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Original Article

LEPTIN AND GABA INTERACTIONS ON THERMOREGULATION OF RATS

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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, $GABA_B$-agonist baclofen and $GABA_B$-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 extracellulary by conventional electrophysiological equipment, using brain slice preparations. The separate intraperitoneal injection of leptin as well as $GABA_B$-antagonist CGP35348 produced significant hyperthermia in rats while the $GABA_B$-agonist baclofen caused a decrease in the core body temperature. The probable synergy between the hyperthermic effects of leptin and $GABA_B$-antagonist did not occur. On the contrary, the effect of this combination was lower as compared to the result of the separate administration of $GABA_B$-antagonist. When leptin was applied just prior to $GABA_B$-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, $GABA_B$-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

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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 $GABA_A$, $GABA_B$ and $GABA_C$ [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 $GABA_A$ and $GABA_B$ 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 $GABA_B$-receptors and this effect has been restricted to temperature-sensitive neurons [7].

The aim of this study was to investigate interactions between leptin and $GABA_B$-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 μM) and CGP35348 (10 μM), 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 1C. 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 (Δ) values (average changes in temperature compared to the initial one) (mean Δ 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 μm) 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 ± 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 30th and 60th min after leptin injection. $GABA_B$ receptor antagonist CGP35348 (5 mg/kg i.p.) produced significant ($P<0.001$) long lasting hyperthermia occurred at 30th, 60th, 90th, 120th and 150th min after application. Injection of $GABA_B$ receptor agonist baclofen in a dose 5 mg/kg i.p. caused significant decrease ($P<0.001$) in core body temperature in rats between 30th and 120th min (Fig. 1).

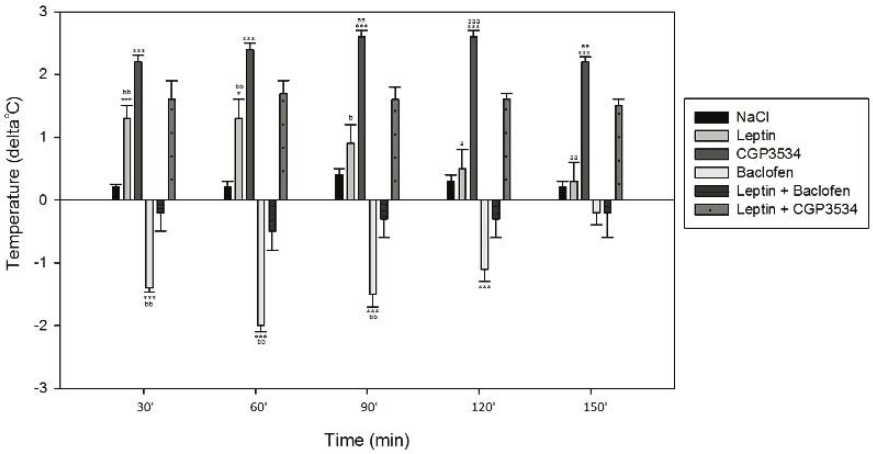

Figure 1. Effects of intraperitoneal (i.p.) injection of Leptin, Baclofen and CGP35348 on the core body temperature of rats, applied separately and in combinations. Mean changes (temperature deltaC) after i.p. administration of Leptin (0.5 mg/kg), Baclofen (5 mg/kg) and CGP35348 (5 mg/kg), separately 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: $^{a}P<0.05$; $^{aa}P<0.01$; $^{aaa}P<0.001$. Significant differences in comparison with a combination of Leptin+Baclofen: $^{b}P<0.05$, $^{bb}P<0.01$.

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 $GABA_B$-antagonist CGP35348 (10 μM), applied separately or in combination by different order, as well as by Leptin (10 nM) and $GABA_B$-agonist baclofen (1 μM). We used an administration in bolus of the experimental drugs during constant perfusion of the slice preparation with ACSF.

Leptin, like $GABA_B$-antagonist CGP35348 increased firing rate in rat PO/AH neurons, while the $GABA_B$-agonist baclofen caused a dose-response decrease in firing rate (Fig. 2). The probable synergy between the effects of leptin and $GABA_B$-antagonist was not registered. On the contrary, the effect of this combination was lower as compared to the result of separate administration of $GABA_B$-antagonist. When leptin was applied in combination with $GABA_B$-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).

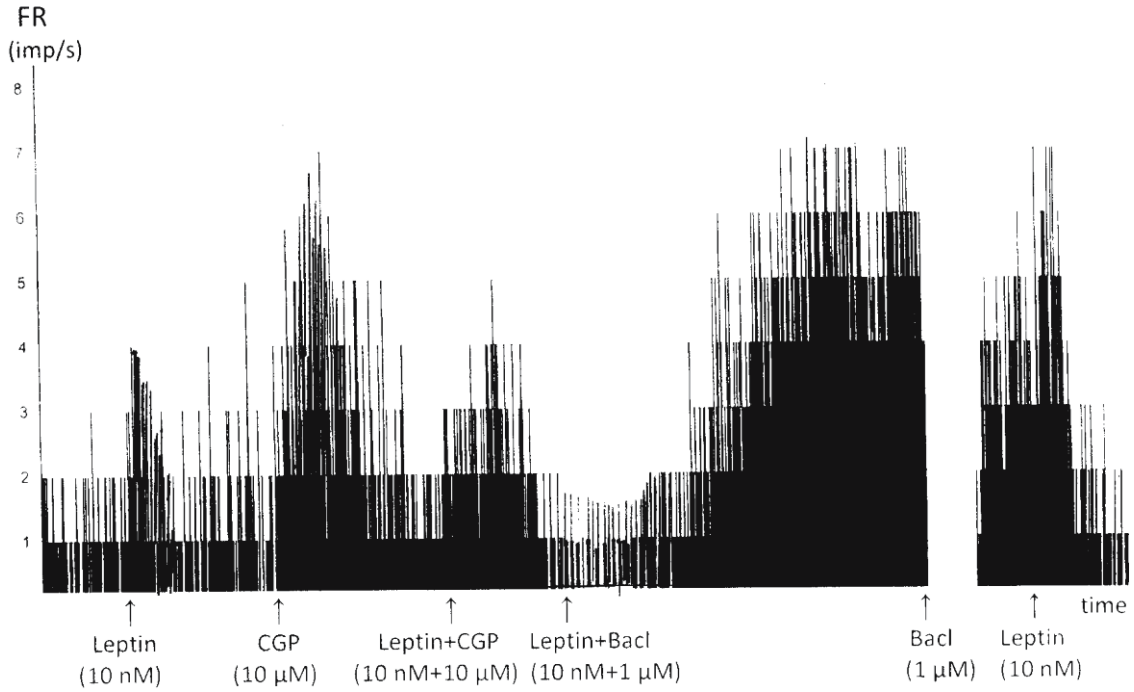

Figure 2. Interactions of Leptin and CGP35348 or Baclofen on firing rate of rat PO/AH neuron. Original recordings of firing rate (FR, imp/s) from neuron of the medial preoptic area in rat slice preparation. Leptin, CGP35348 and Baclofen (Bacl) (arrows, bolus injection 0.1 ml of 10 nM, 10 μM and 1 μM, 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 $GABA_B$-antagonist CGP35348, produced significant hyperthermia in rats, while the $GABA_B$-agonist baclofen caused a decrease in core body temperature of rats. However, there was no synergism in the hyperthermic effect of leptin and $GABA_B$-antagonist. Also, neither hyperthermic effect of leptin nor hypothermic effect of $GABA_B$-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 $GABA_B$-antagonist or $GABA_B$-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.

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