Effects of Leptin on activity and temperature-sensitivity of rat PO/AH neurons

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ABSTRACT

Leptin, the obese gene peptide, is involved in the regulation of feeding behavior and energy balance. Extracellular recordings were made from neurons in slices of the preoptic area/anterior hypothalamus (PO/AH) of rats, to investigate the effects of Leptin on neuronal response characteristics on the level of central temperature controller. Leptin increased dose-dependently tonic activity (firing rate) in all type of PO/AH neurons, but decreased temperature sensitivity (temperature coefficient, TC) in warm-sensitive neurons. Our results are step of understanding the complicated mechanisms of action of neuromodulatory acting peptides on the level of central temperature controller – the neurons of the PO/AH.

INTRODUCTION

Leptin, the obese gene peptide, is involved in the regulation of feeding behavior and energy balance. Leptin regulates energy balance largely through isoform B leptin receptor-expressing neurons (LepR neurons) in the brain and leptin activates one subset of LepR neurons (leptin-excited neurons) while inhibiting the other (leptin-inhibited neurons)[1]. It has been found that leptin acts on the brain to increase postprandial heat production in skeletal muscle of sheep[2]. The control of brown fat cell development and activity is physiologically ensured by the sympathetic nervous system (SNS), which densely innervates brown adipose tissue (BAT). SNS-mediated thermogenesis is largely governed by hypothalamic and brainstem neurons. With regard to energy balance, the leptin-melanocortin pathway appears to be a major factor in controlling brown adipocyte thermogenesis[3]. Bechtold et al., 2012[4] have found that altered thermoregulation in Gpr50(-/-) mice is associated with attenuated responses to leptin and a suppression of thyrotropin-releasing hormone.

Temperature regulation is controlled by a hierarchy of neural structures. The preoptic area of the anterior hypothalamus (PO/AH) plays a prominent role in thermoregulation and strongly influences each of the lower effector areas. There are warm- and cold-sensitive neurons, as well as temperature-insensitive neurons in the PO/AH and they are known to be affected by local and peripheral temperatures. The neurons in PO/AH are supposed to build a neuronal network which takes part in the central control of body temperature[5]. These neurons are affected by different neurotransmitters and neuromodulators[6]. It has been shown long time ago...
that many neurotransmitters and neuromodulators take part in the process of thermoregulation\(^7\). In order to contribute to understanding the mechanisms of action on the level of the central temperature controller, the effects of leptin on activity (firing rate) and temperature sensitivity were studied on rat PO/AH neurons in a brain slice preparation.

**MATERIALS AND METHODS**

**Extracellular recordings**

Slices (400 µm) from the preoptic area/anterior hypothalamus (PO/AH) of male Wistar rats (200-220 g) were prepared and stored as previously described Schmid and Pierau, 1993\(^8\). Extracellular recordings of the neuronal activity were made with glass-covered platinum-iridium electrodes during continuous perfusion with oxygenated ACSF at a rate of 2 ml/min. The temperature of the tissue slice was kept constant at 38°C. Sinusoidal temperature changes within the range of 35-41°C were performed with the aid of a Peltier thermoassembly (rate 0.02°C/s). 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 was 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\(^9\). The slope of the steepest regression line was used as the temperature coefficient (TC) of the unit. Temperature-sensitive neurons are defined by a TC ≥ 0.8 imp/s/°C for warm sensitivity and TC ≤ -0.6 imp/s/°C for cold sensitivity; all other neurons are by this definition temperature insensitive. 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 leptin. All data are presented as means ± S.E.M. For statistical evaluation a paired t-test was performed.

**Substances and design of experiments**

Leptin (OB) Rat Recombinant (Sigma, Germany) was used in this study.

Regarding to firing rate investigations, Leptin previously prepared as stock solution (1 nM, 10 nM and 100 nM), was added as bolus to the perfusion with oxygenated ACSF.

Leptin (100 nM) was diluted in ACSF just prior to the application and applied by superfusion, regarding to temperature sensitivity investigations. Before application of the test substance, the temperature sensitivity of a given neuron was determined using two sinusoidal temperature stimuli at intervals of 5 minutes. Superfusion of test substance was started 5 min after the last control temperature stimulus; Leptin was applied for 5 min before the next temperature stimulus was performed. Superfusion returned to ACSF after this stimulus was completed and a further temperature stimulus was given after a delay of at least 10 min. An additional temperature stimuli were applied in anticipation of complete recovery. Only one neuron per slice was tested.

**RESULTS**

Extracellular recordings were obtained from 39 neurons in slices of the hypothalamic medial preoptic area of rats. Fifteen extracellular recorded neurons, regardless of their type of temperature sensitivity were used to investigate the changes in firing rate by Leptin in

![Figure 1: Dose-response effect of Leptin on firing rate of rat PO/AH neurons](image-url)
concentrations of 1 nM, 10 nM and 100 nM, applied by different order. Leptin increased firing rate of rat PO/AH neurons in a dose-dependent manner (Figure 1 and Figure 2).

Whereas the neuronal activity was generally increased in all types of PO/AH neurons, the temperature sensitivity was significantly changed only in a warm-sensitive neurons. Twelve warm-sensitive neurons, as well as twelve temperature-insensitive neurons were treated with Leptin 100 nM for investigation the changes made in temperature sensitivity. The decrease of temperature coefficient (TC) among the warm-sensitive neurons was found, while the TC of temperature-insensitive neurons was not significantly changed (Figure 3).

Recovery in neuronal temperature sensitivity (TC) was observed after wash out of the substance (Figure 3).

**DISCUSSION**

Neuronal models of hypothalamic control of body temperature predict that an increase in activity of warm-sensitive neurons causes hypothermia by activating heat loss mechanisms, while the reduction of firing rate reduces the heat loss mechanisms and activates heat production resulting in hyperthermia. In the present study the application of Leptin on rat hypothalamic slices...
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caused dose-dependent increase of firing rate in all types of PO/AH neurons. Davidowa and Plagemann, 2000[10] have shown that Leptin increases firing rate in neurons of the ventromedial hypothalamic nucleus in normal rats. However, the effect on spontaneous activity could not be considered as the only mechanism by which the neuronal circuitry may change the signal to the thermoregulatory effector sites[11,12]. Various substances, such as bombesin, prostaglandin E2, vasopresin, TRH and GABA, which produce a pronounced change in body temperature when injected into the PO/AH in vivo, also affect the temperature sensitivity of hypothalamic neurons[6,11,12]. For instance, the hyperthermia induced by μ-opioid agonists could be easily explained by the decrease in temperature sensitivity of PO/AH neurons and this effect was restricted to warm-sensitive neurons[13]. It has been shown that about 30% of the spontaneously firing neurons in the PO/AH are warm-sensitive[14]. Electrophysiological studies reported that substances, which induced hyperthermia, increase temperature sensitivity of warm-sensitive rat PO/AH neurons[6]. In the present study the application of Leptin on rat hypothalamic slices caused significant decrease in temperature sensitivity (TC) of warm-sensitive PO/AH neurons. Our in vivo investigations made have shown that Leptin increased core body temperature in rats between 30th and 90th min after injection[15].

The results from this study suggest that Leptin causes a dose-dependent increase of firing rate in rat PO/AH neurons, but decrease of TC in the warm-sensitive neurons in PO/AH. These results are step of understanding the complicated mechanisms of action of neuromodulatory-acting substances on the level of central temperature controller – the neurons of PO/AH.

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