechanisms (see below).

LTF without serotonin

More recent studies challenged the idea that serotonergic inputs are necessary to induce LTF. For example, activation of α1-adrenergic receptors can induce phrenic LTF independently of 5-HT receptors via a pathway that is mediated by protein kinase B (Akt) instead of ERKs [41,42]. Interestingly, both α1-adrenergic receptors and 5-HT receptors are coupled to G_q-proteins, a class of g-protein that is linked to the activity of phospholipase C [43], suggesting that these two types of receptor may converge on a common pathway and serve as common activators of LTF. It has been proposed that these two mechanisms form a pathway termed the "Q" Pathway (Fig. 2) [44].

Figure 2. New model for phrenic long-term facilitation with multiple signaling pathways



The **G_q pathway** (blue arrows) proceeds as described in Fig. 1A but can also be activated by α1-adrenergic receptors (α_1R) and less severe hypoxia than the G_s pathway [47]. The **G_s pathway** (green arrows) can be induced by the activation of adenosine type 2A receptors (A_{2A}R) or serotonin type 7 receptors (5-HT$_7$R), which are coupled to G_s proteins. G_s signaling activates protein kinase A (PKA), which stimulates immature TrkB to modulate phospho-protein kinase B (pAkt). In other systems, this phosphorylates glutamatergic N-methyl-D-aspartate receptors (NMDARs) and increases sensitivity to pre-synaptic glutamate release. Recently, additional pathways (dashed arrows) have been described wherein vascular endothelial growth factor receptor-2 (VEGFR-2) or erythropoietin receptor (EPOR) induce LTF via phosphoinositide 3-kinase (PI3K) and pAkt, and perhaps pERK. Potential effects of reactive oxygen species on G_s and G_q pathway interactions are not shown. Figure 2 is modified from [44].

Recently, another signaling pathway capable of inducing phrenic LTF has been reported, and it is mediated by spinal cord adenosine type 2A receptors (A_{2A}R) [45-47] and 5-HT$_7$Rs [45-48]. A_{2A}Rs signal through adenylate cyclase-coupled G_s proteins, and, accordingly, this pathway has been termed the "S" pathway (Fig. 2) [44]. Support for the idea that G_s signaling has a more general role in LTF comes from the observation that 5-HT$_7$Rs, which also utilize G_s, can induce long-lasting phrenic motor facilitation [48]. It is possible that 5-HT$_7$Rs play a role in the enhanced phrenic LTF observed with chronic intermittent hypoxia (see above). Interestingly, the S Pathway involves activation of immature TrkB independently of BDNF synthesis and this pathway proceeds through the activation of PI3K/Akt, but does not involve ERKs [45]. The S and Q pathways are simultaneously initiated by intermittent hypoxia but they tend to limit each other, since blocking only one pathway increases phrenic LTF [44,46]. This interaction is typical of G_s and G_q proteins, which interfere with each other via a well-described cross-talk mechanism in other systems [49]. This cross-talk may involve reactive oxygen species [46], which are involved in phrenic LTF and ventilatory LTF [50-54] (the effects of reactive oxygen species on LTF are beyond the scope of this brief review). The physiological significance of the dual G protein mechanisms for the hypoxic ventilatory response may relate to the recent discovery that different levels of hypoxia induce different pathways for phrenic LTF, such that more severe hypoxic episodes (25-30 mmHg Pa_{O2}) preferably induce the S Pathway, whereas during moderate hypoxia (45-55 mmHg Pa_{O2}), the Q pathway is favoured [47].

In addition to these pathways, signaling mechanisms mediated by vascular endothelial growth factor [55], and erythropoietin [56], have also recently been described, and both pathways interact with ERK and Akt signaling [44,55-58]. It remains to be seen how these mechanisms utilize components of the Q and S pathways, or if they represent entirely new signaling mechanisms that mediate phrenic LTF.

Beyond LTF, the Q and S pathways are important modulators of a variety of respiratory and non-respiratory circuits that mediate both sensory and motor systems. For example, the G_s pathway has been implicated in several related processes: the regulation of heart rate by sympathetic and vagal nerve β–adrenergic inputs to cardiac pacemaker cells [59]; the control of respiratory depression during rapid eye movement (REM) sleep via a mechanism involving adenylyl cyclase [60]; and the sensitivity of inhibitory glycine receptors, which play important roles in motor control, pain, and ventilation [61,62]. Similarly, the Q pathway has been implicated in central pattern generation of respiratory control in the brainstem of juvenile rats [63], while interactions between the Q pathway and other G protein receptors mediate Purkinje cell signaling in the coordination of motor control [64]. In general, the activity of the various G proteins and their interactions occur via highly conserved signaling pathways, so research in other areas will likely prove valuable for understanding of the roles of these pathways in LTF, and *vice versa*.

Age, gender, and strain in the manifestation of LTF

An important caveat to this research is that the majority of studies have been undertaken in young male Sprague-Dawley rats. Sex hormones regulate plasticity in the CNS, including those for ventilatory responses to intermittent hypoxia [65,66]. Research has demonstrated that the magnitude of phrenic LTF is markedly reduced in old versus young male Sprague-Dawley rats (13 vs. 3-4 months), and LTF of the hypoglossal nerve is abolished in the older population [67]. This effect has been linked to the expression of sex hormones and LTF of the phrenic and hypoglossal nerves is similarly abrogated in gonadectomised or aged male Fischer 344 rats relative to young intact animals; the decrease in hypoglossal LTF correlates with decreased expression of the sex hormones testosterone, progesterone, and oestradiol [68], and testosterone supplementation reverses the effects of gonadectomy [69], or aging [70]. Furthermore, the expression of LTF has also been shown to vary between different strains of rats, such that acute intermittent hypoxia-induced changes in 5-HT signaling, phrenic LTF, and LTF of the hypoglossal nerve are not observed in Brown Norway rats, and are more pronounced in Lewis rats than in Fischer 344 rats [71,72]. This indicates that genetic and epigenetic differences may also contribute to the extent to which LTF is induced by intermittent hypoxia.

Conclusions

New experiments demonstrate multiple pathways for the physiological expression of LTF of respiratory motor activity following intermittent hypoxia. Hence, a given neurochemical mechanism cannot be used to define a given time domain of the hypoxic ventilatory response. The physiological significance for different mechanisms for LTF remains to be determined and, in particular, differences in sensitivity of the Q versus S pathways to various patterns of intermittent hypoxia remain to be tested. Also, the idea that LTF and ventilatory acclimatization to chronic sustained hypoxia use different signaling mechanisms can be questioned; a critical argument supporting different mechanisms for these two forms of plasticity has been that the mechanism of LTF requires serotonin, while ventilatory acclimatization to hypoxia does not [2]; however, the recent discovery of serotonin-independent LTF nullifies this distinction. Finally, the clinical significance of LTF and its role in sleep-disordered breathing remains to be determined. Depending on how LTF affects individual ventilatory and upper airway muscles, loop gain, and ventilatory thresholds, LTF might stabilize or destabilize breathing with intermittent hypoxia during sleep-disordered breathing [73-75].

Abbreviations

5-HT, 5-hydroxytryptamine; $A_{2A}R$, adenosine type 2 receptors; Akt, protein kinase B; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; ERK, extracellular signal-regulated kinases; LTF, long-term facilitation; REM, rapid eye movement; TrkB, high-affinity receptor tyrosine kinases.

Disclosure

The authors declare that they have no disclosures.

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