The Carotid Body Does Not Mediate the Acute Ventilatory Effects of Leptin

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Abstract

Leptin is a hormone produced mostly in adipose tissue and playing a key role in the control of feeding and energy expenditure aiming to maintain a balance between food intake and metabolic activity. In recent years, it has been described that leptin might also contributes to control ventilation as the administration of the hormone reverses the hypoxia and hypercapnia commonly encountered in ob/ob mice which show absence of the functional hormone. In addition, it has been shown that the carotid body (CB) of the rat expresses leptin as well as the functional leptin-B receptor. Therefore, the possibility exists that the ventilatory effects of leptin are mediated by the CB chemoreceptors. In the experiments described below we confirm the stimulatory effect of leptin on ventilation, finding additionally that the CB does not mediate the instant to instant control of ventilation.

Keywords

Leptin • Carotid body • Ventilation • Catecholamine hypoxia

43.1 Introduction

Leptin is a hormone expressed in adipose tissue and secreted in direct proportion to adipose tissue mass (Cao 2014). Leptin function is to adjust food intake to energy expenditure. Circulating

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leptin traverses blood brain barrier and reaches all peripheral tissues. Acting centrally, leptin tends to decrease food intake activating hypothalamic satiety centers, and acting peripherally facilitates nutrients oxidation. Centrally, leptin also activates the sympathetic system (acting at the nucleus arcuatus and at the nucleus tractus solitarius (NTS; Mark 2013), and this activation also contributes to augment the oxidative metabolism in peripheral tissues (Aguer and Harper 2012) as well as to augment blood pressure and to create a certain status of insulin resistance. Also, recent research has been published concerning the role of leptin in the respiratory system, with leptin's involvement in the most common disorders of the respiratory system including obstructive sleep apnoea-hypopnoea syndrome (OSAHS), asthma, chronic obstructive pulmonary disease (COPD) and lung cancer (Malli et al. 2010).

The CB are small arterial chemoreceptor organs which sense blood O_2 and CO_2/pH levels and are formed by clusters of cells surrounded by a dense net of capillaries that facilitates the presentation of the blood-borne stimuli to chemoreceptor cells. Chemoreceptor cells are synaptically connected with the sensory nerve endings of the carotid sinus nerve (CSN), whose central projections terminate in the brainstem, in the nucleus of solitary tract (Katz et al. 1997; Gonzalez et al. 1994), and activate adaptive responses in the respiratory and cardiovascular systems, involving also the participation of endocrine and sympathetic nervous systems (Kumar 2009). Models of CB functioning consider that chemoreceptor express O_2-sensor(s) which are coupled to certain K^+ channels in such a manner that a decrease in PO_2 leads to inhibition of K^+ channels, cell depolarization, activation of voltage dependent Ca^{2+} channels, and augmentation of the release of neurotransmitters. These drive the chemoreceptor cell-nerve ending synapses to an increase in the action potential frequency in the CSN (see González et al. 1992, 2009; Peers 1997; Kemp 2005). The end result being that in hypoxia (or in hypercapnia/acidosis) the initiation of cardio respiratory reflexes, most significantly hyperventilation, aimed to normalize arterial blood gases.

Recently, two different laboratories have demonstrated the presence of leptin and leptin receptors ObRb in the CB chemoreceptor cells (Porzionato et al. 2011; Messenger and Ciriello 2013; Messenger et al. 2013). This fact, combined with the observation that increased circulating leptin levels induce the expression of phosphorylated signal transducer and activator of transcription 3 (pSTAT3) and the immediate early gene Fra-1 within these chemoreceptor cells (Messenger et al. 2012) suggest that leptin may play an important role in modulating CB function, and thereby ventilation. Along these lines, it has been observed administration of leptin have increases ventilation (Inyushkin et al. 2009; O'Donnell et al. 1999; Chang et al. 2013). In this context, the aim of our study has been to investigate the contribution of the CB to the ventilatory responses elicited by leptin. To achieve this aim, we have combined *in vivo* and *in vitro* experiments to explore effects of leptin on ventilation and direct effects of leptin in the CB chemoreceptor cells.

43.2 Methods

43.2.1 Animals and Anesthesia

Experiments were performed in adult Wistar rats 12 weeks of age following the European Community Council directive of 24 of November 1986 (86/609/EEC) for the Care and Use of Laboratory Animals. Rats were housed four per cage, with free access to food and water and maintained in the vivarium of the University of Valladolid and at the vivarium of NOVA Medical School under controlled conditions of temperature and humidity and a stationary light–dark cycle.

43.2.2 Intravenous Administration of Leptin in Anaesthetised Animals

Animals were anaesthetized with sodium pentobarbital (60 mg/kg, i.p.) dissolved in physiological saline. Anaesthetized rats were given

intravenous boluses of leptin at doses of (300, 600 and 900 ng/Kg) spaced 15 min from each other.

We recorded respiratory frequency (bpm) and tidal volume (TV, ml/kg) and basal minute ventilation (ml/kg/min) was calculated. The stimulation of the CB was achieved by 5 and 15 s of carotid bilateral occlusion applied immediately prior to each leptin injection and 15 min after the last. The 5 s occlusion would represent an almost pure hypoxic stimulus while the 15 s occlusion would be more an ischemic stimulus combining hypoxia, hypercapnia and acidosis in the CB and likely some central effects (Monteiro et al. 2011). We performed a bilateral carotid occlusion during 5 and 15 s prior to leptin infusions. Once recovered basal respiratory patterns a blood sample was taken to measure blood glucose and leptin levels; leptin was assayed by an ELISA provided by Cusabio Biotech; bioNova Cientifica, Madrid, Spain, following the instructions of the supplier**.** Blood pressure and heart rate was continuously monitored all along the experiments.

43.2.3 ^{3}H-Catecholamine (^{3}H-CA) Release Experiments Using Freshly Isolated Intact CBs

General procedures used to label chemoreceptor cells ^{3}H-catecholamine (^{3}H-CA) (CA) deposits and later to study their release have been described in previous publications (Vicario et al. 2000; Gonzalez-Martín et al. 2011). And analytical methods have been described in detail in Conde et al. (2006). The experiments included pairs of control *vs.* experimental CBs. Solutions were pre-equilibrated at 37 °C with a water vapour-saturated gas mixture (21 % O_2, normoxia, or 7 % O_2, hypoxic stimulation, 5 % CO_2, balanced N_2) and all along the experiments the vials atmospheres were gassed with the same gas mixture. Hypoxic and depolarizing stimuli consisted of 10 min incubations, with low PO_2-equilibrated (7 % O_2; $PO_2 \approx 46$ mmHg) and high K^+-containing solutions (35 mM; equiosmolar Na^+ was removed). Control organs were stimulated with the hypoxic and high external potassium stimulus and experimental CBs were similarly stimulated but the solutions contained 40 ng of leptin from 20 min before hypoxic stimulation and leptin was kept until the end of the experiments. Data were presented as means ± SEM of release evoked (% content). Statistical significance of differences was assessed using a two-tailed t-test for unpaired data.

43.3 Results

43.3.1 Effects of Leptin in Ventilation

Figure 43.1a depicts typical recordings of respiratory rate and TV in basal conditions and in response to ischemic hypoxia, induced by occlusions of common carotid artery (CCO) lasting 5 and 15 s in a control rat. Recordings were made before any leptin injection and 15 min after leptin bolus injection (300 ng/Kg). Control MV amounting to 365.2 ± 26.67 mL/min/kg was dose-dependently increased by leptin (Fig. 43.1b). Leptin increased in about the same percentage breathing frequency and TV. Figure 43.1c shows the increase in MV produced by bilateral carotid artery occlusions during 5 and 15 s. Note that the 5 s occlusion augmented MV by approximately by around 50 % and again leptin, injected 15 min in advance, further increased ventilation in a dose-related manner reaching 25–30 % with the highest dose employed. Fifteen seconds occlusion augmented MV by over 150 % and again leptin augmented the occlusion effect.

43.3.2 Plasma Leptin and Glucose Levels

Figure 43.2a depicts the levels of leptin found in blood during our experimental protocol. As it can be seen, the basal leptin levels were 11.3 ± 2.0 ng/ml (n = 6), being these levels surprisingly higher than the levels reported by other laboratories (2.2 ng/ml, Zeng et al. 1997; ca. 2 ng/ml, Simler et al. 2007; 0.78 ng/ml, Chaiban et al. 2008; ca. 4 ng/ml, Messenger et al. 2012). In additional studies from our laboratory and using ELISA kits of the same suppliers we have obtained the basal

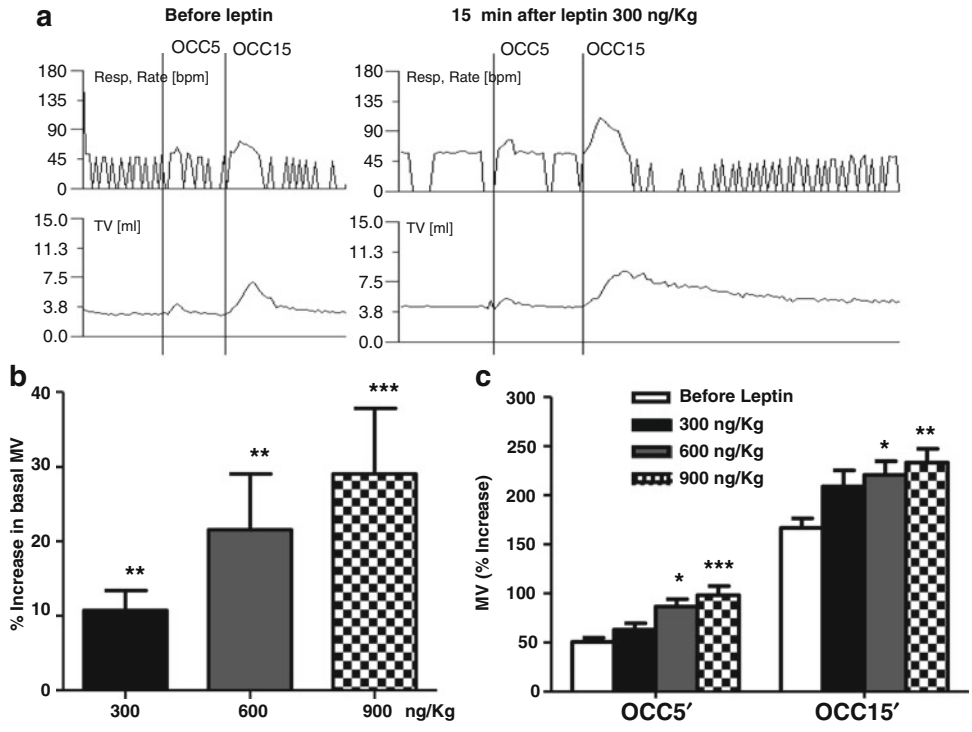

Fig. 43.1 (**a**) Typical recordings of respiratory rate (Resp. Rate) (bpm) and tidal volume (TV; mL), in basal conditions and in response to ischemic hypoxia, induced by occlusions of common carotid arteries (*CCO*) in a control rat. Recording was made before and after leptin infusion. (**b**) Percent increase in basal (normoxic) ventilation after leptin infusion in awake control rat. (**c**) MV (% increase) resulting from both common carotid arteries occlusion (*CCO*) in basal conditions and after leptin infusions. Data are means ± SEM of eight individual values (*p < 0.05, ** p < 0.01, ***p < 0.001)

levels of leptin found have been 8.9 ± 0.5 ng/ml (Olea et al. 2014) being these values statistically identical to the values found in the present study.

Also, herein we found that with increasing doses of leptin there was a trend for the hormone levels to increase, although only with the highest dose of leptin infused the levels reached a statistical significance (16.8 ± 2.8 ng/ml; n = 6; p = 0.05). None of the doses of leptin tested in the present study altered glycaemia (Fig. 43.2b).

43.3.3 Leptin Does Not Activate Basal Nor Hypoxic and High K^+ Induced Release of ^3H Catecholamine in CBs

Figure 43.3a, b show the general protocol and mean time course of ^3H-CA release experiments in four control and in four experimental CBs incubated with leptin (40 ng/ml) as marked by dashed bars. No apparent differences were observed in the time course of ^3HCA release in control vs. experimental organs, being the transition of the release from the leptin-free to leptin-containing media identical to control CBs. Similarly, the hypoxia and high K^+ induced release showed no apparent differences in the time course or in the magnitude of the evoked responses (Fig. 43.3b).

43.4 Discussion

Herein we have found that leptin increased spontaneous and ischemic hypoxia-induced ventilation in a dose-dependent manner although without affecting basal or stimuli-evoked catecholamines release from the CB. Leptin is a pleiotropic hormone whose actions extend from

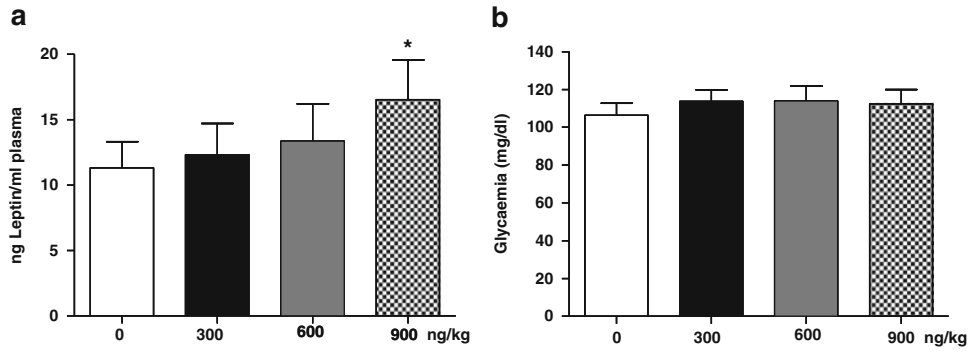

Fig. 43.2 Plasma leptin levels (**a**) and glycaemia (**b**) in basal conditions and after leptin infusions. Means ± SEM of six individual values (*p = 0.05)

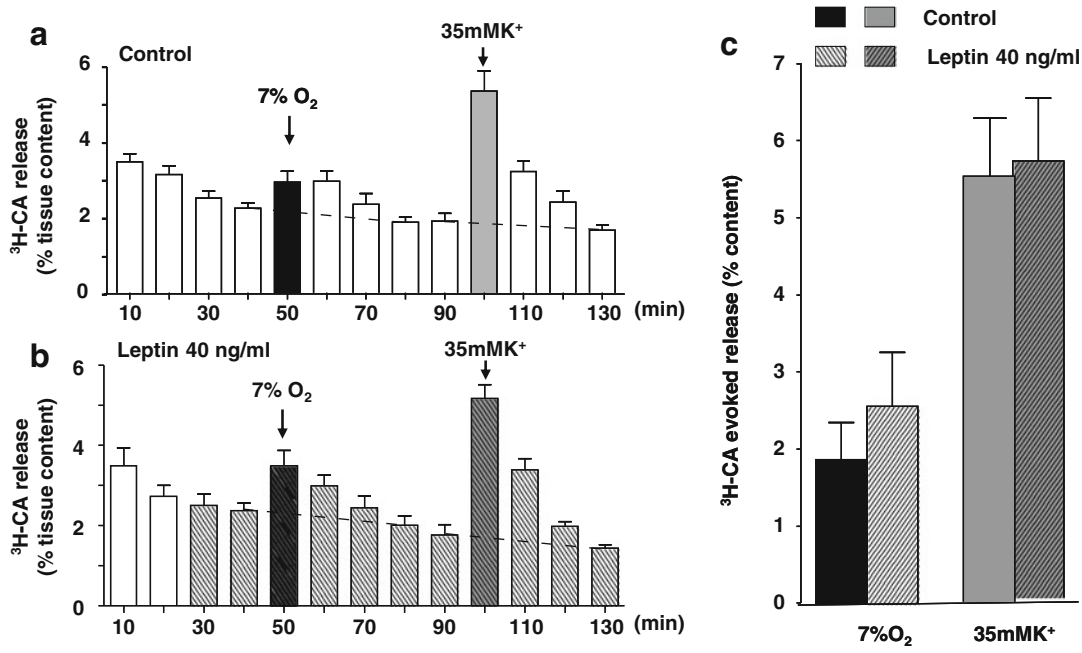

Fig. 43.3 (**a**) and (**b**) show the time course of the release of ^{3}H-catecolamines by the CB of control rats in control incubating solution (**a**) and in solution containing leptin (40 ng/ml; **b**). (**c**) shows the evoked release of ^{3}H-catecolamines induced by hypoxia and high external K+

immune system homeostasis to reproduction and angiogenesis, but in the past years, a growing number of studies have examined the potential role of leptin in the respiratory system (Malli et al. 2010). These studies further suggest a significant impact of leptin on specific respiratory diseases, indicating that the pathophysiological significance of leptin regarding respiratory function in humans remains to be clarified.

Early on, obesity and leptin lack in *ob/ob* mice was related to hypoventilation encountered in obese humans, with its maximal expression in the obesity hypoventilation syndrome (Pickwick syndrome) defined by the combination of obesity, night hypoxia and permanent hypercapnia (Bickelmann et al. 1956; see Olson and Zwillich 2005).

Laboratory studies of O'Donnell and colleagues in animal models have provided evidence

indicating that systemic administration of leptin to leptin deficient Ob/Ob mice increased breathing frequency, tidal volume and minute ventilation, also leading to a significant decrease in the pre-treatment elevated arterial P_aCO_2 when compared to wild-type mice (O'Donnell et al. 1999). Also, quite recently it has been shown that systemic intravenous administration of leptin in rats also augments ventilation in a CO_2-independent manner (Chang et al. 2013).

These results along with the fact that has been demonstrated the presence of leptin and leptin receptors ObRb in the CB chemoreceptor cells (Porzionato et al. 2011; Messenger and Ciriello 2012; Messenger et al. 2013) suggested that leptin might control ventilation via the CB.

In this work, we have found that administration of leptin in anaesthetised animals augmented basal VE and potentiated the ischemic hypoxia-induced VE in a dose-dependent manner, with a latency of several min (6–8 min) and a long lasting effect (≥15 min). We should mention that the dose of leptin administered to our animals is low as evidenced by the slight modifications of basal leptin levels. In this regard we should clarify that the experiments were designed based on the lower basal leptin levels reported in the literature (Zeng et al. 1997; Simler et al. 2007; Chaiban et al. 2008; Messenger et al. 2012) and the thorough study on whole body leptin kinetics and renal metabolism in vivo by Zeng et al. (1997).

These findings indicate that leptin does indeed augment the ventilatory responses elicited by hypoxia as well as basal ventilation. To explore direct effects of leptin in the CB, we studied in vitro the time course of the release of ^{3}H-CA using intact CBs, and we observed that leptin at concentrations ×4 basal endogenous levels does not affect the release of ^{3}H-CA either in basal conditions or during hypoxic or high external K^+. Owing that the release of CA is very reliable index of chemoreceptor cell activation (González et al. 1992) data would indicate that leptin must exert its ventilatory effects acting centrally, probably at the nucleus of the tractus solitarious where the sensory nerve of the CB projects and where Ciriello and Moreau (2013) have demonstrated the existence a set of neurons that expressing leptin receptors receive inputs from CB chemoreceptors. It is worth noting, that these neurons expressing leptin receptors project to the vasopressor sites of the rostroventrolateral medulla, and thereby contribute with the hypothalamic nuclei with neurons (arcuate, ventromedial, paraventricular and dorsomedial nuclei) to control sympathetic activity (particularly renal sympathetic activity; Ciriello and Moreau 2012), heart rate and blood pressure (Harlan and Rahmouni 2013). Thus, the NTS provides a direct link between CB chemoreceptor activity and sympathetic activation.

In this line, Inyushkin et al. (2009) showed that leptin microinjections into the NTS in the brain of rats is associated with increased pulmonary ventilation and respiratory volume and enhanced bioelectrical activity of the inspiratory muscles suggesting that leptin may be implicated in ventilatory control through direct effects on respiratory control centres.

In conclusion, our data indicate that leptin augments basal and hypoxic-ischemic ventilation in a dose manner acting centrally, presumably at the NTS. Leptin does not appear to participate in the minute to minute chemoreception process in the CB, but owed the expression of the hormone and its receptors in chemoreceptor cells, it surely participates in long term regulation of CB function, probably contributing to the sensitization of this chemoreceptor know to occur in chronic hypoxia.

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