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Effects of Orexin A on Thermal Behaviour: Substantial Evidences for Thermoregulatory Role of Orexin A

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Abstract

Orexin A is a hypothalamic neuropeptide produced in the dorsal and lateral hypothalamus, and orexin-producing cell have widespread anatomical projections within the central nervous system. Orexin A is involved in multiple physiological functions, including eating behavior, thermoregulation, and sleep-regulation. The aim of this work was to study the thermal preference induced by orexin A. A thermal preference task with floor thermal gradient from 16°C to 25°C, divided into 10 equal segments, was designed to evaluate the thermal preferences in rats. Male rats (n=10, divided into two groups of five animals) received an intracerebroventricular injection of 1.5 nmol of orexin A or vehicle and were subsequently tested for thermal preference for 4 h. The results showed that the rats injected with orexin A had increased motor activity compared to the control group, particularly after the second hour of the test. Moreover the group treated with orexin A preferred hotter temperatures ranging from 24 to 25°C compared to the control group that preferred temperature of 22°C, which is near room temperature for rat housing (22 ± 1°C). No significant correlation was seen between thermal preference and time (hours). Since orexin A induces thermal preference, this study indicates that this neuropeptide plays a key role in the thermoregulation.

Keywords: orexin A; motor activity; thermal preference

Introduction

Orexin A is a hypothalamic neuropeptide whose name was formerly introduced because of its putative role in eating behavior [1,2]. Actually, studies on orexin A have demonstrated its role in the regulation of different physiological functions-such as blood pressure [3], metabolic rate [2], body temperature [4], awakening [5,6], heart rate [7]-suggesting, overall, a role of orexin A in the control of vegetative functions. Moreover, orexin A has an important role in thermoregulation; in fact, an Intra Cerebro Ventricular (ICV) injection of orexin A can increase body thermal. This effect is present both in anesthetized rats and in animals without access to food, and suggests that thermogenesis induced by orexin is not only a consequence of food intake or motor activity [8,9]. It has been hypothesized that orexin A modulates body thermal through the medial preoptic area [10,11]-a region involved in central thermoregulation—and numerous orexin projections are present in this area [12]. In addition, the inhibitors of the synthesis of prostaglandins, which act on this region, block the hyperthermia induced by orexin A in anesthetized rats [13]. Alternatively, orexin A could modulate thermogenesis directly through activation of the autonomic system; in fact, an ICV injection of orexin A increases the sympathetic output to inter scapular brown adipose tissue (IBAT) [14], which is the major source of non shivering-termogenesis and energy balance [15,16]. Several studies on the interaction between antipsychotic drugs and the orexin system showed that the thermogenic effect of orexin A also involve serotoninergic and dopaminergic systems. The increase in the sympathetic activity induced by orexin A is reduced by antipsychotic drugs, such as haloperidol, that are antagonist of D_2 receptors [7,17]. Furthermore atypical antipsychotic drugs, such as olanzapine, that are 5-HT_2 receptor and D_2 receptor antagonists [18,19], decrease or block hyperthermic effects of orexin A [20]. Because thermoregulation depends on both vegetative and behavioral mechanisms, the aim of the present study was to evaluate the thermal behaviour induced by orexin A, namely, whether an ICV injection of orexin A can modify the thermal preference in rats. To this end, a cage with a gradient thermal along the floor and a transparent wall was used to record, through a computerized visual system, the overall time spent moving or resting at each thermal ranging from 16 to 25°C.

Methods

Animals

Male Sprague Dawley rats (n=10, divided randomly into two groups of five animals) weighing between 250 and 300 g (age: 9-10 weeks) were used for the experiments. The rats were housed in pairs at controlled thermal (22 ± 1°C) and humidity (70%) with a 12:12 h light–dark cycle with light from 07:00 to 19:00, and with access to food and water *ab libitum*. The experiments were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by Animal Research Committee.

Surgery

All animals were anesthetized with intra-peritoneal injection of chloral hydrate (0.6 g/kg). A stainless guide cannula (0.9 mm in diameter) was stereotaxically placed 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 [21]. The rats

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were given 7-10 days to recover from surgery before the experiments.

Orexin A injection

A dose of 1.5 nmol of orexin A (Sigma–Aldrich, Italy), dissolved in 5 mL of 0.9% NaCl sterile solution, was used for the ICV injection. Previous experiments showed that this is a submaximal dose for the induction of hyperthermia [22]. Orexin A (or saline) was injected unilaterally into the left cerebral ventricle by gravity flow (1 min) over 2 min. The injected volume was controlled using a transparent polyethylene tube with a graduation of microliters. The cannula (0.8mm in diameter) used for the injection was 0.4 mm longer than the guide cannula. The flow of the fluid, controlled by gravity, confirmed the correct position of the cannula into the lateral cerebral ventricle.

Thermal preference task

The thermal preference was evaluated with a custom cage that consisted of a rectangular copper floor of 150 cm×15 cm and four walls (30 cm in height). Three of the walls were constructed with black-painted wood, and a transparent Plexiglas wall was used for one of the longer sides of the cage. Underneath the copper floor, two adjacent copper tubes produced a thermal gradient due to heat exchange between cold water flowing in one tube and hot water flowing in the other one. The flow rate and the prime thermals of the water were adjusted to have a thermal gradient on the floor of the cage between 16°C at one extremity and 25°C at the other extremity (Figure 1).

Procedure

After recovery, the experiments were conducted during the light phase to minimize the effect of the endogenous orexin A that is high during the dark phase [23]. Orexin A (or saline) was injected into the left cerebral ventricle by gravity over 2 min. The injected animal was then placed in the thermal-preference apparatus for 4 h. The position of the animal along the long axes of the cage was automatically determined and recorded continuously by a computerized visual system that consisted of a simple web-cam framing the cage through the transparent wall and attached to a PC. Custom software written with Lab View (National Instruments, Texas, USA) recognized the animal due to the high contrast between his white coat and the black background (Figure 1). The cage was divided into ten segments and the time spent resting on each segment was calculated. Resting was defined as staying in the same segment for at least one minute. The time spent moving was also calculated.

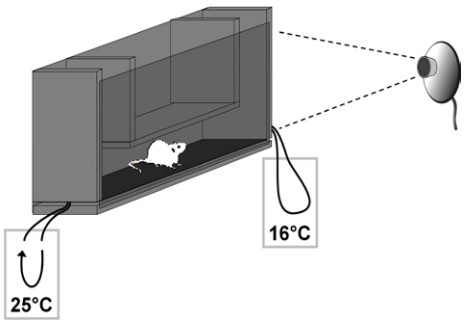

Figure 1: Schematic of the apparatus for the evaluation of thermal preference. The cage consisted of a rectangular copper floor with two adjacent copper tubes that produced a thermal gradient due to heat exchange between cold water flowing in one tube and hot water flowing in the other one. The cage was divided into ten segments with a 1°C difference between one other. The thermal gradient ranged from 16°C at one extremity to 25°C at the other extremity.

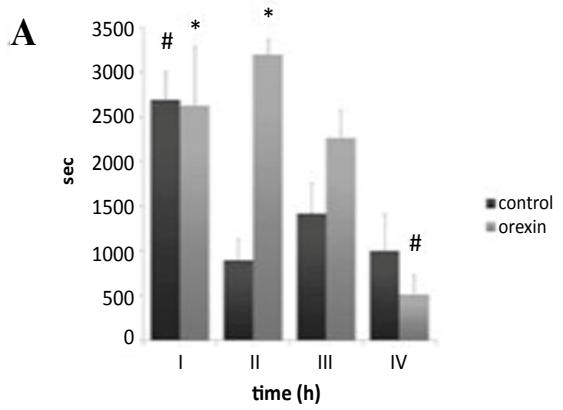

Figure 2a: Total moving time spent by the animals at 1, 2, 3 and 4 hours after ICV injection of vehicle (dark gray) or orexin A (light gray). The control animals showed high locomotor activity in the first hour, whereas rats treated with orexin A showed high locomotor activity in the first two hours compared to the fourth hour and in the second hour compared to the other time. The locomotor activity of the orexin A group is high in the first and second hour compared to the control group at 2, 3 and 4 hours.

Statistical analysis

Data are presented as mean ± standard error of mean. Statistical analysis was performed using the Analysis Of Variance (ANOVA). Multiple comparisons were performed using the Fisher-LSD post hoc test. Parameters were analyzed using SPSS 10.0 for Windows (SPSS Inc., Chicago, IL). A P value of less than 0.05 was considered significant.

Results

Orexin A injection caused a significant increase in the time spent moving

The ANOVA for the time spent moving showed a significant difference for the following factors: treatment (orexin A, saline) [$F(1,40)=6.474$; $P<0.05$], time (1, 2, 3, 4 hour) [$F(3,40)=9.667$; $P<0.01$], and the interaction term treatment*time [$F(3,40)=5.878$; $P<0.01$]. The LSD Fisher post hoc test showed a significant difference for the time spent moving between the following: 1h and the other hours in the control group ($P<0.05$); 4h and the other hours in the orexin A group ($P<0.01$); orexin A at 1-2h and control group at 2, 3 and 4 h ($P<0.01$). The total time spent moving by the control group was higher in the first hour compared to the other hours. The total time spent moving by the orexin A group was higher in the first two hours compared to the control group (Figure 2A). Moreover, the total time spent moving of the orexin A group was significantly higher (43.2 ± 13.1%) than the control group.

Orexin A injection caused a significant shift of the thermal preference toward higher thermals

The ANOVA for the time spent resting at each temperature showed a significant difference for the following factors: temperature [$F(9,400)=3.295$; $P<0.01$], the interaction treatment*temperature [$F(9,400)=3.438$; $P<0.01$] and the interaction temperature*time [$F(27,400)=2.035$; $P<0.01$]. No significant difference was showed for the following factors: treatment [$F(1,400)=0.979$; $P=0.323$], time [$F(3,400)=2.574$; $P=0.054$], interaction treatment*time [$F(3,400)=2.529$; $P=0.057$], the interaction treatment*temperature*time [$F(27,400)=0.936$; $P=0.56$]. The LSD Fisher post hoc test showed a

significant difference between the orexin A group and the control group for the time resting at 25-24 ($P<0.05$) and 22°C ($P<0.01$). The orexin A group spent significantly more time at 24-25°C compared to the control group, while the control group spent significantly more time at 22°C compared to the orexin group (Figure 2B).

Discussion

The present study demonstrates that the ICV injection of orexin A causes an increase in locomotor activity and a modification of the thermal preference in rats. Both rats treated with the saline (control) and orexin A showed higher locomotor activity in the first hour as compared with the other hours. This result suggests simply a response to the novel environment. In fact, the rats explore for a time period ranging between 30 and 90 min with phases of progression and stopping if placed in a new environment. Subsequently, they stop in one preferred place—called 'home place'-for very long periods [24,25]. The orexin A group also showed an increase in motor activity during the second hour compared to the third and fourth hour, and compared to the control group at 2, 3 and 4 h. This result suggests that orexin A immediately increases locomotor activity, and that this effect is long-lasting. This data agrees with previous studies that show that ICV injection of orexin increases motor activity- in particular, spontaneous motor activity, locomotor activity and the grooming until 90 min [26]. Also, orexin A presents a decrease in motor activity during the dark phases, when motor activity is normally increased compared to the light phase [27,28].

The present study also showed that non-treated animals preferred temperatures of 22°C, which is near room temperature for rat housing (22 ± 1°C), while the orexin A group preferred temperatures of 24-25°C. This preference did not depend by the time. This result suggests an effect of orexin A on thermal behavior. Thermal preference depends on body temperature, sleep-wakefulness, locomotor activity, and whether they are housed alone or within groups. In particular, rats have exhibited decreased body temperature, motor activity, wakefulness with increased frequency of sleep episodes, and prefer higher temperatures during the light (non-activity) phase. During the dark (activity) phase, they have exhibited increased body temperature, motor activity, and wakefulness, and prefer lower temperatures [29]. Using an environmental chamber with three interconnected compartments at 24, 27 or 30°C, respectively, some studies reported that rats preferred an ambient temperature of 24°C at night and 27°C during the day [29-31]. Also, the maximum REM sleep time was calculated at 29-30°C [29-31]. Another study demonstrated that rats preferred to stay at the 24°C chamber for most of the time during both day and night [32]. In both cases the animals were housed at a controlled temperature of 25 ± 1°C. Moreover, the thermo neutrality- or the ambient temperature in which metabolic rate is minimal, and when rats show normal activity-ranges from 18 ± 1.9 to 28.1 ± 1°C in rats [33]. In our work we used a thermal preference task with a larger range of temperatures, and the rats were housed at a lower temperature (22 ± 1°C). Therefore, the preference for lower temperatures compared to the other studies could depend on the lower temperature at which the rats were habituated, and could also depend on the higher range of temperatures used in the thermal task. However, the orexin group showed a preference for higher temperatures compared to the control group. Therefore, we suggest that this thermal behavior is ultimately dependent on the effect of orexin A. In fact, during the dark phase- when the rats exhibited an increase in motor activity and awake time, and preferred low temperatures [29,34]-the level of orexin A is higher than during the light phase [23]. Moreover, orexin knockout mice shows, in particular during the dark phase, less motor activity, less body temperature, and less wakefulness compared to wild type [35-37]. The reason for the higher temperature preference in the rats treated with orexin A is difficult to explain. We hypothesized that this is another mechanism of thermogenesis induced by orexin A. Orexin A ICV determines an increase in thermogenesis, and a consequent increase in body temperature through different mechanisms. These mechanisms include the following: increases of the IBAT temperature [38] through a direct innervation of the intermedio-lateral column of spinal cord by orexin-fibers [39], and an increase of sympathetic activity [40]; increase of food intake [41,42]; and increase of motor activity [26,43]. These mechanisms determined an increase of body temperature for several hours, and suggest a long-lasting effect of the orexin. Usually the thermal behavioral response to hyperthermia would be the preference to return from lower temperatures to the baseline temperature; however, in our experiments we find that rats prefer hotter temperatures after the ICV administration of orexin A. Previous studies also showed that orexin A acts on the pre optic area, and probably increase the thermostat setting. Therefore, we hypothesize that the ICV administration of orexin A increases the thermostat setting through the activation of different pathways, and increases the body temperature to match the thermostat setting. In particular orexin A, in addition to the reported mechanisms, immediately increases locomotor activity until 2 h. After the effect on locomotor activity ends, the rats prefer hotter temperatures in order to increase their body temperature.

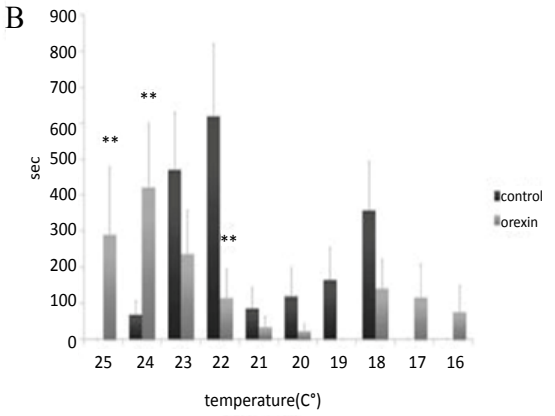

Figure 2b: Resting time spent by the animals for each segment of the thermal preference apparatus in the first (A), second (B), third (C) and fourth (D) hour after ICV injection of vehicle (dark gray) or orexin A (light gray). The orexin A group spent more time at 24-25°C compared to the control group. The values are expressed as mean percentage±SEM. Asterisk indicates a significant difference ($P<0.05$).

compared to the other hours; * compared to the control group at 2, 3 and 4 hours; ** compared to the control group

The present experiment is a very valid demonstration that orexin A is a thermoregulatory peptide. This idea has been already supposed by the authors of this experiment, by demonstrating a paradoxical eating behavior (hyperphagia and hypophagia) induced by orexin A. Indeed, Monda et al. [44] report an experiment where food intake and body temperature were monitored in 24h-fasting male Sprague-Dawley rats for 15 h after food presentation during the dark period. Orexin A was injected into the lateral cerebral ventricle 6h before food presentation. Food intake and body temperature were controlled also in rats receiving orexin A at the same time of food presentation.

Orexin A caused the same elevation of body temperature in

both groups, while food intake was significantly lower in the group receiving orexin A 6h before food presentation in comparison to the other group. This study demonstrated that the effects on food intake induced by orexin A depend on the time of food presentation. This induces us to revise the role of orexin A in the control of food intake. The name assigned to this peptide was due to the strong increase in food intake after an orexin A administration, assigning a fundamental role in the induction of food intake [45,46]. The results of the above publication call for a re-discussion of this role, underlining the importance of orexin A in the control of the sympathetic activity and body temperature, which in turn affects food intake. An icv injection of orexin A induces an increase in the sympathetic activity and in the body temperature independently of food ingestion that is reduced in the rats with a delayed presentation of food. This suggests that the effects on body temperature are prevalent with respect to eating behavior. Then, orexin A can induce hyperphagia, but also hypophagia, contradicting the significance of this name that assign a primary hyperphagic effect to this peptide. Other substances with primary hyperphagic effect, as neuropeptide Y or galanin, induce a reduction of the sympathetic discharge and a decrease in body temperature [20,47-52].

Conversely, substances with a primary hypophagic effect cause an increase in the sympathetic activity. For example, leptin induces reduction of food intake [53-55], along with an increase in the firing rate of the sympathetic nerves to IBAT and a rise in T_{IBAT} [56, 57]. For this reason, orexin A cannot be considered a substance with a primary hyperphagic effect. The orexin A can induce hypophagia or hyperphagia [45], but it always induces an activation of thermogenesis. We can suppose that this peptide elevates the thermoregulatory set-point, inducing the reactions to reach the new level of body temperature. The increase in food intake, obtained in the rats with a non-delayed presentation of food, could be a reaction aimed to reach an elevated body temperature. Indeed, food ingestion induces a rise in body temperature due to post-prandial thermogenesis [58-62]. The hyperphagic effect of orexin A disappears when the body temperature is already increased, so that a reduction in food intake can happen in this condition.

Since thermoregulation is highly coordinated with sleep and cardiovascular control, further experiments could be carried out with analysis of these physiological parameters. Furthermore, additional experiment should be performed using animals lacking orexin A and blocking orexin A receptors.

In conclusion, the thermoregulatory role of orexin A is substantially demonstrated by the findings reported in this paper.

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