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Interactions of leptin and gaba-acting drugs on firing rate of PO/AH neurons

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ABSTRACT

GABA is the main inhibitory neurotransmitter in the central nervous system. Leptin, the obese gene peptide, is involved in the regulation of feeding behavior and energy balance. The present study was set to determine the changes of firing rate in the neurons of the preoptic area/anterior hypothalamus (PO/AH) in male Wistar rats after administration of leptin, $GABA_B$-agonist baclofen and $GABA_B$-antagonist CGP35348 applied separately or in combinations. *In vitro* experiments were made on PO/AH neurons by conventional electrophysiological equipment, using brain slice preparation. To trace the changes in neuronal firing rate we used administration in bolus of the experimental substances during constant perfusion of the slice preparation with artificial cerebrospinal fluid (ACSF). The separate bolus injection of leptin as well as $GABA_B$-antagonist CGP35348 produced significant increase of firing rate in rat PO/AH neurons, while the $GABA_B$-agonist baclofen caused a decrease in firing rate. The probable synergy between the effects of leptin and $GABA_B$-antagonist was not occurred. In the opposite, effect of this combination was lower compared to the result of the separate administration. When leptin was applied in combination with $GABA_B$-agonist baclofen neither one of their separate effects appeared.

The data from this study provide a new point of view concerning the interactions of leptin and GABA. These results are step of understanding the complicate mechanisms involved in the interactions of neurotransmitters and neuromodulators in the central nervous system.

KEYWORDS

Leptin;
$GABA_B$-agonist;
$GABA_B$-antagonist;
Firing rate;
Interaction;
PO/AH neurons;
Rat;
Brain slices.

INTRODUCTION

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 neuronal network which takes part in the central control of body temperature[1]. These neurons are affected by different neurotransmitters and neuromodulators[2].

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the central nervous system. GABA could also modify neurotransmission processes on the level of central temperature controller - PO/AH neurons.

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The action of GABA is mediated by receptors belonging to three distinct types, termed $GABA_A$, $GABA_B$ and $GABA_C$ [3]. $GABA_A$ and $GABA_C$ receptors form membrane channels (ionotropic receptors), while $GABA_B$ receptors belong to the family of G-protein-coupled receptors (metabotropic receptors). The substances altering body temperature induce specific changes in the activity and/or temperature sensitivity of neurons in the preoptic area of the anterior hypothalamus (PO/AH). 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[4].

Leptin, the obese gene peptide, is involved in the regulation of feeding behavior and energy balance. The action of peripherally released leptin at long-form leptin receptors within the brain represents a fundamental axis in the regulation of energy homeostasis and body weight[5]. In the last years leptin is widely studied not only because of its relatively recent discovery but also because of the variety of its own effects and the interaction with other neuromodulators or neurotransmitters. Leptin regulates energy balance largely through isoform B leptin receptor-expressing neurons (LepR neurons) in the brain[6].

In order to contribute to understanding the mechanisms of action on the level of the central temperature controller - PO/AH neurons, the interactions between leptin, $GABA_A$-agonist and $GABA_B$-antagonist on neuronal activity (firing rate) were studied in a brain slice preparation of rat PO/AH neurons.

MATERIALS AND METHODS

Extracellular recordings

Slices (400 μm) from the preoptic area/anterior hypothalamus (PO/AH) of male Wistar rats (200-220g) were prepared and stored as previously described Schmid and Pierau, 1993[7]. 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).

Temperature sensitivity of neurons was determined by application of sinusoidal temperature stimulus and calculated by a computer program relating the discharge rate of the neuron (bin width = 5 s) to the actual temperature, and fitting either one linear or two piecewise regression lines through the data[8]. The slope of the steepest regression line was used as the temperature coefficient (TC) of the unit. Changes in neuronal activity (firing rate, FR) were calculated with the aid of the same computer program, providing information on the mean value of firing rate for the duration of 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.

Substances

Leptin (OB) Rat Recombinant (Sigma, Germany), Baclofen (Sigma, Germany) and CGP35348 (Sigma, Germany) were used in this study. The doses used during the investigation were defined by literature data as well as own previously made experiments.

Regarding to firing rate changes, Leptin (100 nM), Baclofen (0.1 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.

RESULTS

Extracellular recordings were obtained from 24 neurons in slices of the hypothalamic medial preoptic area of rats. Twelve extracellulary recorded neurons, regardless of their type of temperature sensitivity were used to investigate the changes in firing rate by Leptin (100 nM) and $GABA_B$-antagonist CGP35348 (10 μM), applied separately or in combination by different order, as well as the same design of experiment was used about investigation of Leptin (100 nM) and $GABA_B$-agonist baclofen (0.1 μM).

Leptin increased firing rate as well as $GABA_B$-antagonist CGP35348 produced significant increase of

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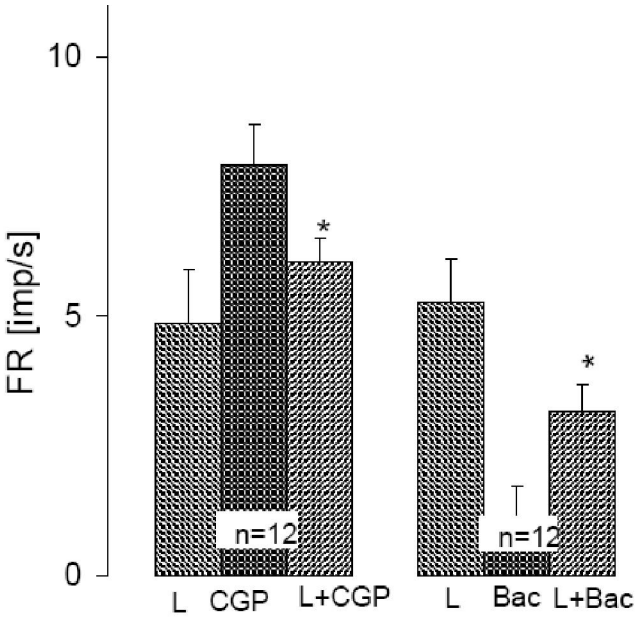

Figure 1 : Changes in firing rate of rat PO/AH neurons after application of Leptin CGP35348 and Baclofen

Original recordings of firing rate (FR, imp/s) from neuron of the medial preoptic area in rat slice preparation. Leptin and Baclofen (arrows, bolus injection 0.1 ml of 100 nM and 0.1 μM, respectively), applied separately and in combination.

DISCUSSION

There are many evidences for leptin–GABA interactions on different levels. Leptin acts in the brain to prevent obesity. Remarkably, the vast majority of leptin's antiobesity effects are mediated by GABAergic neurons. 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[9].

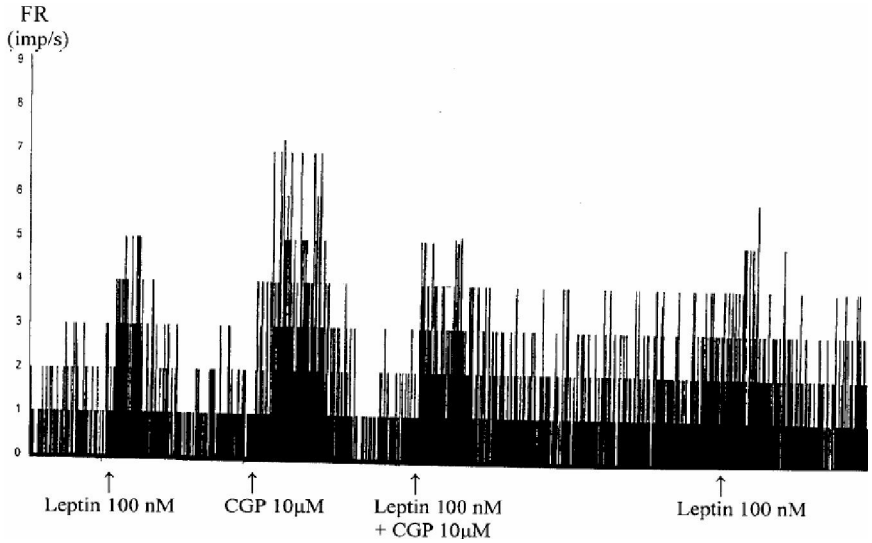

Figure 2 : Interactions of Leptin and CGP35348 on firing rate of rat PO/AH neuron

firing rate in rat PO/AH neurons (Figure 1 and Figure 2), while the $GABA_B$-agonist baclofen caused a decrease in firing rate (Figure 1 and Figure 3). The probable synergy between the effects of leptin and $GABA_B$-antagonist was not occurred. In the opposite, effect of this combination was lower compared to the result of the separate administration (Figure 1 and Figure 2). When leptin was applied in combination with $GABA_B$-agonist baclofen neither one of their separate effects appeared (Figure 1 and Figure 3).

Average change of firing rate (FR) [imp/s] in extracellulary recorded PO/AH neurons after bolus injection 0.1 ml of Leptin 100 nM (L), CGP35348 10 μM (CGP) and Baclofen 0.1 μM (Bac) (separate and combine administration, respectively). Significant values: *$p<0.05$ (in comparison with separate application of substances); means ± S.E.M; n - number of neurons investigated.

Original recordings of firing rate (FR, imp/s) from neuron of the medial preoptic area in rat slice preparation. Leptin and CGP35348 (arrows, bolus injection 0.1 ml of 100 nM and 10 μM, respectively), applied separately and in combination.

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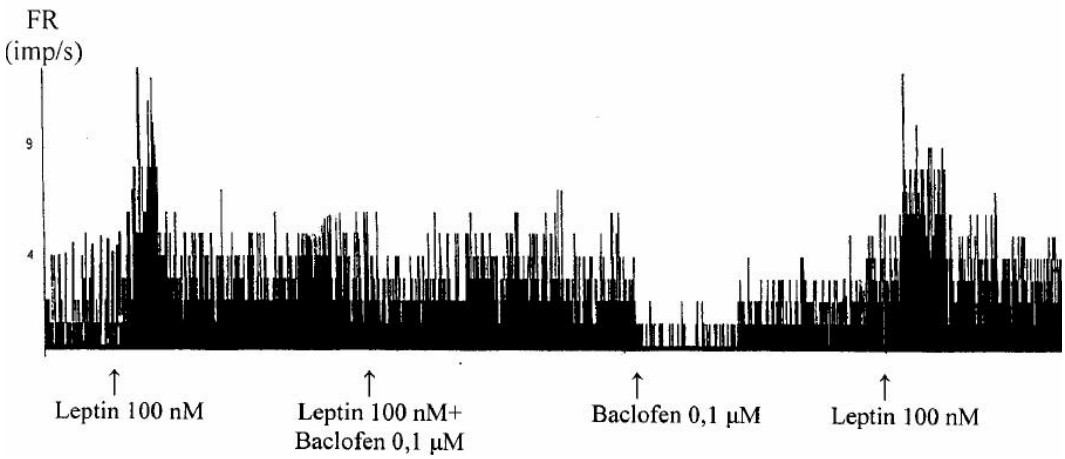

Figure 3 : Interactions of Leptin and Baclofen on firing rate of rat PO/AH neuron

Recently, a strong GABA-ergic modulation of leptin was postulated. The data show a strong association between leptin levels and doses of GABA-mimetic active substance clomethiazole[10].

Leptin inhibited norepinephrine (NE) efflux from the hypothalamus in a dose-dependent manner. Recent results demonstrate 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[11].

In vitro changes of firing rate observed in PO/AH neurons were in correlations with *in vivo* effects determined. *In vivo* results suggested that systemic administration of leptin produced significant hyperthermia in rats, as well as the $GABA_B$-antagonist CGP35348, while the $GABA_B$-agonist baclofen caused decrease in core body temperature. However, there wasn't synergism in hyperthermic effect of leptin and $GABA_B$-antagonist. The effect of combination was lower than the effects of substances applied alone. Neither hyperthermic effect of leptin nor hypothermic effect of $GABA_B$-agonist occurred when leptin was applied just prior baclofen[12].

CONCLUSION

There was not synergism between leptin and $GABA_B$-antagonist or $GABA_B$-agonist on firing rate of PO/AH neurons in rats. These results are step of understanding the complicated interactions of neuromodulatory-acting substances on the level of central temperature controller – the neurons of PO/AH.

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