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Ibotenate Lesion of the Ventromedial Hypothalamus Lowers Hyperthermic Effects of Prostaglandin E_1

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Summary

This experiment tested the effects of an intracerebroventricular injection of prostaglandin E_1 on the sympathetic activation and the thermogenic changes in rats with ibotenate lesions of the ventromedial hypothalamus. Under pentobarbital anesthesia, twelve Sprague-Dawley male rats were lesioned bilaterally in the ventromedial hypothalamus with an injection of ibotenic acid (30 nmol into each side). Sham lesions were carried out in other twelve control rats. After 48 h, all animals were anesthetized with ethyl-urethane. The firing rate of the sympathetic nerves innervating the interscapular brown adipose tissue and the colonic and interscapular brown adipose tissue temperatures were monitored before and after an intracerebroventricular injection of prostaglandin E_1 (500 ng) or saline. Prostaglandin E_1 induced an increase in the firing rate of sympathetic nerves and the colonic and interscapular brown adipose tissue temperatures. These effects were reduced by the ventromedial hypothalamic lesion. Since ibotenic acid destroys cell bodies, the findings indicate that neurons of the ventromedial hypothalamus play a considerable role in the control of sympathetic activation and the thermogenic changes during prostaglandin E_1 hyperthermia.

Key words

Brown adipose tissue • Central nervous system • Hyperthermia • Pyrogen • Rat • Sympathetic activity

Introduction

In response to a pyrogen, such as prostaglandin E_1 (PGE_1), animals reduce their heat loss and increase heat production in order to raise body temperature to a new, elevated level (Kluger 1990). The increase in heat production is also obtained through enhanced discharges of the sympathetic nerves to interscapular brown adipose tissue (Monda *et al.* 1994a), the principal effector of non-shivering thermogenesis (Cannon *et al.* 1998). Although the major brain site where PGE_1 has its effect has been identified as the preoptic/anterior hypothalamus (PO/AH) (Stitt 1986), other hypothalamic areas are also involved in the induction of PGE_1 hyperthermia. An intracerebroventricular administration of PGE_1 increases the firing rate of posterior hypothalamic neurons (Monda *et al.* 1994b), while pretreatment with muscimol in the posterior hypothalamus reduces the hyperthermic responses to PGE_1 (Monda *et al.* 1995b).

The ventromedial hypothalamus (VMH) is involved in the control of non-shivering thermogenesis. Electrolytical lesions of the VMH decrease IBAT activity (Monda *et al.* 1993), while a stimulation of the VMH increases IBAT temperature (T_{IBAT}) (Thornhill and Halvorsan 1994).

The aim of the present experiments was to evaluate the effects of VMH ibotenate lesions on the sympathetic and thermic changes induced by PGE_1, injected 48 h after the neurotoxic lesion, and to distinguish the role of ventromedial hypothalamic

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neurons from fibers of passage in this hyperthermic phenomenon.

Methods

Animals

We used male Sprague-Dawley rats (n=24), weighing 280-320 g. These were housed in pairs at a controlled temperature (22±1 °C) and humidity (70 %) with a 12:12 h light-dark cycle from 07:00 to 19:00 h. Laboratory standard food (Mil, Morini, Italy) and water were available at all times. The experiments were in conformity with the European Convention for the Protection of Vertebrate Animals used for Experimental and Scientific Purpose (Council of Europe No. 123, Strasbourg 1985).

Apparatus

The firing rate of nerves to IBAT was recorded by a pair of silver wire electrodes. The electrical pulses were amplified by a condenser-coupled amplifier and were filtered by band-path filters (NeuroLog System, Digitimer). The raw pulses were displayed on an oscilloscope (Tektronix) and passed to a window discriminator. Square waves from the discriminator were sent to an analog-digital converter (DAS system, Keithley) and stored on a PC every 5 s. Furthermore, a rate meter with a reset time of 5 s was used to observe the time course of the nerve activity recorded by a pen recorder (Vitatron). Because signal-to-noise ratio depended on the number of nerve filaments and the contact between nerve and electrodes, the basal burst rates were different for each rat. The threshold level of the event detector was fixed during the experiment at 50 % of the peaks of the largest pulses and above background noise.

Colonic temperature (Tc) and T_{IBAT} were measured with thermocouples (Ellab). Signals were sent to an analog-digital converter (DAS system, Keithley) and stored on a PC every 1 min.

Drug

Ibotenic acid and PGE_1 were purchased from Sigma (St. Louis, USA). Drugs were dissolved in a pyrogen-free phosphate-buffered saline solution.

Surgery

All animals were anesthetized with i.p. pentobarbital (50 mg/kg b.w.) and a 20-gauge stainless guide cannula was positioned stereotaxically (Pellegrino *et al.* 1979) above a lateral cerebral ventricle at the following coordinates: 1.7 mm lateral to the midline, 0.4 mm posterior to the bregma, 3.0 mm from the cranial theca. The guide cannula was secured to the skull by screws and dental cement. A stylet was inserted into the guide tube and removed only during drug administration. Rats were given 7-10 days to recover from surgery as judged by the recovery of preoperative body weight.

Procedure

After the recovery, all animals were anesthetized with i.p. pentobarbital (50 mg/kg b.w.) and placed in a stereotaxic frame. In 12 animals, ibotenic acid (30 nmol in 0.5 µl into each side) was injected bilaterally at the following coordinates: 1.0 mm lateral to the midline, 0.2 mm anterior to the bregma, 9.9 mm below the cranial theca (Pellegrino *et al.* 1979). The drug was delivered over a 10-min period. Other rats received saline injection only. The ibotenic acid diffused into the VMH within several minutes, according to the Fick law (Schwarcz *et al.* 1979).

After 48 h, 6 sham-lesioned animals (first group) were anesthetized with ethyl-urethane (1.2g/kg b.w.) and mounted in a stereotaxic frame. The level of anesthesia was kept constant as evaluated by skeletal muscle relaxation, eye and palpebral responses to stimuli (Soma 1971). Nerve activity was recorded from small nerve bundles dissected from intercostal nerves supplying the right side of IBAT. Nerve filaments were isolated from the central cut end of these nerve bundles under a dissecting microscope to record the efferent activity with a pair of silver wire electrodes. The nerve filaments were covered with a mixture of vaseline and liquid petroleum (paraffin oil) at 37 °C to avoid dehydration. The firing rate was recorded over 20 min (baseline period) before and 40 min after an injection of 500 ng PGE_1, which was introduced into the left cerebral ventricle by gravity flow over 1 min. PGE_1 was injected directly into a lateral cerebral ventricle so that its dilution in the cerebrospinal fluid was very rapid. The cannula for the injection was 0.4 mm longer than the guide cannula. Furthermore, Tc and T_{IBAT} were monitored at the same time as the nerve recording. Tc was measured by inserting the thermocouple into the colon at a depth of 7 cm, while T_{IBAT} was monitored by inserting the thermocouple into the left side of IBAT. The same variables were recorded in the other 6 VMH-lesioned animals (second group) receiving PGE_1. The same procedure used with the first group was carried out with the other 6 sham-lesioned animals (third group) except that saline was injected into

the lateral ventricle. Saline was injected in 6 VMH-lesioned rats (fourth group) and the same variables were monitored. The baseline values of Tc from all animals used were maintained constant by a heating pad. The electrical energy supplying the pad was not altered during the entire experimental period.

Histology

At the end of the experiment, the lesion and the location of the cannula were identified. A stain (bromophenol blue) was injected into the lateral ventricle in the same volume used for drug administration. The rats then received an overdose of pentobarbital (150 mg/kg b.w.) and were perfused with 0.9 % NaCl followed by 10 % (vol/vol) formalin solution. The brain was removed and stored in formalin solution. After a few days, 50 μm coronal sections of the fixed brain were cut and stained with neutral red. The position and the extent of the lesion were evaluated by an imaging analyzer (Biovision).

Statistical analysis

The values are presented as means ± S.E.M. Statistical analysis was performed using analysis of variance and multiple comparisons were performed by the Newman-Keuls *post hoc* test.

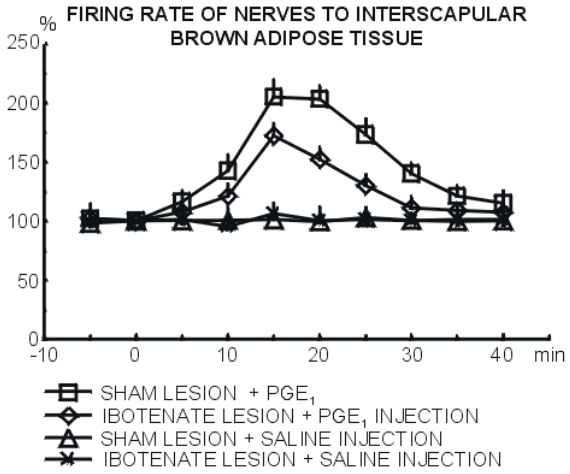

Fig. 1. *Changes in firing rate of nerves to interscapular brown adipose tissue in lesioned or sham lesioned rats with intracerebroventricular (i.c.v.) injection of PGE_1 or saline. I.c.v. injection at time 0. Data are means ± S.E.M.*

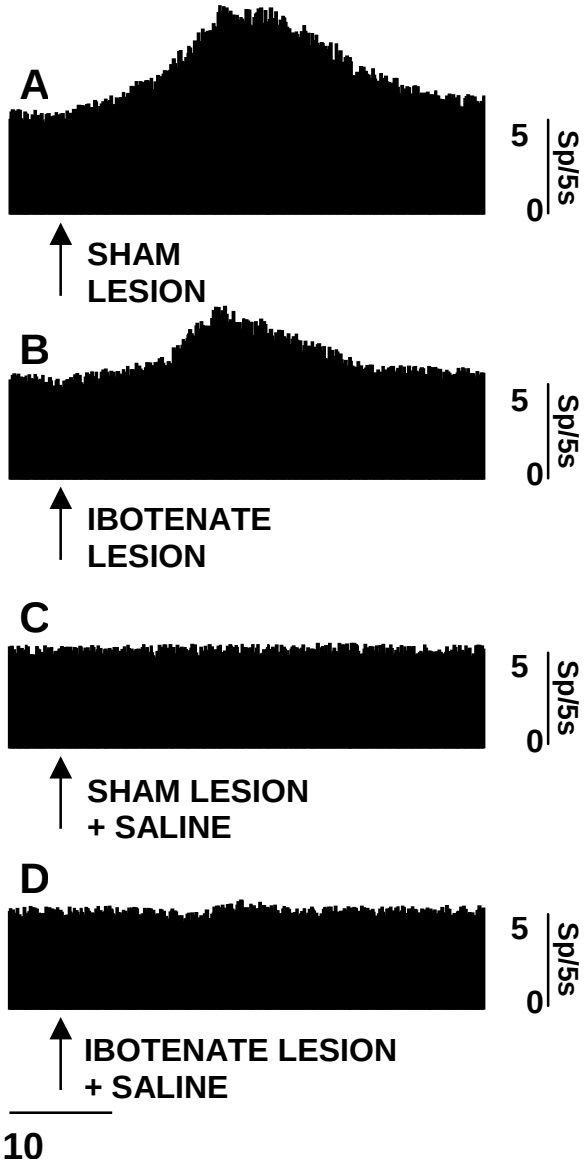

Fig. 2. *Actual firing rate changes in a sham-lesioned rat receiving PGE_1 (panel A) or saline (panel C) and in a lesioned rat receiving PGE_1 (panel B) or saline (panel D). The arrow indicates the time of i.c.v. injection.*

Results

Figure 1 shows the percentage changes in firing rate of nerves supplying IBAT. PGE_1 increased the firing rate that peaked at 15 min in rats with the sham lesions. This rise was reduced in the lesioned animals. Saline injection did not produce any modification in the sham or lesioned rats. Analysis of variance showed significant effects for PGE_1 [$F(1, 20) = 96.408$, $p<0.01$], for lesion [$F(1, 20) = 11.078$, $p<0.01$], for time [$F(8, 160)$ 21.032,

p<0.01], for interaction PGE_1 x lesion [F(1, 20) = 13.180, p<0.01], PGE1 x time [F(8, 160) = 19.849, p<0.01], and PGE_1 x lesion x time [F(8, 160) = 2.638, p<0.01]. The *post hoc* test showed that the sham lesion+PGE_1 group was different from the lesion+PGE_1 group by 15 to 30 min and from other groups by 10 to 35 min. Differences were demonstrated between the lesioned group receiving PGE_1 and groups receiving saline by 15 to 25 min.

The absolute values (spikes per 5 s) at time 0 were: a) 38.62±5.27 in the sham-lesioned rats injected with PGE_1; b) 37.32±4.89 in the lesioned rats injected with PGE_1; c) 38.72±5.74 in the sham-lesioned rats injected with saline; d) 39.19±5.72 in the lesioned rats injected with saline. Examples of changes in actual firing rate of the nerves to IBAT are depicted in Figure 2.

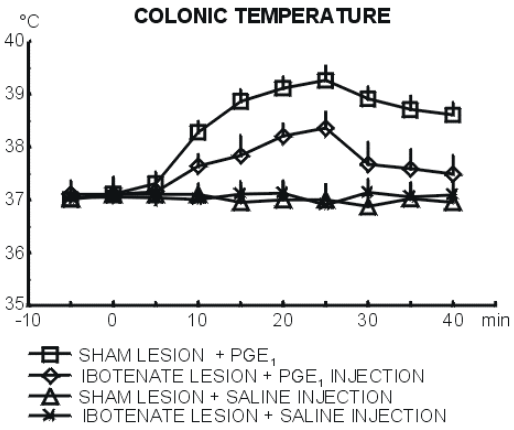

Fig. 3. *Changes in temperature of interscapular brown adipose tissue in lesioned or sham-lesioned rats with i.c.v. injection of PGE_1 or saline. I.c.v. injection at time 0. Data are means ± S.E.M.*

Figure 3 illustrates T_{IBAT} changes. PGE_1 caused a rise that peaked at 20 min in the sham-lesioned rats. This increase was reduced in the lesioned animals. In the lesioned or sham-lesioned rats, the saline injection did not cause any changes. Analysis of variance showed significant effects for PGE_1 [F(1, 20) = 14.217, p<0.01], for time [F(8, 160) = 10.121, p<0.01], and for interaction PGE_1 x time [F(8, 160) = 9.567, p<0.01], and PGE_1 x lesion x time [F(8, 160) = 2.270, p<0.05]. The *post hoc* test showed that sham lesion+ PGE_1 group differed from other groups by 10 to 40 min. Differences were demonstrated between lesion+ PGE_1 group and groups receiving saline by 15 to 25 min. Furthermore, the changes in firing rate of IBAT nerves preceded the changes in temperature.

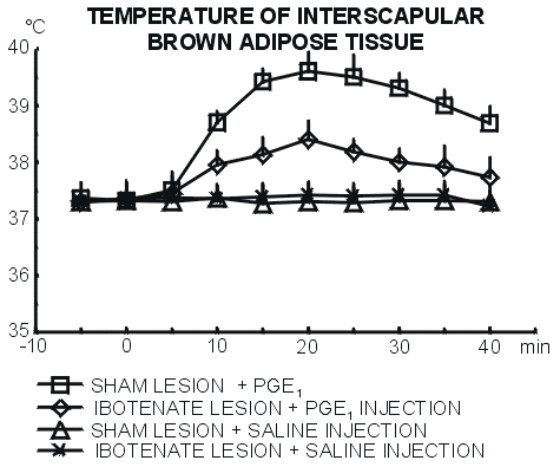

Fig. 4. *Changes in colonic temperature in lesioned or sham-lesioned rats with i.c.v. injection of PGE_1 or saline. I.c.v. injection at time 0. Data are means ± S.E.M.*

Changes in Tc are shown in Figure 4. PGE_1 induced a rise with a peak at 25 min in the sham-lesioned rats. The VMH lesion reduced the increase induced by PGE_1 injection. No changes occurred in the lesioned or sham-lesioned animals with saline administration. Analysis of variance showed significant effects for PGE_1 [F(1, 20) = 15.065, p<0.01], for time [F(8, 160) = 8.526, p<0.01], for interaction PGE_1 x time [F(8, 160) = 10.138, p<0.01], and PGE_1 x lesion x time [F(8, 160) = 2.770, p<0.01]. The *post hoc* test showed that the sham lesion+PGE_1 group differed from the other groups at 10 to 40 min. Differences were demonstrated between the lesion+PGE_1 group and groups receiving saline at 20 and 25 min. There were no differences in baseline parameters of all three groups.

The injection of ibotenic acid did not cause damage of the preoptic cells, because lesions did not extend to the peri-VMH area and their size was almost identical. The mean area of lesions measured at the maximum extent was 0.33±0.05 mm^2 on the right side and 0.38±0.07 mm^2 on the left side.

Discussion

Our findings indicate that an ibotenate lesion of ventromedial hypothalamic neurons reduces the effects of PGE_1. This suggests that the neurons of VMH play an important role in the control of sympathetic activation and thermogenic changes by stimulating the prostaglandinergic system. It has been demonstrated that an intracerebroventricular injection of PGE_1 increases sympathetic nerve discharge to IBAT (Monda *et al.* 1994a). This increase is modified by some experimental manipulations (Monda *et al.* 1995c). Intracerebroventricular injections of PGE_1 induce a dose-dependent increase in body temperature, which mimics the fever due to physiopathological levels of PGE_2 in the cerebrospinal fluid (Gollman *et al.* 1988, Komaki *et al.* 1992).

It has been demonstrated here for the first time that ibotenate lesion of the VMH reduces the rise in sympathetic discharge of nerves to IBAT during PGE_1-induced hyperthermia. The VMH was destroyed by excitotoxic lesions. Since the ibotenic acid destroys cell bodies, it permits us to distinguish the role played by cells from fibers of passage, and it indicates that the neurons of the VMH play an important role in the PGE_1 hyperthermia. The VMH lesion does not abrogate completely the response to PGE_1. It shows that the VMH controls this phenomenon only partly. Other hypothalamic structures are involved in the control of PGE_1 hyperthermia. Lesions of the paraventricular nucleus (Horn *et al.* 1994) and injection of muscimol into the posterior hypothalamus (Monda *et al.* 1995b) reduce, but do not block the hyperthermic responses to pyrogens. For these reasons, a VMH lesion attenuates the response to PGE_1, but does not abrogate it.

This experiment emphasizes the role of VMH neurons in the activation of non-shivering thermogenesis, by using a stimulus other than food intake. Indeed, the functions of VMH in the control of thermogenesis related to food intake have been extensively studied (Baskin *et al.* 1998, Schwartz *et al.* 1995, Wolf 1998). We also showed that the heat production is reduced by VMH lesions in the preabsorbtive phase of postprandial thermogenesis (Monda *et al.* 1997). On the other hand, these findings reveal an aspect of VMH that could link cerebral stimulation of prostaglandinergic system to the control of food intake. We recently showed that an activation of thermogenesis induced by an i.c.v. injection of PGE_1 reduces food intake (Monda *et al.* 1999) and an inhibitor of prostaglandin synthesis increases food intake in rats with lateral hypothalamic lesions (Monda *et al.* 1996b). It would be interesting to investigate the effects of PGE_1 administration on food intake in VMH lesioned rats. This could reveal a role played by VMH in prostaglandinergic control of food intake.

The present findings suggest that PGE_1 injected into a cerebral ventricle acts on the PO/AH, which in turn influences VMH activity. PO/AH is a responsive structure to PGE_1, whereas this neural mediator slightly stimulates the other hypothalamic areas, including the VMH. Indeed, the increase in IBAT temperature due to the PGE_1 injection into the VMH is much lower than that induced by the same dose of PGE_1 injected into PO/AH (Simpson *et al.* 1994). On the other hand, this does not exclude the possibility that a VMH lesion destroys a likely site of PGE_1 direct action (Kuriyama *et al.* 1990).

We report direct evidence of sympathetic nerve discharge to IBAT after PGE_1 injection. IBAT activity is controlled by the sympathetic nervous system, and factors, which influence thermogenesis, appear to act centrally to modify the sympathetic outflow to IBAT (De Luca *et al.* 1987, Monda *et al.* 1995a). The significant role of IBAT in the hyperthermia induced by PGE_1 is confirmed by these findings.

In conclusion, the destruction of VMH neurons reduces the PGE_1 hyperthermia, indicating that these neurons play a considerable role in the control of sympathetic discharge, which regulates the thermogenic changes induced by PGE_1. Further experiments should be carried out to investigate if responses to an injection of interleukins or other pyrogens are similarly attenuated by the VMH lesion.

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Reprint requests

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