

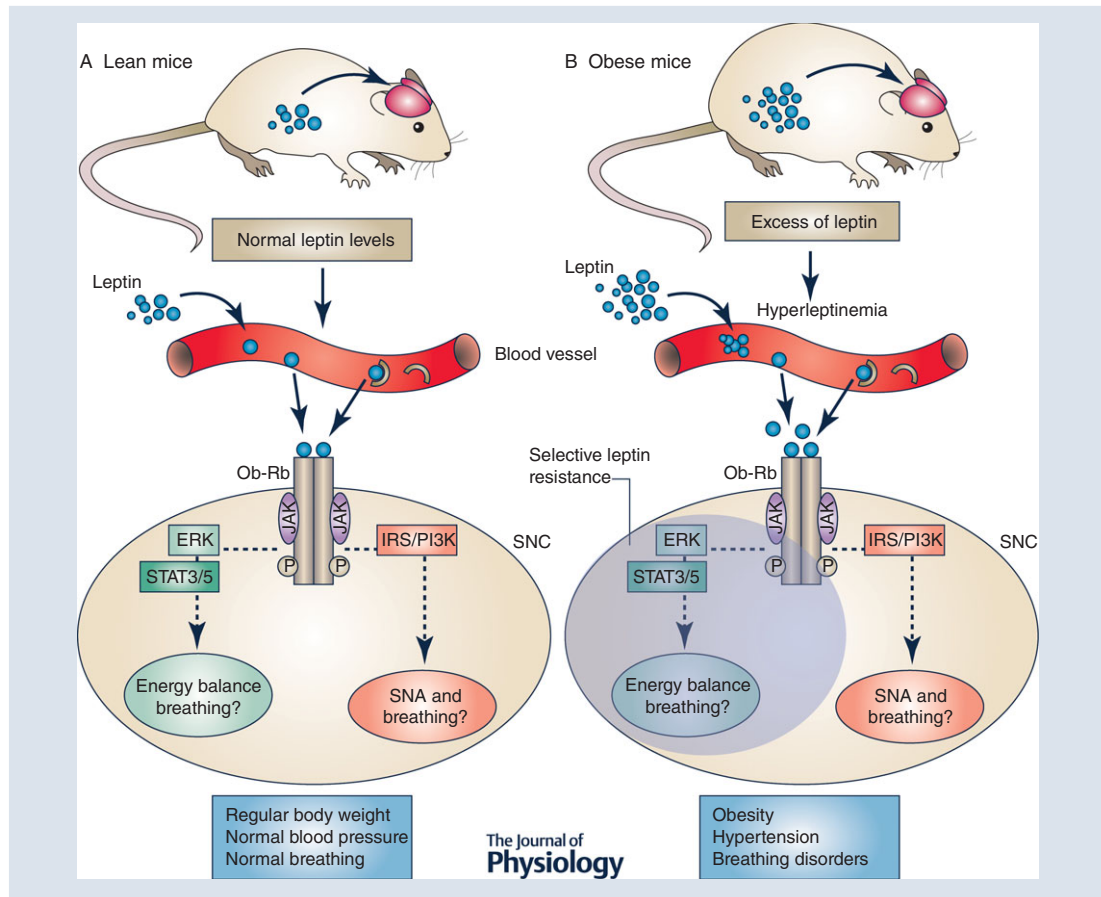
SYMPOSIUM REVIEW

# Facilitation of breathing by leptin effects in the central nervous system

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**Abstract** With the global epidemic of obesity, breathing disorders associated with excess body weight have markedly increased. Respiratory dysfunctions caused by obesity were originally attributed to mechanical factors; however, recent studies have suggested a pathophysiological component that involves the central nervous system (CNS) and hormones such as leptin produced by adipocytes as well as other cells. Leptin is suggested to stimulate breathing and leptin deficiency causes an impairment of the chemoreflex, which can be reverted by leptin therapy. This facilitation of the chemoreflex may depend on the action of leptin in the hind-brain areas involved in the respiratory control such as the nucleus of the solitary tract (NTS), a site that receives chemosensory afferents, and the ventral surface of the medulla that includes the retrotrapezoid nucleus (RTN), a central chemosensitive area, and the rostral ventrolateral medulla (RVLM). Although the mechanisms and pathways activated by leptin to facilitate breathing are still not completely clear, evidence suggests that the facilitatory effects of leptin on breathing require the brain melanocortin system, including the POMC–MC4R pathway, a mechanism also activated by leptin to modulate blood pressure. The results of all the studies that have investigated the effect of leptin on breathing suggest that disruption of leptin signalling as caused by obesity-induced reduction of central leptin function (leptin resistance) is a relevant mechanism that may contribute to respiratory dysfunctions associated with obesity.

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**Abstract figure legend** A. Normal blood levels of leptin in a lean mice: Leptin action on Ob-Rb receptors in the central nervous system (SNC) activates different intracellular pathways like ERK/STAT3/5 and IRS/PI3K that modulate energy balance and sympathetic nerve activity (SNA), respectively. The same pathways may also modulate breathing. B. Excess of leptin in obese mice: hyperleptinemia causes selective resistance to leptin in the CNS affecting energy balance (which intensify obesity) and breathing (causing breathing disorders). The activation of SNA is preserved and intensified causing hypertension.

**Abbreviations** ARC, arcuate nucleus; CNS, central nervous system; LR, leptin receptor; MC3/4R, melanocortin 3 and 4 receptors; NTS, nucleus of the solitary tract; POMC, proopiomelanocortin; RSNA, renal sympathetic nerve activity; RTN, retrotrapezoid nucleus; RVLM, rostral ventrolateral medulla.

## Introduction

With the global epidemic of obesity, the prevalence of breathing disorders associated with excess body weight has markedly increased (Leung & Bradley, 2001; Gilat *et al.* 2014). The most common types of breathing disorder exhibited by obese persons are obesity hypoventilation syndrome (OHS) and obstructive sleep apnoea (OSA) (Malhotra & White, 2002; Olson & Zwillich, 2005). These conditions are not mutually exclusive and the majority of the patients with OHS also have severe OSA (Malli *et al.* 2010). Furthermore, a pathological consequence of OSA is the development of sympathetic overactivity and arterial hypertension (Malli *et al.* 2010; Mansukhani *et al.* 2014). The impact of obesity on breathing disorders was originally thought to be due to excessive fat tissue in the upper airways that increases the risk of obstruction during sleep (Malhotra & White, 2002; Olson & Zwillich, 2005). However, recent studies have suggested that an excess of adipose tissue can contribute to the genesis of

these syndromes (OHS and OSA) through its exaggerated production of adipokines (Makinodan *et al.* 2008; Malli *et al.* 2010; Cundrle *et al.* 2013).

Leptin, a hormone produced by adipocytes as well as other cells, is a respiratory stimulant which raises the possibility that obese patients may exhibit hypoventilation as a consequence of, at least in part, reduced leptin action in the brain (Morris & Rui, 2009; Malli *et al.* 2010). In agreement with this hypothesis, experimental studies have demonstrated that leptin-deficient (*ob/ob*) mice have an impaired ventilatory response to hypercapnia, a deficiency that can be rescued by systemic or central leptin replacement independently of changes in body weight (O'Donnell *et al.* 2000; Bassi *et al.* 2012). Additionally, intravenous infusion of leptin for 60 min elicited a long-lasting increase in the amplitude of phrenic nerve discharge in rats (peaked at 90 min, 30 min after terminating leptin infusion), supporting the role of leptin as an important facilitator of ventilation (Chang *et al.* 2013).

**Table 1. Ventilatory responses to leptin in different conditions**

Species	$\dot{V}_E$	Body weight	Leptin therapy and breathing responses	References
ob/ob mice ( <i>in vivo</i> )	↑ or ↔ basal $\dot{V}_E$ ↓ $\dot{V}_E$ -CO <sub>2</sub>	↑↑	Systemic or 4V (3 days) ↑ basal $\dot{V}_E$ ↑ $\dot{V}_E$ -CO <sub>2</sub>	O'Donnell <i>et al.</i> (2000) Bassi <i>et al.</i> (2012)
ob/ob mice ( <i>in vivo</i> )	↔ basal $\dot{V}_E$ ↓ $\dot{V}_E$ -CO <sub>2</sub>	↑↑	RTN/pFRG (3 days) ↑ basal $\dot{V}_E$ ↑ $\dot{V}_E$ -CO <sub>2</sub>	Bassi <i>et al.</i> (2014a)
Mice LepR/POMC-cre LepR/Nestin-cre MC4R <sup>-/-</sup>	↓ basal $\dot{V}_E$ ↓ $\dot{V}_E$ -CO <sub>2</sub>	↑↑	—	Bassi <i>et al.</i> (2014b)
Rats (anesthetized)	↔	↔	NTS (bolus) ↑ basal $\dot{V}_E$	Inyushkin <i>et al.</i> (2009) Inyushkina <i>et al.</i> (2010)
Rats (anesthetized)	↔	↔	NTS (bolus) ↑ AP ↓ bradycardia ↑ chemoreflexic response	Ciriello & Moreau, (2012)
Rats ( <i>in vitro</i> )	↔	↔	Brainstem slices ↔ RTN-CO <sub>2</sub> sensitivity	Bassi <i>et al.</i> (2013)
Rats ( <i>in vivo</i> )	↔	↔	LV (6 days) ↑ basal $\dot{V}_E$ ↑ $\dot{V}_E$ -CO <sub>2</sub>	Bassi <i>et al.</i> (2014b)
Rats ( <i>in vivo</i> )	↔	↔	LV + MC3/4R blockade (6 days) ↔ basal $\dot{V}_E$ ↔ $\dot{V}_E$ -CO <sub>2</sub>	Bassi <i>et al.</i> (2014b)

$\dot{V}_E$ , ventilation; RTN/pFRG, retrotrapezoid nuclei/parafacial respiratory group; NTS, nucleus of the solitary tract; AP, arterial pressure; LV, lateral ventricle; 4V, fourth ventricle; MC3/4R, melanocortin 3 and 4 receptors. ↑ increased or elevated; ↑↑ extreme elevated; ↓ reduced; ↔ no change.

### Where does leptin act in the CNS to modulate ventilation?

**Leptin might act in the nucleus of the solitary tract (NTS) to stimulate breathing and the chemoreflex.** Leptin circulates freely in the plasma and crosses the blood–brain barrier via a saturable receptor-mediated transport system (Morris & Rui, 2009) to enter the central nervous system (CNS) and regulate neural pathways that control appetite (Grill *et al.* 2002), sympathetic nerve activity (SNA), thermogenesis (Rahmouni & Morgan, 2007; Mark *et al.* 2009) and potentially ventilatory function (Inyushkina *et al.* 2010; Bassi *et al.* 2012, 2014a).

The hypothalamic arcuate nucleus (ARC) was initially considered the main site of leptin actions in modulating energy balance and sympathetic activity; however, increasing evidence suggests that leptin may also act on a more extensive brain network that includes hindbrain areas such as the NTS and the ventral surface of the medulla (Grill *et al.* 2002; Ciriello & Moreau, 2013; Barnes & McDougal, 2014; Arnold & Diz, 2014; Ciriello & Caverson, 2014; Bassi *et al.* 2014a). The presence of functional leptin receptors (LRs) was demonstrated on cell bodies within the NTS and systemic or focal injections of leptin into the NTS enhanced c-fos expression in the caudal NTS

subnuclei, suggesting that the NTS is an area activated by leptin (Mercer *et al.* 1998; Elias *et al.* 2000; Hosoi *et al.* 2002; Ciriello & Moreau, 2013). Injections of leptin into the NTS of anaesthetized rats acutely increased basal renal sympathetic nerve activity (RSNA) and the pressor response to peripheral chemoreflex activation (Mark *et al.* 2009; Ciriello & Moreau, 2012, 2013; Ciriello & Caverson, 2014). In addition, leptin in the NTS attenuated the arterial baroreflex response (Arnold *et al.* 2009; Arnold & Diz, 2014) and enhanced basal respiration activity (Inyushkin *et al.* 2009; Inyushkina *et al.* 2010). These data are evidence that the NTS might be a site of leptin action to control chemoreflex responses (Table 1, and Figs 1 and 2).

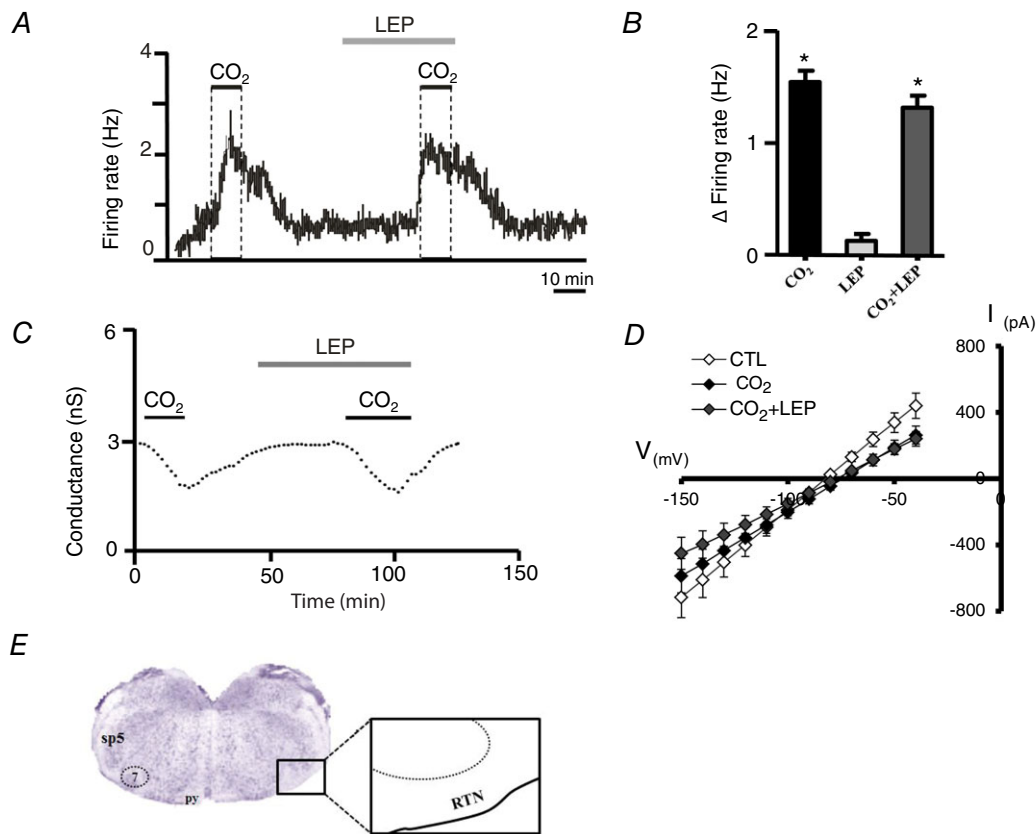
The effects of LR stimulation are suggested to be mediated by cytokine receptor-like signals, including the activation of Janus kinases (JAKs) and signal transducers and activators of transcription (STATs) (Ghilardi & Skoda, 1997). In the CNS, leptin increases the activity of JAK2 to trigger three major intracellular pathways: (1) phosphorylation of the tyrosine (Tyr) residue 1138 of LR, resulting in phosphorylation and nuclear translocation of STAT3; (2) phosphorylation of insulin receptor substrate 2 (IRS2), activating phosphatidylinositol 3-kinase (PI3K); and (3) phosphorylation of Tyr985 of LR, which recruits



2010; Gourine *et al.* 2010; Huckstepp *et al.* 2010) and LRs were found in astrocytes in the hypothalamus (Kim *et al.* 2014). Tests *in vitro* also demonstrated that leptin did not affect basal conductance and  $\text{CO}_2/\text{H}^+$ -sensitive current of chemosensitive RTN astrocytes (Fig. 2C and D; Bassi *et al.* 2013). Furthermore, we found no evidence that leptin receptors are present on chemosensitive RTN neurons or astrocytes. Therefore, these results suggest that the activity of RTN chemoreceptors (neurons or astrocytes) is not modulated by leptin on a short time scale.

In summary, *in vivo* experiments demonstrated that leptin stimulated the ventilatory response to  $\text{CO}_2$  when chronically administered into the rostral ventrolateral region of the medulla in mice, suggesting that leptin may facilitate chemorespiratory mechanisms located in this region. On the other hand, tests *in vitro* showed

no effect of acute treatment with leptin on the basal level of the  $\text{CO}_2/\text{H}^+$  sensitivity of chemosensitive RTN neurones or astrocytes in slices of rat pups. These contradictory results might be related to the different species tested or because the effects of leptin on chemorespiratory mechanisms might depend on its chronic long time scale action in the ventrolateral region of the medulla. It is also possible that leptin does not directly act in the chemosensitive RTN neurons or astrocytes. Leptin may act in other neurones (not chemosensitive) that are part of the neural circuitry involved in chemoreflex, such as those located in the RVLM and Bötzing complex in which LRs are present (Barnes *et al.* 2010; Bassi *et al.* 2012) (Fig. 3). The potential action of leptin in the ventrolateral medulla to facilitate chemoreflex and the effects of leptin on central chemoreception are questions that need more studies in the future.



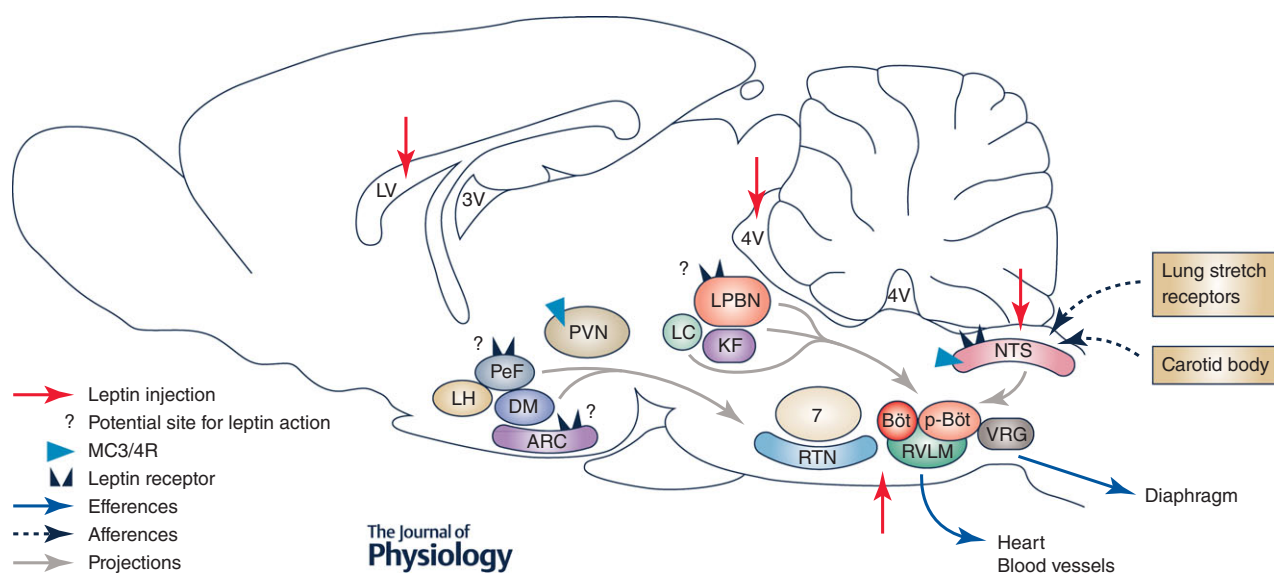
**Figure 2. Acute leptin application had no effect on the activity of chemosensitive RTN neurones or astrocytes**

A, firing rate trace showing the response of a chemosensitive RTN neurone to 15%  $\text{CO}_2$  under control conditions and in the presence of 100 nM leptin (LEP). B, summary data showing the firing rate response of RTN chemoreceptors ( $n = 10$ ) to 15%  $\text{CO}_2$ , leptin and 15%  $\text{CO}_2$  plus leptin. C, conductance trace (holding potential =  $-80$  mV, in TTX to block neuronal activity) showing the response of a chemosensitive RTN astrocyte to 15%  $\text{CO}_2$  under control conditions and after 30 min incubation in 100 nM leptin. D, summary data ( $n = 9$ ) showing the current–voltage relationship under control conditions, during 15%  $\text{CO}_2$  and in 15%  $\text{CO}_2$  plus leptin. \*Different from baseline conditions ( $P < 0.05$ ). After recording, the cells were filled with biocytin for later immunohistochemical detection of leptin receptors. E, coronal brain section showing the location of RTN; py, pyramidal tract; sp5, spinal trigeminal tract.

Leptin also increases RSNA and, therefore, may affect blood pressure contributing to the development of hypertension (Hall *et al.* 2010). Leptin may affect blood pressure and RSNA by directly acting on LRs present on adrenergic/noradrenergic C1/A1 cells located in the ventrolateral medulla, which possibly are part of the pre-autonomic neurones of the RVLM involved in cardiovascular regulation (Barnes & McDougal, 2014). Activation of either the hypoxic or hypercapnic chemoreflex elicits hyperventilation combined with sympathetic activation. These responses are controlled by a neuronal network within the ventrolateral medulla and depend on the modulation of the pre-sympathetic neurons in the RVLM by the respiratory pattern generator (Haselton & Guyenet, 1989; Dempsey *et al.* 2002). Considering that leptin administered into the ventrolateral medulla enhances basal ventilation and the hypercapnic ventilatory response (see above), it is possible that leptin acting on the ventral surface of the medulla might also influence blood pressure by contributing to this coupling of respiratory and cardiovascular modulation (Figs 2 and 3). This is a further area ripe for investigation.

**Leptin actions in the hypothalamus may also contribute to the chemoreflex.** The brain melanocortin system mediates the majority of leptin effects on appetite, metabolism and cardiovascular function, especially in the hypothalamus (Greenfield, 2011; Dubinion *et al.* 2013; Li *et al.* 2013). Leptin acts on POMC neurons in the ARC and promotes the release of  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ -MSH) that, in turn, activates the melanocortin 3 and 4 receptors (MC3/4R) (Fig. 1) (Cowley *et al.* 2001; Morton & Schwartz, 2011).

Leptin acting on POMC neurones and recruiting melanocortin system also appears to be involved in leptin effects on breathing function. Agouti yellow ( $A^y$ ) mice, a model overexpressing the MC3/4R-inhibiting agouti protein, exhibited a reduced CO<sub>2</sub> ventilatory responsiveness during non-REM sleep, but not during wakefulness (Polotsky *et al.* 2004). Similarly, central MC3/4R blockade reduced the stimulatory effects of intracerebroventricular leptin administration on the ventilatory response to central chemoreceptor activation (Bassi *et al.* 2014b), supporting the involvement of



**Figure 3. Potential sites of leptin action to modulate breathing**

Schematic view showing sites where leptin was administered (red arrows) into the CNS in previous studies (Inyushkin *et al.* 2009, 2010; Bassi *et al.* 2012, 2013, 2014; Ciriello & Moreau, 2012) and possible sites of leptin action for breathing modulation. Leptin administered into the lateral ventricle (LV) might act in different nuclei of the hypothalamus, including in perifornical (PeF), dorsomedial (DM) and arcuate (ARC) nuclei that express leptin receptors (LR) and MC3/4R and which are known to affect ventilation via projections to nuclei in the pons and medulla involved in respiratory control, among them the lateral parabrachial nuclei (LPBN) and the retrotrapezoid nuclei (RTN). In addition, leptin may also directly act in brainstem nuclei that control breathing and chemoreflex function, such as the nucleus of the solitary tract (NTS), the rostral ventrolateral medulla (RVLM) and the RTN. However, the respiratory network is complex and leptin effects on respiratory control are still poorly understood. Other respiratory nuclei that leptin might act on are locus coeruleus (LC), Kölliker-Fuse (KF), Bötzing complex (Böt), pre-Bötzing complex (p-Böt) and ventral respiratory group (VRG). Other abbreviations: 7, facial nuclei; LH, lateral nucleus of hypothalamus; PVN, paraventricular nucleus of hypothalamus; 3V, third ventricle; 4V, fourth ventricle.

the melanocortin system in mediating leptin effects on ventilation (Table 1). In addition, mice with LR deletion in the entire CNS (Lept-Nestin-cre) or specifically in POMC neurons (LepR/POMC-cre) also exhibited attenuated baseline and ventilatory responses to CO<sub>2</sub> (Bassi *et al.* 2014*b*). Taken together, these results suggest that leptin facilitates ventilatory function, at least in part, via the brain melanocortin system, including the POMC–MC4R pathway (Figs 1–3).

The MC3/4R is a major pathway in the hypothalamus and the expression of LR and/or MC4R is present in many hypothalamic nuclei, including the paraventricular nucleus (PVN), dorsomedial nucleus (DMH) and peri-fornical region (PeF) of the hypothalamus (Kishi *et al.* 2003). Activation of the PVN region with bicuculline or glutamate increased phrenic and hypoglossal outflows in some studies (Yeh *et al.* 1997; Mack *et al.* 2002; Fortuna *et al.* 2009). Additionally, disinhibition of neurones in the DMH increased phrenic nerve activity (McDowall *et al.* 2007), whereas micro-injection of gabazine into the PeF increased blood pressure, phrenic nerve discharge and firing rate of the chemosensitive RTN neurones in isoflurane-anaesthetized rats (Fortuna *et al.* 2009; Li *et al.* 2013). Therefore, perhaps leptin might also modulate respiratory responses activating the melanocortin system in these hypothalamic nuclei (Fig. 3), a hypothesis that needs future investigation.

Besides this hypothalamic effect of leptin and MC4R, extra-hypothalamic autonomic and respiratory control by leptin–MC4R has been suggested. MC4R-specific hybridization was evident in the central nucleus of the amygdala, the periaqueductal grey, the lateral parabrachial nucleus (LPBN), the NTS, the dorsal motor nucleus of the vagus (DMV), and the intermediolateral nucleus of the spinal cord (IML), among others (Kishi *et al.* 2003). In addition, the presence of MC4R in the RVLM is also suggested. The direct administration of picomolar concentrations of MC3/4R agonist (5 pmol of MTII) into the RVLM elicited hyperthermia, tachycardia, hyperactivity and body weight loss (Skibicka & Grill, 2009). In the same study, the effects of the activation of melanocortin receptors in other hindbrain nuclei, such as the NTS and LPBN, indicate that the effects of CNS MC3/4R stimulation are mediated by an anatomical distributed network of melanocortin receptors. However, the involvement of MC3/4R on chemorespiratory control in the ventral area of medulla has to be investigated.

It is important to note that in some studies leptin was injected in a relatively high concentration and, therefore, it is not possible to exclude actions outside the sites of injection such as the NTS and RTN and/or that the actions are more pharmacological than physiological. Thus, more studies are necessary to clarify this potential physiological action of leptin in specific areas of the hindbrain involved in cardiorespiratory control.

## Perspectives and conclusions

Emerging evidence clearly shows that leptin is a relevant modulator of breathing. In the last few years, an increasing number of studies have investigated the effects of this peptide in the modulation of the respiratory activity, especially on central chemorespiratory control as recent results have shown. Leptin may act in different areas of the CNS modulating breathing by acute and chronic mechanisms. The rapid effects of leptin were demonstrated in the NTS and attributed to a direct effect of leptin on membrane potential. Conversely, in ventral areas of the medulla, such as the RTN and RVLM, it seems that the modulation of breathing depends on chronic effects of leptin. The POMC–MC3/4Rs are also a potential mechanism activated by leptin to modulate breathing. However, more studies are necessary for a definitive conclusion about the involvement of these mechanisms on the effects of leptin on respiratory responses.

Although few studies about the action of leptin in the complex network involved with breathing control can be found in the literature, the information that currently exists is enough to emphasize the important relationship between obesity and excessive production of leptin, which can generate dysfunction in the action of this peptide in the CNS (leptin resistance), promoting not only an excess accumulation of fat tissue and cardiovascular injury, but also serious respiratory disorder. Given this, a range of possibilities is open for future experiments to clarify the role of leptin in respiratory function.

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## Additional information

### Competing interests

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