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Activation of the brain melanocortin system is required for leptin-induced modulation of chemorespiratory function

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Abstract

Melanocortin receptors (MC3/4R) mediate most of the metabolic and cardiovascular actions of leptin.

Aim—here we tested if MC4R also contributes to leptin's effects on respiratory function.

Methods—after control measurements, male Holtzman rats received daily microinjections of leptin, SHU9119 (MC3/4R antagonist) or SHU9119 combined with leptin infused into the brain lateral ventricle for 7 days. On the 6th day of treatment, tidal volume (V_T), respiratory frequency (f_R) and pulmonary ventilation (V_E) were measured by whole-body plethysmography during normocapnia or hypercapnia (7% CO_2). Baseline mean arterial pressure (MAP), heart rate (HR) and metabolic rate were also measured. V_E, V_T and f_R were also measured in mice with leptin receptor deletion in the entire central nervous system (LepR/Nestin-cre) or only in proopiomelanocortin neurons (LepR/POMC-cre) and in MC4R knockout ($MC4R^{-/-}$) and wild-type mice.

Results—leptin (5 μg/day) reduced body weight (~17%) and increased ventilatory response to hypercania, whereas SHU9119 (0.6 nmol/day) increased body weight (~18%) and reduced ventilatory responses compared to control-PBS group (Lep: 2119 ± 90 $ml.min^{-1}.kg^{-1}$and SHU9119: 997 ± 67 $ml.min^{-1}.kg^{-1}$, vs PBS: 1379 ± 91 $ml.min^{-1}.kg^{-1}$). MAP increased after leptin treatment (130 ± 2 mmHg) compared to PBS (106 ± 3 mmHg) or SHU9119 alone (109 ± 3 mmHg). SHU9119 prevented the effects of leptin on body weight, MAP (102 ± 3 mmHg) and ventilatory response to hypercania (1391 ±137 $ml.min^{-1}.kg^{-1}$). The ventilatory response to hypercania was attenuated in the LepR/Nestin-cre, LepR/POMC-cre and $MC4R^{-/-}$ mice.

Conclusion—these results suggest that central MC4R mediate the effects of leptin on respiratory response to hypercapnia.

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CONFLICT OF INTEREST

We have no conflict of interest to disclose.

Keywords

blood pressure; central chemoreception; hypercapnia; leptin; MC3/4 receptor; melanocortin system

Introduction

Previous studies suggest that leptin, an adipocyte derived cytokine, is an important modulator of respiration. In addition to its effects to reduce food intake and body weight and to increase sympathetic nervous system activity (SNA) and blood pressure (BP), central leptin administration also enhances baseline respiratory activity and chemorespiratory responses to carbon dioxide (CO_2) (Bassi *et al*., 2012, Bassi *et al*., 2014, Hall *et al*., 2010, Inyushkina *et al*., 2010). However, the central mechanisms activated by leptin to facilitate respiratory responses are still unclear.

Previous studies have demonstrated an important role of the brain melanocortin system in mediating most of leptin's actions on appetite, metabolism and cardiovascular function. Leptin depolarizes proopiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus (ARC) inducing the release of alpha-melanocyte stimulating hormone (α-MSH) which, in turn, activates the melanocortin 3 and 4 receptors (MC3/4R) located in several hypothalamic nuclei as well as in other regions of the brain including the brainstem (Morton & Schwartz, 2011, Cowley *et al*., 2001). Deletion of leptin receptors specifically in POMC neurons abolished leptin's ability to raise BP and to reduce fasting glucose and insulin levels (do Carmo et al., 2011). Moreover, pharmacological blockade of MC3/4R completely prevented the anorexic and antidiabetic effects of leptin (da Silva *et al*., 2004, da Silva *et al*., 2009). However, the role of brain MC3/4R in mediating the actions of leptin on respiratory function is not clear.

Support for a role of the brain melanocortin system in modulating ventilatory function comes from previous observations that obese agouti yellow mice, a model overexpressing the agouti protein which inhibits MC3/4R, exhibit attenuated ventilatory responses to CO_2 (Polotsky et al. 2004). This finding is consistent with the possibility that MC3/4R may also be part of the mechanisms activated by the central chemoreflex that elicits respiratory responses to stimuli such as hypercapnia. Nevertheless, there have been no studies, to our knowledge, that evaluated the potential contribution of central MC3/4R on breathing.

To test the hypothesis that activation of brain MC3/4R is necessary for leptin to increase ventilatory responses to hypercapnia, we studied the effects of leptin on ventilation after chronically inhibiting MC3/4R using a pharmacological antagonist. We also examined the ventilatory responses to hypercapnia in mice with MC4R deficiency, leptin receptor deletion in the entire central nervous system (CNS), and in mice with leptin receptor deletion specifically in POMC neurons. The results suggest that central MC4R play a key role in leptin-induced increase in ventilatory response to hypercapnia.

METHODS

The experimental procedures and protocols of this study were approved by the Animal Experimentation Ethics Committee of the School of Dentistry of Araraquara, São Paulo State University, UNESP, Brazil and by the University of Mississippi Medical Center Institutional Animal Care and Use Committee, and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Animals

Male 9-week-old Holtzman rats (n = 24) obtained from the colony of the São Paulo State University, Brazil, and male 20-week-old C57BL/6J wild-type (WT, n = 5), $MC4R^{-/-}$ (n = 5), LepR/POMC-cre (n = 3) and LepR/Nestin-cre (n = 3) mice were used. LepR/POMC-Cre mice were generated as previously described (do Carmo *et al*., 2011). LepR/Nestin-cre mice were generated by crossing Nestin-Cre mice (Jackson Laboratories, Bar Harbor, Maine) with $LepR^{flox/flox}$ mice (generously provided by Dr. Jeffrey Friedman, Rockefeller University, New York, NY). $MC4R^{-/-}$ and WT mice were purchased from the Jackson Laboratories. All animals were maintained on a 12 h–12 h light–dark cycle in a room with controlled temperature (23 ± 2 °C) and humidity (55 ± 10%). Standard rat chow and tap water were available ad libitum.

Intracerebroventricular cannula implantation

The rats were anesthetized with an intraperitoneal injection of ketamine (80 mg/kg; Cristalia, Itapira, SP, Brazil) combined with xylazine (7 mg/kg; Agener União, Embu-Guaçu, SP, Brazil) and placed in a stereotaxic apparatus (Kopf, Tujunga, CA, USA). Under aseptic conditions a stainless steel cannula (10 × 0.7 mm) was implanted into the brain right lateral ventricle (LV) using the following stereotaxic coordinates: 0.4 mm caudal to bregma, 4.0 mm below the dura-mater and 1.6 mm to the right of the midline. The guide cannula was anchored to the skull with stainless steel screws and dental acrylic resin. A metal stylet was inserted to seal the guide cannula when not in use.

At the end of the surgery the rats received an intramuscular injection of benzylpenicillin (80.000 IUs) plus streptomycin (33 mg; Pentabiótico Veterinário – Pequeno Porte, Fort Dodge Saúde Animal Ltda., Campinas, Brazil (0.2 ml/rat) and a subcutaneous injection of analgesic/anti-inflamatory Ketoprofen 1% (Ketoflex, Mundo Animal, São Paulo, Brazil (0.03 ml/rat). The rats were allowed to recover for 7 days before accuracy of the cannula placement was tested by measuring the dipsogenic response (immediate drinking of at least 5 mL of water in 10 min) to a LV injection of angiotensin II (50 ng/1 μl).

Arterial catheter implantation

On the 6^{th} day of treatment (one day before recording mean arterial pressure - MAP and heart rate - HR), the rats were anesthetized with ketamine and xylazine, as described above, and under aseptic conditions the femoral artery were isolated and cannulated with polyethylene tubes (PE-10 connected to a PE-50) filled with saline. The catheter were exteriorized between the scapulae and fixed on the back of the animal to allow recording of baseline MAP and HR in freely moving animals.

Daily i.c.v. microinjections

Leptin (5 μg/3 μl, purchased from the National Hormone & Peptide Program, CA, USA) and/or the melanocortin receptor antagonist SHU9119 (0.6 nmol/3 μl, Phoenix Pharmaceuticals, CA, USA) were administered into the LV of rats for 7 consecutive days. The doses of leptin and SHU9119 were chosen based on previous studies (Choi et al., 2003, Fendt et al, 2009, Inyushkin et al., 2009, Mark et al., 2009). The rats were divided into the following groups: 1) daily leptin treatment alone, 2) daily SHU9119 treatment alone; 3) daily SHU9119 treatment combined with leptin treatment (30 min interval between injections); and 4) Vehicle control group that received daily phosphate buffer saline injections (PBS, pH=7.4, 3 μl). The LV injections were administered at 24-h intervals for 7 days between 11:00 am and noon. The injections were made with a Hamilton syringe (5 μl capacity) coupled with a polyethylene tubing (PE-10) connected to a needle (0.3 mm in diameter and 12 mm in length). Each injection was administered into the LV over 30 s and the injector was left in place for additional 30 s to allow the drug to diffuse in the CSF, followed by removal of the injector and replacement of the stylet into the guide cannula.

Ventilation Measurements

Pulmonary ventilation (V_E) was measured using whole-body plethysmography as previously described (Malan, 1973, Bassi *et al*., 2012). Briefly, rats and mice were acclimatized to the plethysmography chambers (Bonther, Ribeirao Preto, SP, Brazil), 5 L for rats and 1 L for mice, at room temperature (25° C) for ~40 minutes. The ports for gas exit or entrance in the chamber were closed to produce an internal constant volume. Chamber temperature was less than 1° C above room temperature (~25° C) with no significant variations over the period that all measurements were made (5 min). Rectal temperature, measured using a digital thermometer (Omron - MC-245, China) lubricated with vaseline, was constant and no significant changes were observed between groups. Signals of breathing frequency (f_R, breathins.min^{-1}) and tidal volume (V_T, mV) were measured by changes in the pressure inside the chamber caused by inspiratory and expiratory temperature fluctuations using a spirometer (model ML141, AD Instruments, Colorado Springs, USA). Signals were analyzed using PowerLab data acquisition, AD Instruments, Colorado Springs, CO, USA. The system was calibrated with injections of 1 mL for rats or 0.2 mL for mice of room air with the animal inside the plethysmography chamber. V_E was calculated as the product of f_R and V_T.

The tidal volume (VT) was calculated using the formula:
$$VT = VK \times (PT/PK) \times (TA/TR) \times (PB - PC)/(PB - PC) - (TA/TB) \times (PB - PR),$$ where:

PT is the pressure deflection (mV) associated with each VT,

PK is the pressure deflection (mV) associated with the calibration volume (VK) injection,

TB is the body core temperature (Kelvin degree),

TA is the air temperature in the animal chamber (Kelvin degree),

PB is the barometric pressure (mmHg),

PR is the water vapor pressure at Tc (mmHg),

PC is the vapor pressure of water vapor in the animal chamber (mmHg),

TR is the room temperature (Kelvin degree).

VE and VT are represented at the ambient barometric pressure, Tc, saturated with water vapor at this temperature (BTPS). The PC (the water vapor pressure in the animal chamber) was calculated indirectly by using an appropriate table (Dejours, P., 1981).

Measurements of metabolic rate

Oxygen consumption (VO_2), carbon dioxide production (VCO_2) and respiratory quotient ($RQ = VCO_2/VO_2$) were calculated using indirect calorimetry. Briefly, on the 6th day of LV treatment, the rats were acclimatized to the plethysmography chamber (5 L) at room temperature (25 °C) for ~30 minutes. The chamber was connected to a gas analyzer (model ML206, ADInstruments) to measure variations of O_2 and CO_2 inside the chamber during 1 minute.

Experimental protocol in rats

Rats were housed individually in standard metabolic cages for the duration of the study. Food and water intake were measured daily between 11:00 am and noon. Body weight and urinary excretion of Na^+ and K^+ were evaluated before starting and on day 7 of treatment. For the injections, each rat was removed from its home cage and while gently held, the stylet was removed and the injector inserted in the guide cannula. *(see details of injections in Daily i.c.v. microinjections item)*. The rats were returned to their home cages following each injection. On the 6th day of LV injections, after receiving the injections, the rats were transferred to the plethysmography chamber for measurements of metabolic rate (VO2, VCO2 and RQ) and pulmonary ventilation under normocapnia and during central chemoreflex activation by hypercapnia (5 minutes breathing a gas mixture containing 21% O2 plus 7% CO2). To avoid the interference of the surgery, only after the end of the respiratory tests, the arterial catheter was implanted to record MAP and HR on the next day (7th day of treatment)

Experimental protocol in mice

The pulmonary ventilation protocol in mice was similar to the pulmonary ventilation protocol in rats described above. However, we did not instrument mice for LV treatment or cardiovascular measurements.

Statistical Analysis

The results are presented as mean ± SEM. Kolmogorov-Smirnov normality test and one-way or two-way ANOVA followed by Bonferroni's post hoc test were used for comparisons using GraphPad Prism, San Diego, CA, USA. Statistical significance was accepted at the level of $p < 0.05$.

RESULTS

Metabolic and cardiorespiratory responses to daily LV leptin treatment

Chronic LV leptin treatment (5 μg/day) for 7 days reduced daily food intake, body weight and water intake with minor changes in urine volume (Figure 1A-B). Leptin also reduced urinary excretion of Na^+ (0.68 ± 0.09 mEq/24 h, vs. PBS: 1.29 ± 0.08 mEq/24 h) and K^+ (1.29 ± 0.16 mEq/24 h, vs. PBS: 3.37 ± 0.66 mEq/24 h), proportionally to the reduction in food intake (Figure 1B).

Under normocapnia (room air), leptin into the LV enhanced baseline V_E (783±36 $ml.min^{-1}.kg^{-1}$, vs. control-PBS: 570±53 $ml.min^{-1}.kg^{-1}$) [$F(6, 80) = 26.3$; $P < 0.0001$] as a consequence of an increase in V_T, whereas f_R was not modified (Figure 2A). LV leptin treatment also enhanced 7% CO_2-induced increase in V_E (ΔV_E = 1335±79 $ml.min^{-1}.kg^{-1}$, vs. PBS: 779±81 $ml.min^{-1}.kg^{-1}$) [$F(3, 16) = 19.2$; $P < 0.0001$] due to an increase in V_T and f_R (Figure 2B).

Although VCO_2 and VO_2 were similar between leptin and PBS treated groups, leptin tended to reduce VCO_2, which resulted in a significant reduction in RQ (0.73±0.02 $ml.kg^{-1}.min^{-1}$, vs. PBS: 0.86±0.04 $ml.kg^{-1}.min^{-1}$) (Table 1). In addition, leptin into the LV increased MAP (130±2 mmHg, vs. PBS: 106±2 mmHg) [$F(6, 37) = 6.12$; $P < 0.0002$] and also tended to increase HR (409±11 bpm, vs. PBS: 369±24 bpm) (Figure 2C).

Metabolic and cardiorespiratory responses to daily LV treatment with SHU9119 alone or in combination with leptin

LV injections of the MC3/4R antagonist SHU9119 (0.6 nmol/day) increased daily food intake (36.5 ± 1.4 g, vs. PBS: 27.8 ± 1.7 g) and water intake (Δ+8.6 ±1.1 mL, vs. PBS: Δ −1.9 ± 1.6 mL). Body weight and urine volume increased proportionally to increases in food intake, which also explains the increased urinary Na^+ and K^+ excretion (Figure 1 A-B).

Baseline V_E, V_T and f_R in normocapnia were not modified by SHU9119 treatment alone (Figure 2). During exposure to 7% CO_2, the treament with SHU9119 reduced V_E (997 ± 67 $ml.min^{-1}.kg^{-1}$, vs. PBS: 1379 ± 91 $ml.min^{-1}.kg^{-1}$) [$F(6, 80) = 26.3$; $P < 0.01$] and V_T (6.83 ± 0.5 $ml.kg^{-1}$ vs. PBS: 8.99 ± 0.89 $ml.kg^{-1}$) [$F(6, 80) = 15.4$; $P<0.05$] (Figure 2-A). However, the increase (delta) of V_E and V_T produced by the exposure to 7% CO_2 in rats treated with SHU9119 i.c.v. was not statistically different compared to control-PBS treatment (Figure 2-B). SHU9119 also did not alter f_R, RQ, MAP or HR (Table 1 and Figure 2).

Although SHU9119 treatment alone for 7 days did not evoke marked changes in respiratory, metabolic or cardiovascular function, it abolished leptin's effects to reduce food intake and body weight (Figure 1 and Table 1). In fact, rats treated with SHU9119 + leptin exhibited an increase of ~27% and ~13%, respectively, of food intake and body weight compared to control (Figure 1 and Table 1). Combined treatment, similar to SHU9119 alone, increased water intake and urine volume (Figure 1 A-B) and also increased urinary Na^+ and K^+ proportionally to the increases observed in food intake (Figure 1 A-B). SHU9119 abolished the increase in V_E and V_T produced by leptin in rats exposed to normocapnia or 7% CO_2

(Figure 2-AB). SHU9119 also prevented the reduction in RQ and the increase in MAP evoked by leptin (Table 1, Figure 2C).

Baseline ventilation and ventilatory response to CO_2 in LepR/Nestin-cre, LepR/POMC-cre and MC4R$^{-/-}$ mice

Baseline ventilation under normocapnia was reduced in LepR/Nestin-cre (1501 ± 105 ml.min^{-1}.kg^{-1}), LepR/POMC-cre (1197 ± 95 ml.min^{-1}.kg^{-1}) and MC4R$^{-/-}$ (1473 ± 124 ml.min^{-1}.kg^{-1}) compared to the wild-type (WT, 2221 ± 170 ml.min^{-1}.kg^{-1}) [$F(3, 20)= 66.9$; $P < 0.0001$]. LepR/Nestin-cre, LepR/POMC-cre and MC4R$^{-/-}$ mice had also reduced V_T. LepR/POMC-cre mice also showed reduced f_R, whereas LepR/Nestin-cre mice increased f_R compared to WT (Figure 3A).

The ventilation under 7% CO_2 was markedly attenuated in LepR/Nestin-cre (2569 ± 215 ml.min^{-1}.kg^{-1}), LepR/POMC-cre (3923 ± 112 ml.min^{-1}.kg^{-1}) and MC4R-/- mice (2843 ± 24 ml.min^{-1}.kg^{-1}) compared to WT (7825 ± 373 ml.min^{-1}.kg^{-1}) [$F(3, 20) = 66.9$; $P < 0.001$] and this was mainly caused by a smaller increase in V_T (Figure 3-A). LepR/POMC-cre and MC4R$^{-/-}$ mice also exhibited the smallest increase in f_R under 7% CO_2 compared to WT mice (Figure 3A). LepR/Nestin-cre mice presented f_R similar to WT (Figure 3). The changes in ventilatory response during 7% CO_2 were also attenuated when compared to WT values (Figure 3-B).

DISCUSSION

The present study demonstrates that chronic MC3/4R blockade with SHU9119 administered into the LV of rats reduced ventilatory responses to hypercapnia and abolished leptin-induced increase in baseline ventilation and in the respiratory response to central chemoreceptor activation. This suggests that the ventilatory effects of leptin depend on the activation of central MC3/4R. Moreover, the present results also showed that mice with leptin receptor deletion in the entire CNS or specifically in POMC neurons as well as mice with MC4R deficiency exhibit attenuated baseline and ventilatory responses to CO_2, which reinforces the notion that leptin-induced improvement in ventilatory function is mediated by leptin's direct actions on the CNS and requires functional brain melanocortin system including POMC-MC4R pathway.

The present results also corroborate previous studies demonstrating that chronic CNS MC3/4R blockade increases appetite, promotes rapid weight gain, abolishes leptin's ability to raise BP (Kuo *et al*, 2003, da Silva *et al*., 2004) and to reduce RQ (Dubinion *et al*., 2010). LV leptin administration for 7 days decreased daily food intake and water intake and consequently body weight, without altering urine volume. However, as expected, due the marked reduction in food intake, daily urinary Na^+ and K^+ excretion were also reduced during leptin treatment. Similar to previous studies (Wang *et al*., 1999, Hogberg *et al*., 2006, Dubinion *et al*., 2010) our results also demonstrate that leptin decreases RQ due to a decreased VCO_2. In spite of the reduction of VCO_2, leptin improved the ventilatory response, which suggests that the effects of leptin on ventilation are due to a direct action on central mechanisms that control breathing and not to metabolic changes.

Previous studies have suggested that leptin facilitates ventilatory responses mainly by acting on brainstem areas involved in breathing control including the nucleus of solitary tract (NTS) (Inyushkina *et al*., 2010, Ciriello & Moreau 2013) and ventrolateral medulla (Bassi *et al*., 2012 and 2014). In the present study leptin was delivered into the LV and may have reached forebrain or hindbrain regions that contribute to breathing control. Leptin receptor deletion specifically in POMC neurons resulted in reduced ventilatory responses to hypercapnia, but given that POMC neurons are present in many areas of brain it is difficult to determine which groups of POMC neurons are most important in modulating ventilatory function and perhaps mediating, at least in part, leptin's effects on breathing. Thus, additional studies are needed to address these questions.

Although an action of leptin in the forebrain to facilitate ventilatory responses is possible, these responses to chemical stimulation like hypercapnia and hypoxia result from an increased activity of the medullary neuronal network responsible for the generation and maintenance of the basic respiratory rhythm. However, since the 1960s and 1970s hypoxia or hypercapnia, as well as the electrical stimulation of the carotid sinus nerve, have been shown to activate the caudal hypothalamic region (Cross, 1963, Thomas, 1972) as well other areas of the brain like periaqueductal grey mesencephalic region (Lopes et al., 2014). Moreover, acid-sensing ion channels (ASICs) and transient receptor potential ankyrin subfamily member 1 (TRPA1), both described as a mediators of chemorespiratory responses can be found in the hypothalamus (Song *et al*., 2012, Pokorski *et al*., 2014). Elevated CO_2 markedly increases c-Fos positive cells in PVN (Kc *et al*., 2002) and electrical stimulation of the PVN promotes an increase in ventilation (Duan *et al*., 1996, Yeh, 1997). Additionally, the disinhibition of neurons in the dorsomedial hypothalamus (DMH) promotes an increase in phrenic nerve activity (Mc Dowwall *et al*., 2007), while microinjection of gabazine into the perifornical region (PeF) in the hypothalamus, a region that express leptin receptors (Laque *et al*., 2013) promotes an increase of blood pressure, phrenic nerve discharge and firing rate of the chemosensitive retrotrapezoid neurons (Fortuna *et al*., 2009, Li & Nattie., 2013).

Therefore, with the present data it is not possible to determine the specific area of the brain where the melanocortin receptors activated by leptin to mediate ventilation are located.

Similar to a previous study (da Silva *et al*., 2004), our data demonstrate that leptin-mediated increase in BP is abolished by pre-treatment with SHU9119, which indicates the involvement of MC3/4R in mediating the actions of leptin on BP regulation. However, contrary to the previous study, we did not observe a reduction in BP or HR during SHU9119 treatment alone. One potential explanation for this difference may be the fact that in the present study we administered SHU9119 via daily LV bolus microinjections (0.6 nmol/day), whereas in the previous study SHU9119 was delivered continuously into the LV using osmotic minipumps at a rate of 1 nmol/h. Although SHU9119 treatment regimen used in the present study did not alter BP or HR, it produced the expected effects on appetite and it also affected ventilatory function.

In conclusion, the present results suggest that the brain melanocortin system, present including POMC neurons and MC3/4R, is a key component of the central mechanism

activated by leptin to facilitate ventilatory responses. This study extends our previous findings and demonstrates that a functional brain melanocortin system is critical for leptin's regulation of appetite, energy homeostasis and cardiovascular regulation and highlights yet another major important physiological function of the melanocortin system - modulation of ventilatory function.

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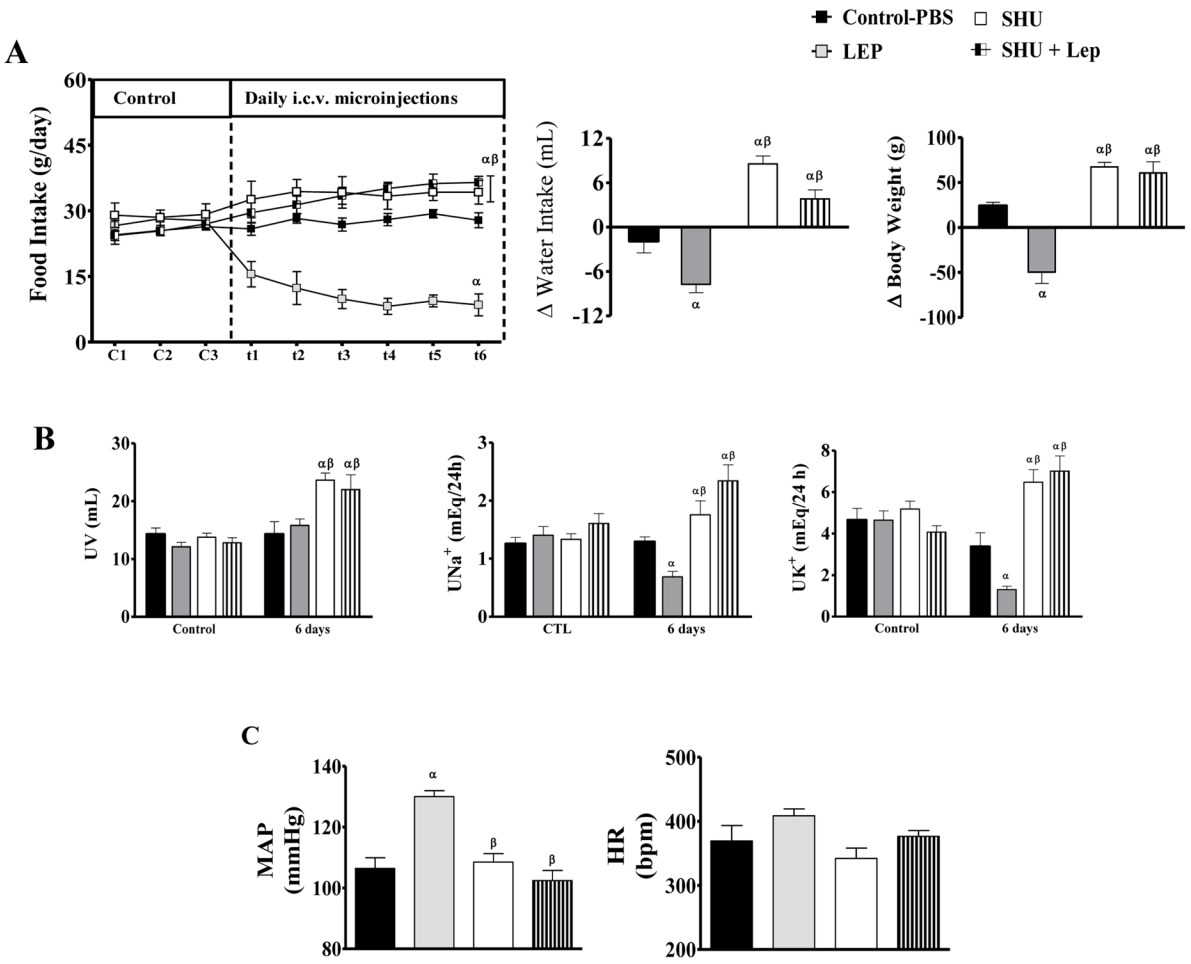

Figure 1.

(A) Daily food intake and changes in daily water intake and body weight. (B) Urinary volume (UV), Na^+ (UNa^+) and K^+ (UK^+) excretion during control period (C1-C3) and during LV treatment (t1-t6) with PBS (control), (LEP, 5 μg/day), SHU-9119 (SHU, 0.6 nmol/day) or SHU-9119 + leptin for 7 days. (C) Mean arterial pressure (MAP) and heart rate (HR) after LV treatments. n = 6/group. α = $p<0.05$ versus control-PBS group. β= $p<0.05$ versus leptin-treated group.

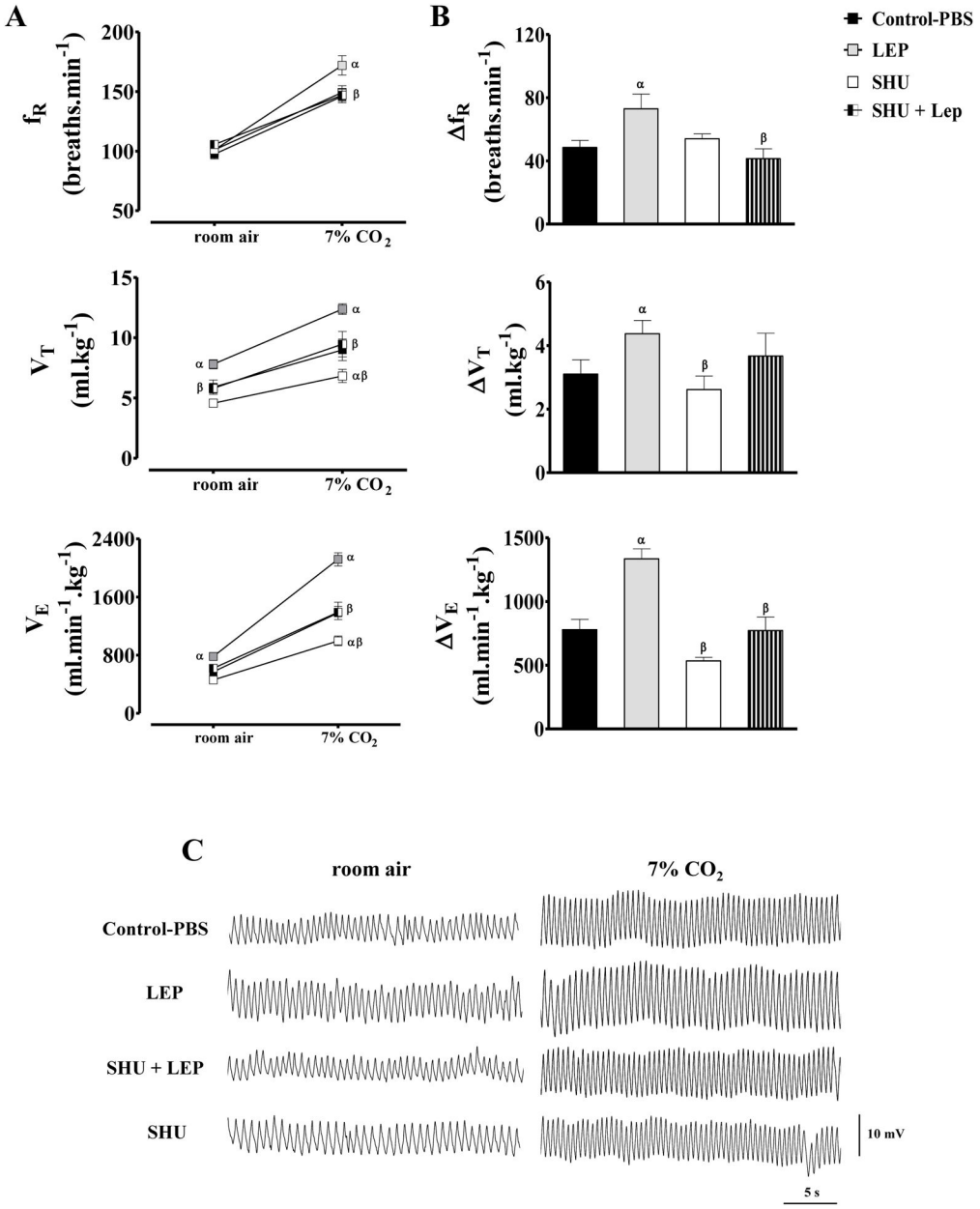

Figure 2.

(A) Respiratory frequency (f_R), tidal volume (V_T) and ventilation (V_E) under normocapnia (room air) or hypercapnia (7% CO_2), (B) changes (Δ) of f_R, V_T and V_E produced by hypercapnia and (C) representative traces of rats breathing room air and 7% CO_2 after receiving LV microinjections of PBS (control), (LEP, 5 μg/day), SHU-9119 (SHU, 0.6 nmol/day) or SHU-9119 + leptin for 6 days. n=6/group. α = $p<0.05$ versus control-PBS group. β = $p<0.05$ versus leptin-treated group.

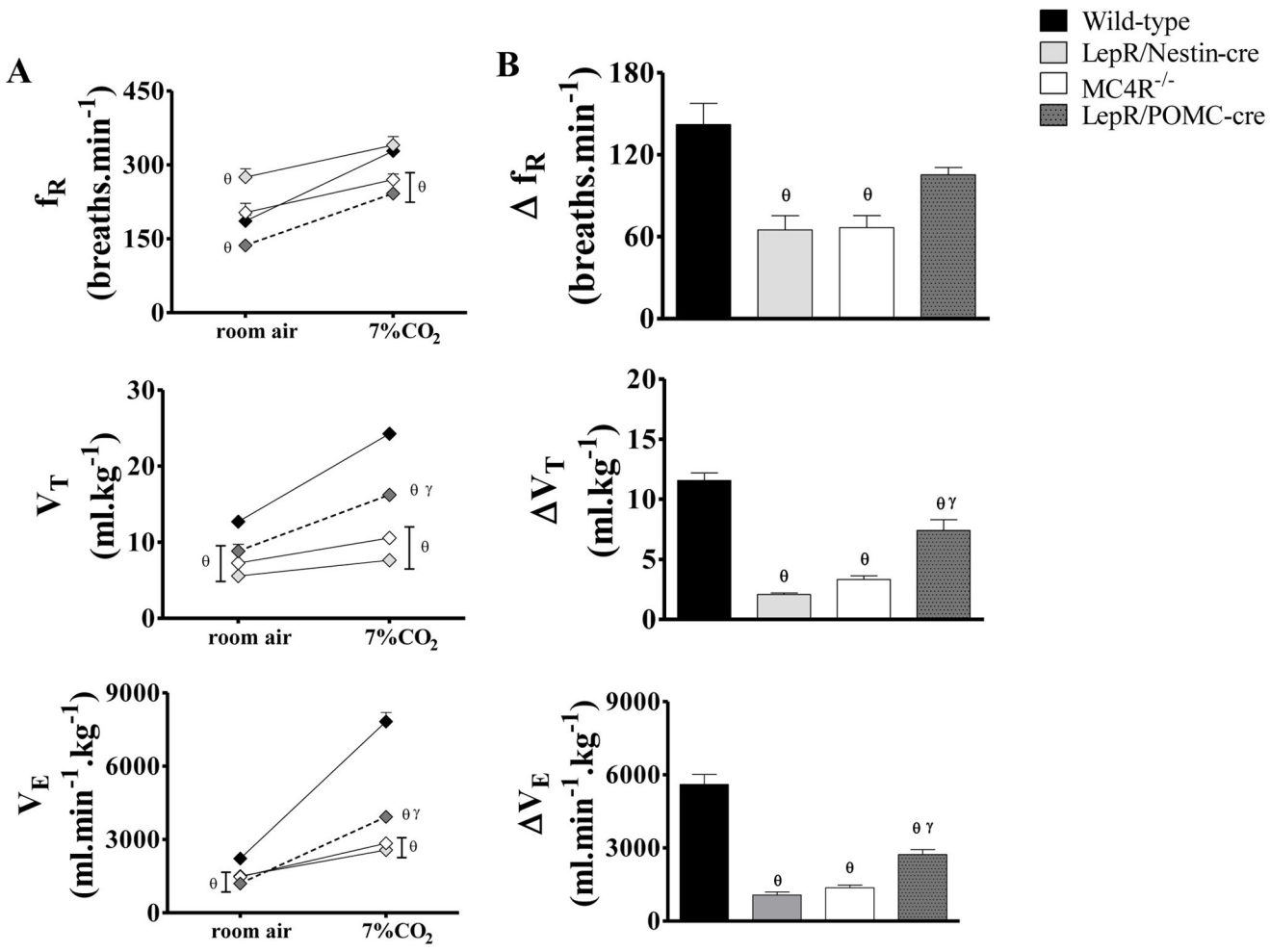

Figure 3.

(A) Respiratory frequency (f_R), tidal volume (V_T) and ventilation (V_E) under normocapnia (room air) or hypercapnia (7% CO_2) and (B) changes (Δ) of f_R, V_T and V_E produced by hypercapnia in wild-type mice, in mice with leptin receptor deletion in the entire brain (LepR/Nestin-cre), in mice with leptin receptor deletion in POMC neurons (LepR/POMC-cre) and in mice with MC4R deficiency ($MC4R^{-/-}$). n=3–5/group. θ = $p<0.05$ versus wild-type group and γ = $p<0.05$ versus LepR/Nestin-cre group.

Table 1

VCO_2, VO_2, RQ, rectal temperature and body weight of rats treated with i.c.v. injections of PBS (control), leptin, SHU-9119 or SHU-9119 combined with leptin for 7 days.

	PBS	LEP	SHU	SHU + LEP
VCO_2 ($ml.kg^{-1}.min^{-1}$)	12.9 ±1	10.9 ±0.7	12.7 ±0.5	11.3 ±0.5
VO_2 ($ml.kg^{-1}.min^{-1}$)	15.1 ±1	15.1 ±0.6	13.8 ±0.6	13.7 ±0.3
RQ (VCO_2/VO_2)	0.86 ±0.04	0.73 ±0.02 $^{\alpha}$	0.92 ±0.01 $^{\beta}$	0.83 ±0.04 $^{\beta;}$
Rectal Temperature (°C)	36.7 ±0.2	36.8 ±0.2	36.6 ±0.2	36.7 ±0.1
Body Weight (g)	342 ±8	286 ±9 $^{\alpha}$	375 ±7 $^{\alpha\beta}$	386 ±12 $^{\alpha\beta}$

Values are means ±SE of metabolic rate (VCO_2, CO_2 consumption; VO_2, oxygen consumption; RQ respiratory quotient) after 6 days of i.c.v. treatments with PBS (3 µl/day), leptin (5 µl/3 µl/day), SHU9119 (0.6 nmol/3 µl/day) or SHU9119+leptin (0.6 nmol/3 µl/day and 5 µg/3 µl/day, respectively). n = 6/group. α different from PBS and β different from leptin. ANOVA one-way ($P < 0.05$).