

## LEPTIN AND GABA INTERACTIONS ON THERMOREGULATION OF RATS

**Krassimira S. Yakimova,  
Rumen P. Nikolov,  
Ivan G. Todorov,  
Milen H. Hristov**

*Department of Pharmacology &  
Toxicology,  
Faculty of Medicine,  
Medical University of Sofia*

### Summary

Leptin inhibits feeding, reduces body weight and increases thermogenesis. Experimental data suggest involvement of GABAergic mechanisms in the regulation of feeding behavior and energy balance. The present study was set to determine the effect of combinations from leptin, GABA<sub>B</sub>-agonist baclofen and GABA<sub>B</sub>-antagonist CGP35348 on thermoregulation of male Wistar rats, using *in vivo* and *in vitro* experiments. The substances used for *in vivo* experiments were administered intraperitoneally (i.p.). The measurement of the body temperature was done via thermistor probes (TX8) and monitored on multichannel recorder Iso-Thermex16. *In vitro* experiments were conducted on rat PO/AH neurons, recorded extracellularly by conventional electrophysiological equipment, using brain slice preparations. The separate intraperitoneal injection of leptin as well as GABA<sub>B</sub>-antagonist CGP35348 produced significant hyperthermia in rats while the GABA<sub>B</sub>-agonist baclofen caused a decrease in the core body temperature. The probable synergy between the hyperthermic effects of leptin and GABA<sub>B</sub>-antagonist did not occur. On the contrary, the effect of this combination was lower as compared to the result of the separate administration of GABA<sub>B</sub>-antagonist. When leptin was applied just prior to GABA<sub>B</sub>-agonist baclofen, neither of their separate effects appeared. *In vivo* effects determined correlated with *in vitro* changes of firing rate observed in PO/AH neurons. The data from this study provide a new point of view concerning the interactions of leptin and GABA on the level of thermoregulation. These results represent a step forward in understanding the complicated mechanisms involved in thermoregulation.

**Key words:** Leptin, GABA<sub>B</sub>-agonist and antagonist, firing rate, PO/AH neurons

### Introduction

Leptin is a 167-aminoacid polypeptide hormone, which is produced by adipocytes. In the brain, leptin inhibits expression of neuropeptide Y and agouti-related peptide by binding to tyrosine-kinase associated receptors. Leptin inhibits feeding, reduces body weight and increases thermogenesis [1]. In the last years leptin has been widely studied not only because of its relatively recent discovery but

### Corresponding Author:

Krassimira S. Yakimova  
e-mail: kyakim@medfac.acad.bg

**Received:** May 15, 2014

**Revision received:** July 18, 2014

**Accepted:** November 24, 2014

also because of the variety of its effects and the interaction with other neuromodulators or neurotransmitters.

The preoptic area of the anterior hypothalamus (PO/AH) plays a prominent role in thermoregulation and strongly influences each of the lower effector areas. The neurons in PO/AH are supposed to build a neural network which takes part in the central control of body temperature [2]. These neurons are affected by different neurotransmitters and neuromodulators [3]. The substances altering body temperature induce specific changes in the activity and/or temperature sensitivity of PO/AH neurons.

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the central nervous system. The action of GABA is mediated by receptors belonging to three distinct types, termed GABA<sub>A</sub>, GABA<sub>B</sub> and GABA<sub>C</sub> [4]. Experimental data suggest involvement of GABAergic mechanisms in the regulation of feeding behavior and energy balance [5, 6].

GABA could modify neurotransmission processes on the level of central temperature controller - PO/AH neurons. Experiments in rat brain slices presented that GABA<sub>A</sub> and GABA<sub>B</sub> agonists dose-dependently decreased the firing rate of warm-sensitive and temperature-insensitive PO/AH neurons, while the temperature sensitivity of rat PO/AH neurons was only changed by ligands of GABA<sub>B</sub>-receptors and this effect has been restricted to temperature-sensitive neurons [7].

The aim of this study was to investigate interactions between leptin and GABA<sub>B</sub>-agonist and antagonist on thermoregulation in rats, using *in vivo* and *in vitro* experiments.

## Materials and Methods

### Substances

Leptin (OB) Rat Recombinant (Sigma, Germany), CGP 35348 (Sigma, Germany) and Baclofen (Sigma, Germany) were used in the study. The doses used were determined on the basis of literature data, as well as on previous experiments of our own.

The substances were administered systemically (intraperitoneally, i.p.) in a volume 0.2 ml/100 g body weight (Leptin - 0.5 mg/kg; Baclofen - 5 mg/kg; CGP35348 - 5 mg/kg) and compared with a control group treated with the same volume of saline (NaCl).

Regarding firing rate changes of PO/AH

neurons, Leptin (10 nM), Baclofen (1  $\mu$ M) and CGP35348 (10  $\mu$ M), previously prepared as stock solutions, were added separately or in combinations as bolus (0.1 ml) to the perfusion with oxygenated ACSF. Only one neuron per slice was tested.

### *In vivo* experiments

#### Experimental animals

The experiments were carried out on male Wistar rats (weight range 180-200 g), divided into groups of 6-8 rats each. The animals were maintained on a standard 12 h light/dark cycle and allowed food and water ad libitum. All experiments started at 10 a.m. and were conducted at ambient temperature 22  $\pm$  1°C. In the handling and care of all animals, the International Guiding Principles for Animal Research were strictly followed.

#### Monitoring of body temperature

Temperature was measured with thermistor probes (TX8) inserted rectally to a depth of 6 cm and monitored on multichannel recorder Iso-Thermex16 (Columbus Instruments, USA). The initial temperature of the animals was taken, and then checked at 30-minute intervals until 150 minutes after injecting substances. The movements of the rats were slightly restricted, as previously described by Rosow et al. [8].

#### Statistical analysis

The results were expressed as delta ( $\Delta$ ) values (average changes in temperature compared to the initial one) (mean  $\Delta$  values  $\pm$  S.E.M.) and analyzed with two-way analysis of variance. For statistical significance a Student's t-test was used.

### *In vitro* experiments

Slices (400  $\mu$ m) from the preoptic area/anterior hypothalamus (PO/AH) of male Wistar rats (200-220g) were prepared and stored as described by Schmid and Pierau, 1993 [9]. Extracellular recordings of the neuronal activity were made with glass-covered platinum-iridium electrodes during continuous perfusion with oxygenated artificial cerebrospinal fluid (ACSF) at a rate of 2 ml/min. Neuronal activity and slice temperature were recorded and stored on a personal computer using a CED (Cambridge Electronic Design)-company 1401 interface and the CED software spike 2, and a digital tape recorder (DAT).

Changes in neuronal activity (firing rate, FR) were calculated with Spike 2 data analysis

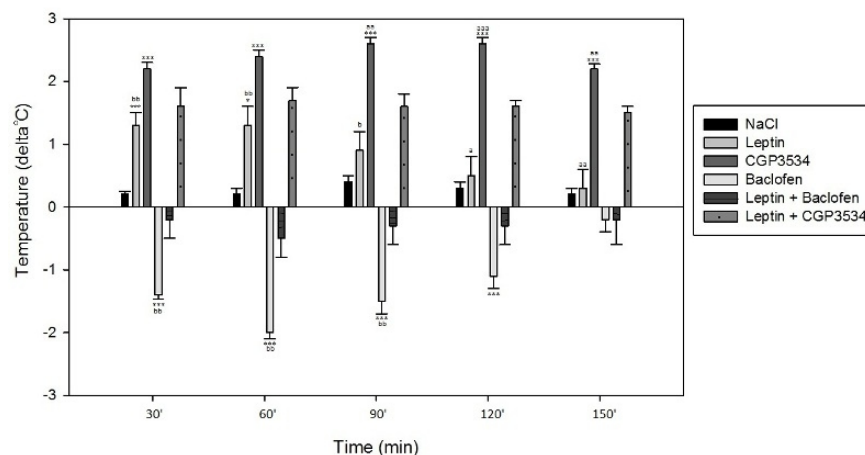
program, providing information on the mean value of firing rate for 1 min, recorded before and after application of the substances. All data are presented as means  $\pm$  S.E.M. For statistical evaluation a paired t-test was performed.

## Results

### *In vivo experiments*

Systemic (i.p.) administration of leptin in a dose

0.5 mg/kg caused moderate increase in body temperature of rats significant at 30<sup>th</sup> and 60<sup>th</sup> min after leptin injection. GABA<sub>B</sub> receptor antagonist CGP35348 (5 mg/kg i.p.) produced significant ( $P<0.001$ ) long lasting hyperthermia occurred at 30<sup>th</sup>, 60<sup>th</sup>, 90<sup>th</sup>, 120<sup>th</sup> and 150<sup>th</sup> min after application. Injection of GABA<sub>B</sub> receptor agonist baclofen in a dose 5 mg/kg i.p. caused significant decrease ( $P<0.001$ ) in core body temperature in rats between 30<sup>th</sup> and 120<sup>th</sup> min (Fig. 1).



**Figure 1.** Effects of intraperitoneal (i.p.) injection of Leptin, Baclofen and CGP35348 on the core body temperature of rats, applied separately and in combinations. Mean changes (temperature delta°C) after i.p. administration of Leptin (0.5 mg/kg), Baclofen (5 mg/kg) and CGP35348 (5 mg/kg), separately and in combinations. Significant differences in comparison with control group (NaCl): \* $P<0.05$ , \*\*\* $P<0.001$ . Significant differences in comparison with combination Leptin+CGP35348: <sup>a</sup> $P<0.05$ ; <sup>aa</sup> $P<0.01$ ; <sup>aaa</sup> $P<0.001$ . Significant differences in comparison with a combination of Leptin+Baclofen: <sup>b</sup> $P<0.05$ , <sup>bb</sup> $P<0.01$ .

Administration of baclofen (5 mg/kg i.p.) just prior leptin (0.5 mg/kg i.p.) altered the effects on body temperature of both leptin and baclofen. Neither hyperthermic effect of leptin nor hypothermic effects of baclofen were registered. (Fig. 1)

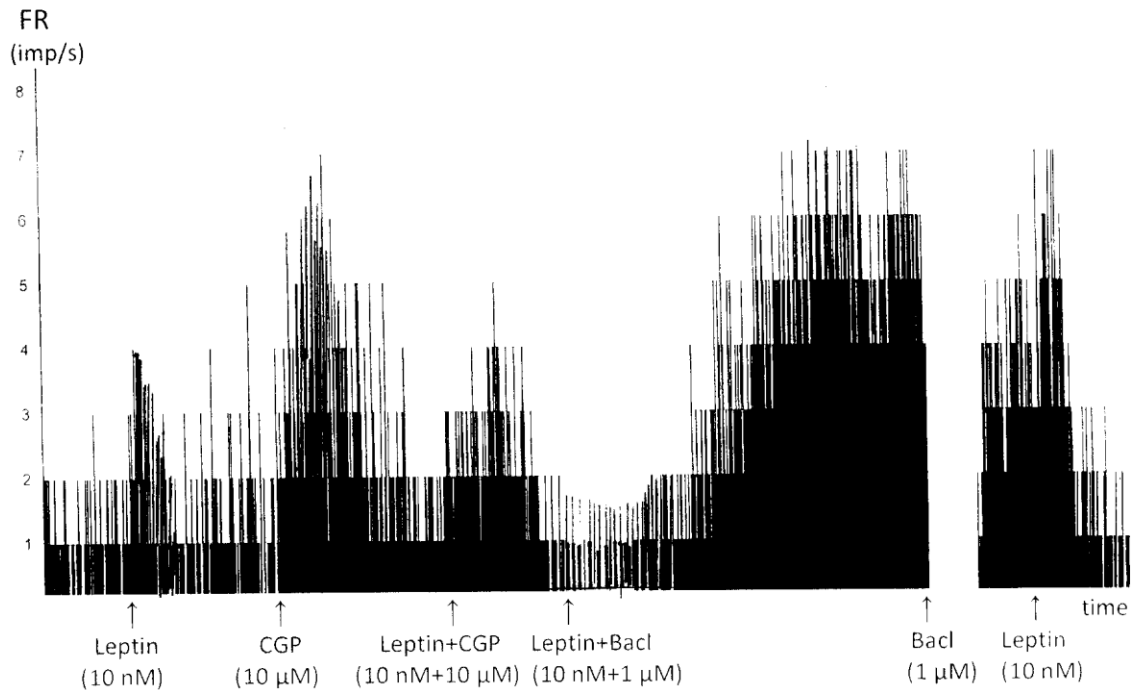
There was no synergism in the hyperthermic effect of leptin and CGP35348. Administration of CGP35348 (5 mg/kg i.p.) just prior leptin (0.5 mg/kg i.p.) caused lower hyperthermic reaction in comparison with CGP35348 (5 mg/kg i.p.) when applied alone (Fig. 1).

### *In vitro experiments*

Extracellular recordings were obtained from neurons (regardless of their type of temperature sensitivity) in slices of the hypothalamic medial preoptic area of rats to investigate the changes in firing rate by Leptin (10 nM) and GABA<sub>B</sub>-antagonist CGP35348 (10  $\mu$ M), applied

separately or in combination by different order, as well as by Leptin (10 nM) and GABA<sub>B</sub>-agonist baclofen (1  $\mu$ M). We used an administration in bolus of the experimental drugs during constant perfusion of the slice preparation with ACSF.

Leptin, like GABA<sub>B</sub>-antagonist CGP35348 increased firing rate in rat PO/AH neurons, while the GABA<sub>B</sub>-agonist baclofen caused a dose-response decrease in firing rate (Fig. 2). The probable synergy between the effects of leptin and GABA<sub>B</sub>-antagonist was not registered. On the contrary, the effect of this combination was lower as compared to the result of separate administration of GABA<sub>B</sub>-antagonist. When leptin was applied in combination with GABA<sub>B</sub>-agonist baclofen, neither of their separate effects appeared (Fig. 2). After treatment with leptin and GABAergic agents the neuronal activity (firing rate) improved (Fig. 2).



**Figure 2.** Interactions of Leptin and CGP35348 or Baclofen on firing rate of rat PO/AH neuron. Original recordings of firing rate (FR, imp/s) from neuron of the medial preoptic area in rat slice preparation. Leptin, CGP35348 and Baclofen (Bacl) (arrows, bolus injection 0.1 ml of 10 nM, 10  $\mu$ M and 1  $\mu$ M, respectively), applied separately and in combinations. Note spontaneous improvement of firing rate of the neuron after treatment with Leptin and GABAacting agents.

## Discussion

Our results suggest that systemic administration of leptin, as well as of GABA<sub>B</sub>-antagonist CGP35348, produced significant hyperthermia in rats, while the GABA<sub>B</sub>-agonist baclofen caused a decrease in core body temperature of rats. However, there was no synergism in the hyperthermic effect of leptin and GABA<sub>B</sub>-antagonist. Also, neither hyperthermic effect of leptin nor hypothermic effect of GABA<sub>B</sub>-agonist occurred when the substances were applied in combinations. *In vitro* changes of firing rate observed in PO/AH neurons were in correlations with the effects determined *in vivo*. There was no synergism between leptin and GABA<sub>B</sub>-antagonist or GABA<sub>B</sub>-agonist on the firing rate of PO/AH neurons in rats.

Data has been published to prove the existence of leptin–GABA interactions on different levels [10]. Leptin acts in the brain to prevent obesity. Remarkably, the vast majority of

antiobesity effects of leptin are mediated by GABAergic neurons [11]. Leptin, working directly on presynaptic GABAergic neurons, reduces inhibitory tone to postsynaptic POMC neurons. As POMC neurons prevent obesity, their disinhibition by leptin action on presynaptic GABAergic neurons probably mediates, at least in part, leptin's antiobesity effects [12].

Recently, a strong GABAergic modulation of leptin was postulated. The data showed a strong association between leptin levels and doses of GABA-mimetic substance clomethiazole [13].

Leptin has been shown to inhibit norepinephrine (NE) efflux from the hypothalamus in a dose-dependent manner. Recent results have demonstrated for the first time that leptin could act directly on the hypothalamus to inhibit NE efflux through GABA. It was concluded that leptin could probably produce its central and neuroendocrine effects by modulating NE and GABA levels in the hypothalamus [14].

## Conclusion

The results presented are a step to understanding the complicated interactions of neuromodulatory acting substances on the level of central temperature controller – the neurons of PO/AH, and provide a new point of view concerning the interactions of leptin and GABA on the level of thermoregulation.

## References

1. Henry BA, Andrews ZB, Rao A, Clarke IJ. Central leptin activates mitochondrial function and increases heat production in skeletal muscle. *Endocrinology*. 2011;152(7):2609-18.
2. Boulant JA. Role of preoptic-anterior hypothalamus in thermoregulation and fever. *Clin Infect Dis*. 2000;31(Suppl 5):S157-61.
3. Pierau F-K, Sann H, Yakimova KS, Haug P. Plasticity of hypothalamic temperature-sensitive neurons. *Prog Brain Res*. 1998;115:63-84.
4. Bormann J. The 'ABC' of GABA receptors. *Trends Pharmacol Sci*. 2000;21(1):16-19.
5. Patel SM, Ebenezer IS. The effects of intraperitoneal and intracerebroventricular administration of the GABA<sub>B</sub> receptor antagonist CGP 35348 on food intake in rats. *Eur J Pharmacol*. 2004;503(1-3):89-93.
6. Arima H, Oiso Y. Positive effect of baclofen on body weight reduction in obese subjects: a pilot study. *Intern Med*. 2010;49(19):2043-7.
7. Yakimova KS, Sann H, Schmid HA, Pierau F-K. Effects of GABA agonists and antagonists on temperature sensitive neurons in the rat hypothalamus. *J Physiol - London*. 1996;494(1):217-30.
8. Rosow CE, Miller JM, Poulsen-Burke J, Cochin J. Opiates and thermoregulation in mice. I. Agonists. *J Pharmacol Exp Ther*. 1980;213(2):273-83.
9. Schmid HA, Pierau F-K. Temperature sensitivity of neurons in slices of the rat PO/AH hypothalamic area: effect of calcium. *Am J Physiol*. 1993;264(2 Pt 2):R440-8.
10. Nakamura Y, Hinoi E, Takarada T, Takahata Y, Yamamoto T, Fujita H, et al. Positive regulation by GABA(B)R1 subunit of leptin expression through gene transactivation in adipocytes. *PLoS One*. 2011;6(5):201-67.
11. Xu Y, O'Brien WG 3<sup>rd</sup>, Lee CC, Myers MG Jr, Tong Q. Role of GABA release from leptin receptor-expressing neurons in body weight regulation. *Endocrinology*. 2012;153(5):2223-33.
12. Vong L, Ye C, Yang Z, Choi B, Chua S Jr, Lowell BB. Leptin action on GABAergic neurons prevents obesity and reduces inhibitory tone to POMC neurons. *Neuron*. 2011;71(1):142-54.
13. Dammann G, Walter M, Gremaud-Heitz D, Wolfersdorf M, Hartmann S, Wurst FM. Association between leptin levels and doses of clomethiazole during alcohol withdrawal: a pilot study. *Eur Addict Res*. 2012;18(1):12-5.
14. Francis J, MohanKumar SM, MohanKumar PS. Leptin inhibits norepinephrine efflux from the hypothalamus in vitro: role of gamma aminobutyric acid. *Brain Res*. 2004;1021(2):286-91.