Orexin-A Regulates Body Temperature in Coordination with Arousal Status

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Orexins, hypothalamic neuropeptides, are involved in modulation of food intake and arousal status. To further examine their physiological roles in brain function, the effect of centrally administered orexin-A on body temperature was investigated in rats. Assessed by a telemetry sensor system implanted into the abdominal cavity, infusion of orexin-A into the third cerebroventricle (i3vt) increased body temperature in a dose-responsive manner. Expression of uncoupling protein 1 (UCP1) mRNA in brown adipose tissue (BAT), as a marker for peripheral thermogenesis, failed to increase after the infusion. Expression of UCP3 mRNA in skeletal muscle was up-regulated, whereas UCP2 in white adipose tissue was unchanged after the infusion. The resulting information indicates that orexin neurons regulate body temperature in coordination with arousal status independently of peripheral thermogenesis, which is regulated by BAT UCP1. [Exp Biol Med Vol. 226(5):468–476, 2001]

Key words: Orexin-A; body temperature; thermoregulation; UCP family; telemetry sensor system

Orexins, hypothalamic neuropeptides, have been identified in neural cell bodies of the lateral hypothalamic area (LHA), a brain site known as a feeding center (1). Widespread distribution of two orexin receptors, orexin 1 receptor (OX1R) and orexin 2 receptor (OX2R) was demonstrated throughout the brain (2). OX1R mRNA was most abundant in the ventromedial hypothalamic nucleus (VMH), a site regarded as a satiety center (2). The specific localization of cell bodies and receptors in the hypothalamus strongly suggested a functional role for orexins in control of food intake. Indeed, central infusion of orexins accelerated feeding behavior (1). The LHA is involved not only in feeding, but also in a variety of physiological functions such as control of circadian rhythm, peripheral metabolism, and related functions (3, 4). These findings make us assume that orexin neurons in the LHA may contribute to such regulatory functions of the LHA. In fact, involvement of orexin and its receptors in control of the sleep/wakefulness cycle has been reported (5). Orexin knockout mice exhibited a phenotype notably similar to human narcolepsy patients (5).

Body temperature cycle, as well as the sleep/wakefulness cycle, is known to be an essential biological rhythm among other circadian rhythms observed in mammals (6). The circadian body temperature cycle is sufficiently dominant to influence other behavioral and physiological parameters such as ingestive and ambulatory behavior, enzyme activities, hormone concentrations, cardiovascular function, and so on (7). Arousal status tonically influences circadian changes in body temperature, i.e., decreasing during sleep to a trough and an increasing during wakefulness. Neuromodulators, including neuronal histamine and serotonin, which are involved in regulation of the sleep/wakefulness cycle, have been shown to change body temperature (8, 9). Evidence is thus emerging that suggests the coordination between arousal and body temperature controlling systems. This viewpoint leads us to assume that orexin neurons may regulate body temperature in coordination with arousal status. Indeed, the following neuroanatomical findings support such an assumption. First, both orexin receptors have been identified with moderate density in the medial preoptic area (MPOA), a specific site of thermoregulation and one of the projection sites for terminals of orexin neurons from the LHA (2, 10). In addition, LHA orexin neurons have abundant projections to the suprachiasmatic nucleus (SCN), a regulatory center for circadian rhythm, and to the reticular formation, including the locus coeruleus, raphe nuclei, pedunclopontine, and lateral dorsaltegmental nucleus—all identified as centers for maintenance of arousal level (11). Second, orexin neurons are found to project to the peripheral autonomic nervous system (10).

Uncoupling protein 1 (UCP1) in brown adipose tissue (BAT) that contributes to nonshivering and diet-induced

This work was supported by a grant-in-aid (10470233) from the Japanese Ministry of Education, Science and Culture and by Research Grants for Intractable Diseases from the Japanese Ministry of Health and Welfare, 1998.

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Received July 18, 2000.
Accepted January 19, 2001.

0037-9727/01/2265-0468$15.00

thermogenesis is mainly controlled by the sympathetic nervous system. In addition, UCP2 and UCP3, which are predominantly expressed in white adipose tissue (WAT) and skeletal muscle (MSL), respectively (12), have been shown to receive sympathetic influence (13, 33). Direct chemical stimulation of feeding-related centers such as the LHA, the VMH, and the paraventricular nucleus (PVN) is known to modulate sympathetic nerve activity innervating BAT (14). Hypothalamic neuropeptides, including corticotropin releasing hormone (CRH) and neuropeptide Y (NPY), which modulate food intake, affect BAT thermogenesis through sympathetic nerves (3, 15). Hypothalamic regions relating to control of feeding and peripheral thermoregulation constitute neural networks modulated by these and other neuropeptides. Among the network, the LHA is one of the major origins of the efferent pathways to the BAT (16). These findings indicate that orexin neurons from the LHA may affect peripheral processes of thermoregulation. To test this assumption, the present study aimed to investigate effects of orexin-A on body temperature. We also examined whether the neuropeptide may be involved in gene expression of the peripheral UCP family.

Materials and Methods

Animals. Mature male Wistar King A rats weighing 270 to 360 g were used. They were housed in a sound-proof room illuminated daily from 07:00 to 19:00 hr (a 12:12-hr light dark cycle) and maintained at 21° ± 1°C with humidity at 55% ± 5%, except as otherwise described. The rats were allowed free access to standard pellet rat chow (CLEA Co., Tokyo, Japan, mean pellet weight 49.9 ± 1.7 mg) and tap water (mean droplet volume 30.3 ± 2.0 μl). All studies were conducted in accordance with the Oita Medical University Guidelines based on the NIH Guide for the Care and Use of Laboratory Animals.

Apparatus. Each rat was tested in a 30 × 25 × 25-cm testing chamber equipped with a pellet-sensing eatmeter, photoresistor drinkometer, and photosensing counters to measure ambulatory activity (Astec Co., Fukuoka, Japan). Each testing chamber was maintained under the same conditions as those in the handling and feeding room mentioned above. Numbers of food pellets and water droplets consumed and an index number indicating locomotor activity measured by crossing a photobeam were automatically recorded in a mini-computer system-equipped testing room. The details of the apparatus were described elsewhere (17).

Reagent. Orexin-A (Phoenix Pharmaceuticals, Mountain View, CA) was dissolved in phosphate-buffered saline (PBS) to concentrations of 0.06, 0.3, and 1.5 mM. Each solution was freshly prepared on the infusion day. The pH of each solution was adjusted 6.4 to 7.2.

Surgery. Under sodium pentobarbital anesthesia (45 mg/kg, ip), each rat was placed in a stereotaxic apparatus (Narishige Co., Tokyo, Japan) and a stainless guide cannula (23-gauge) was chronically implanted into the third cerebroventricle (i3vt), according to the atlas of Paxinos and Watson, at least 1 week before infusion day. A stainless steel wire stylet (29-gauge) was inserted in the guide cannula to prevent leakage of the cerebrospinal fluid, as well as to prevent obstruction of the cannula. A battery-operated biotelemetry device designed to measure body temperature (Model TA11CTA-F40, Data Sciences International, St. Paul, MN) was concomitantly implanted in the peritoneal cavity of each rat tested. Rats used for blood sample assay were chronically implanted with a silastic catheter (No. 00, Shinnetsu, Tokyo, Japan) for serum sample collection. A catheter was inserted through the right jugular vein with the inner end fixed immediately inside the right atrium. The sampling tube was attached to a 23-gauge Multi-Sampling Needle (Termo) to prevent air from being sucked into the system. Details of surgery procedures were described elsewhere (18).

I3vt Infusion. Rats tested were infused with 10 μl of test solution at a rate of 1 μl/min through the i3vt cannula under unanesthetized or anesthetized, but unrestricted, conditions (19). Ultimately, orexin-A at a dose of 0.6, 3.0, or 15.0 nmol, together with the same volume of PBS as the control, was infused i3vt at 10:00 to 10:10 hr. After the completion of the infusion experiment, all the animals tested were decapitated to verify the cannula location histologically.

Measurement of Abdominal Temperature. Body temperature was measured by use of battery-operated biotelemetry transmitters. Output (frequency in Hz) was monitored by a mounted antenna placed under each animal cage (Model RPC-1, Data Sciences International). The data were then fed into a peripheral processor (matrix model no. BCM 100, Data Sciences International) that was connected to a computer (Series 3510V5, Compaq Computer Corp., Houston, TX, IBM compatible). The signal frequency in hertz was automatically converted to body temperature through a set of precalibrated functions. Automatic control of data collection and analysis was performed by a Dataquest 4 data acquisition system (Data Sciences International). Body temperature was monitored and recorded at 5-min intervals. Details of the apparatus have been described elsewhere (20).

Matched on the basis of body weight and food intake for the last 2 days in the adaptation period immediately before the infusion day, 20 rats were equally divided into the four groups, i.e., the orexin-A groups at doses of 0.6, 3.0, and 15.0 nmol, and the PBS control groups. Body temperature was measured for 3 days before and 2 days after i3vt infusion of test solution under unanesthetized condition. Body temperature was expressed as the difference from the corresponding value 1 day before this infusion. On the infusion day, food was removed during the light period when rats tested were ascertained in advance not to eat, because nonspecific effects of orexin-induced feeding behavior on body temperature had to be excluded.

Matched on the basis of mean body weight and food intake, 10 rats were equally divided into two groups: the

orexin-A group at a dose of 15.0 nmol and the PBS control group. Their abdominal temperature was measured under pentobarbital sodium anesthesia (45 mg/kg) during 12 hr after the infusion. The experimental procedures were the same as for the unanesthetized experiment, as applicable.

Preparation of Rat cDNA Probe. The PCR primers 5′-CATCTTCTGGAGGTAGC-3′ and 5′-AAGACAGGGCAGAATGG-3′ were designed to the coding region of the rat UCP2 gene and primers 5′-GTTACCTTTCCACTGGACAC-3′ and 5′-CCGTTTCAGCTGCTCATAGG-3′ were designed to the UCP3 gene. Reverse-transcription of 10 μg of total RNA from the brain and the skeletal muscle of Wistar King A rats was carried out using Molony murine leukemia virus reverse transcriptase (Life Technologies, Gaithersburg, MD). PCR was conducted with Taq DNA polymerase (Amersham, Buckinghamshire, UK) and 20 pmol of the primers. The reaction profile is as follows: denaturation at 94°C for 1min, annealing at 50°C for 1 min, and extension at 72°C for 1min, for 30 cycles. The PCR fragment of 1012-bp was subcloned into pCRTM2.1 vector (TA cloning kit; Invitrogen, San Diego, CA), and the nucleotide sequence of amplified cDNA was confirmed by sequencing. The nucleotide sequences were determined by the dideoxynucleotide chain termination method, using synthetic oligonucleotide primers that were complementary to the vector sequence and the ABI373A automated DNA Sequencing System (Perkin-Elmer, Norwalk, CT). All DNA sequences were confirmed by reaching both DNA strands. The rat UCP1 probe was generated in an analogous fashion. GenBank accession numbers of UCP2, UCP3, and UCP1 were AB005143, AB006614, and X03894, respectively.

Northern Blot Analysis. Following decapitation 6 hr after i3vt infusion of test solution, interscapular BAT, epididymal WAT, and soleus MSL were surgically removed. The samples were immediately frozen in liquid nitrogen and stored at −80°C until thawed for RNA extraction. Total cellular RNAs were prepared from various rat tissues with the use of Isogen (Nippon Gene, Toyama, Japan) according to manufacture's protocol. Total RNAs (20 μg/lane) of samples from BAT, WAT, and MSL were electrophoresed on 1.2% formaldehyde-agarose gels. The separated RNAs were transferred onto a Biodyne B membrane (Pall Canada Ltd., Mississauga, Canada) in 20× standard saline citrate (SSC) by capillary blotting (21) and were immobilized by exposure to UV light (0.80 J). Prehybridization and hybridization were carried out according to the method described by Yang *et al.* (22). Membranes were washed under high stringency conditions (21). After washing the membranes, the hybridization signals were analyzed with a BIO-image analyzer BAS 2000 (Fuji Film Institution, Tokyo, Japan). The membranes were stripped by exposure to boiling 0.1% sodium dodecyl sulfate (SDS) and were rehybridized with a rRNA that was used to quantify the amounts of RNA species on the blots.

Measurement of Serum Concentration of Glucose, Insulin, and Free Fatty Acid (FFA). Matched on the basis of body weight and food intake for the last 2 days in the adaptation period immediately before the infusion day, eight rats were equally divided into the orexin-A and the PBS control groups. Serum samples were collected at a volume of less than 0.5 ml for each at 0, 6, and 12 hr after i3vt infusion of 15.0 nmol orexin-A or PBS through a right atrium-implanted catheter under unanesthesized and unrestrained conditions. The samples were separated into serum and were immediately frozen at −20°C until measurement. Serum glucose, insulin, and FFA were measured by a commercially available kit (WAKO Pure Chemical Industries-Eiken Chemical, Tokyo, Japan).

Statistical Evaluation. Data from thermal responses and blood samples were evaluated by an analysis of variance (ANOVA) for repeated measures followed by Scheffe's *post hoc* test. Northern blot assay was carried out by the Mann-Whitney U test. Evaluation for a dose-response curve was made by a single linear regression and ANOVA.

Results

Thermal Response to i3vt Infusion of Orexin-A. Figure 1 shows a typical pattern of time-course changes in body temperature in response to i3vt infusion of 15.0 nmol orexin-A or PBS. Continuous recording of body temperature by the telemetry sensor system revealed a circadian fluctuation, i.e., reaching to a trough at an early phase of the light period and a peak at a delayed phase of the dark period. I3vt infusion of 15.0 nmol orexin-A elevated rat body temperature after the infusion in an early phase of the light period when body temperature would normally be maintained at a lower level.

Figure 2 shows time-course changes in body temperature in response to orexin-A or the same volume of PBS under unanesthetized conditions. Body temperature was increased to a peak at about 3 hr after orexin infusion. The elevation was maintained for 3 hr, reaching the first trough at around 9 hr and returning to the basal temperature thereafter. The elevation of body temperature caused by orexin-A was greater than that by PBS infusion ($P < 0.05$). The dose-responsiveness of orexin-induced thermogenesis was revealed as shown in Fig. 3. ($Y = 38.272 + 0.914X$, $r = 0.69$, $P < 0.05$). As shown in Fig. 4, even under anesthetized conditions body temperature increased over preinfusion volume, peaking at 3 hr and returning to the basal temperature at 9 hr after the infusion. Time-course changes in body temperature were thus similar to those under unanesthetized status.

Changes in Ambulatory Activity after Orexin-A Infusion. Time-course of changes in ambulatory activity after i3vt infusion of 15.0 nmol orexin-A or PBS are shown in Fig. 5. Ambulatory activity was expressed as the difference from the corresponding value 1 day before the infusion. Ambulatory activity was increased to a peak at 1

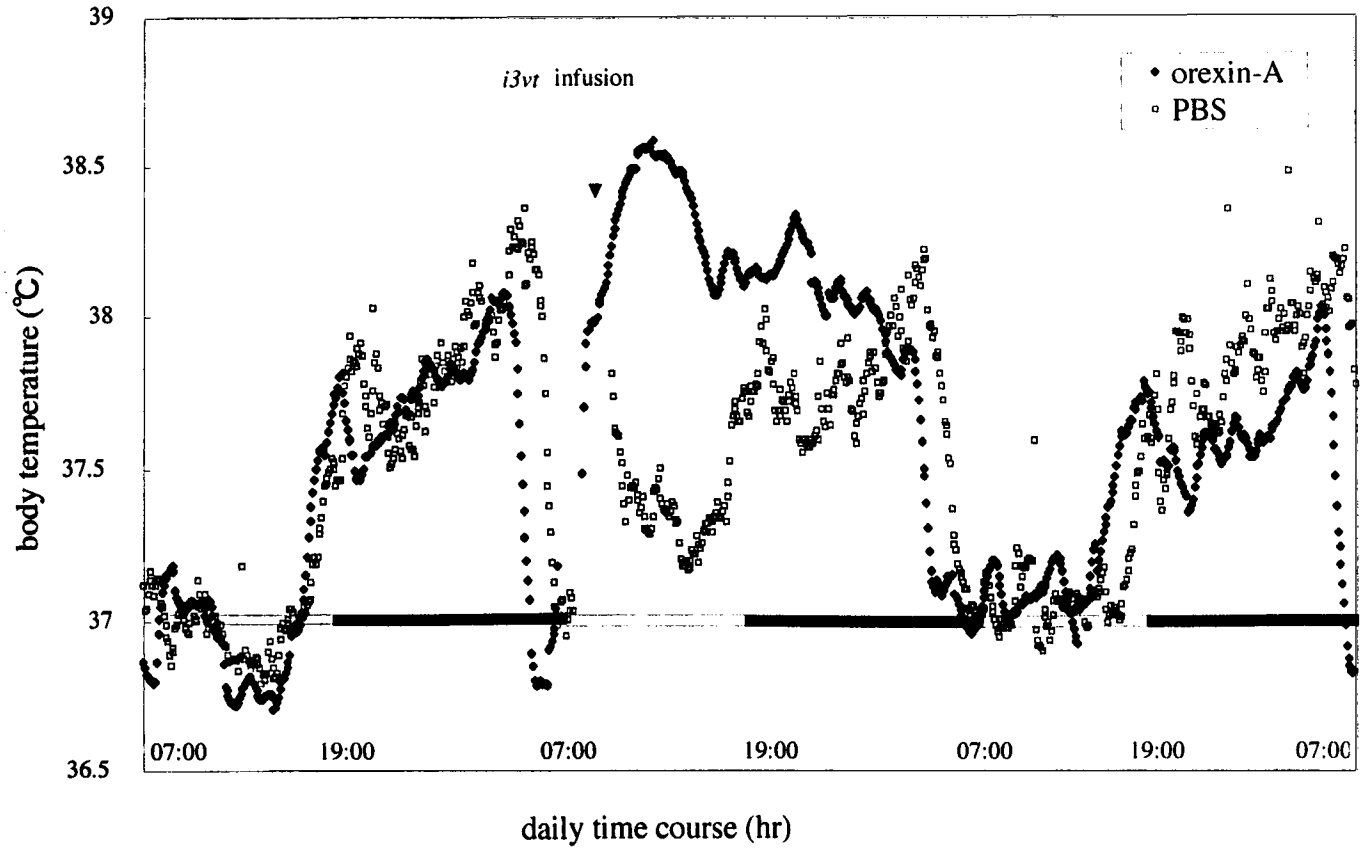

Figure 1. A typical pattern of time-course changes from one rat before, during, and after infusion of orexin-A or PBS into the i3vt. Body temperature showed daily fluctuation at three light-dark cycles, i.e., reaching to a trough at an early phase of the light period and a peak at a delayed phase of the dark period. i3vt infusion, infusion of 15.0 nmol orexin-A or the same volume of PBS at 10:00 hr.

hr after orexin-A infusion and then gradually returned to the basal level. There was a significant difference in ambulatory activity between orexin-A and PBS infusion ($P < 0.05$). Thermogenic behavior, including shivering, was not observed after orexin-A infusion.

Changes in mRNA Expression of the UCP Family after i3vt Infusion of Orexin-A. As shown in Fig. 6, i3vt infusion of orexin-A at a dose of 3.0 or 15.0 nmol induced no remarkable change in mRNA expression of BAT UCP1 or WAT UCP2. Infusion of orexin-A at a dose of 15.0 nmol, but not 3.0 nmol, increased UCP3 mRNA expression in MSL 6 hr after the infusion compared with the corresponding PBS infusion ($P < 0.001$).

Changes in Serum Glucose, Insulin, and FFA after i3vt Infusion of Orexin-A. Table I shows changes in serum glucose, insulin, and FFA after i3vt infusion of 15.0 nmol orexin-A. In response to 15.0 nmol orexin-A-infused i3vt, serum glucose concentration increased to peak at 6hr after the infusion ($P < 0.05$ vs. the corresponding 0-hr value) and returned to the initial level 12 hr after the i3vt infusion. This elevating effect of 15.0 nmol orexin-A on serum glucose concentration was greater than that of PBS ($P < 0.05$).

Akin to the changes in serum glucose, elevation of serum insulin in response to 15.0 nmol orexin-A peaked at 6 hr after the i3vt infusion ($P < 0.05$ vs. the corresponding 0-hr. value). Thereafter, insulin concentration decreased to reach a 12-hr value at a concentration less than the baseline at 0 hr. The elevation of serum insulin concentration induced by the infusion of orexin-A was greater than that after PBS infusion ($P < 0.05$). Unlike glucose or insulin, the effect of 15.0 nmol orexin-A infusion on serum FFA concentration was not significant compared with that of PBS throughout the infusion experiment.

Discussion

The present study demonstrated that i3vt infusion of orexin-A elevated body temperature markedly in rats. In addition, circadian rhythm was detectable in fluctuations of body temperature with an accompanying mesa and trough in the dark and the light periods, respectively. It is well known that circadian rhythm of body temperature that is synchronized with the sleep/wakefulness cycle is regulated endogenously by the SCN in the hypothalamus (24, 25). Circadian rhythms of feeding behavior and ambulatory activity, both of which increase during the dark (26), are also under the control of this system. In viewing the circadian fluctuation of body temperature, the difference between body temperature before and after orexin-A infusion can be differentiated from daily fluctuations of body temperature. Indeed, the present results demonstrate that an orexin-induced increase in body temperature developed and was sustained for about 7 hr after the infusion. Of note, the increasing body tem-

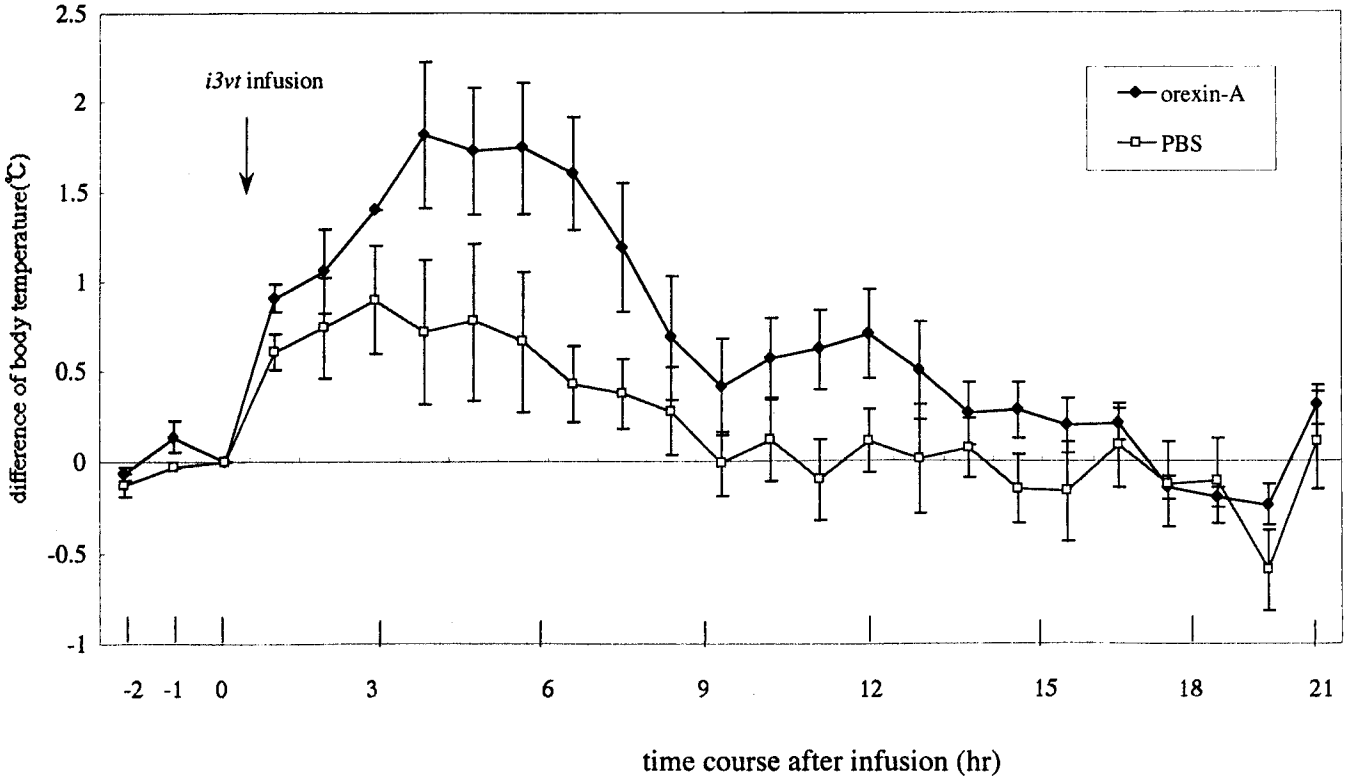

Figure 2. Time-course changes in difference of body temperature in unanesthetized rats following i3vt infusion of orexin-A or PBS. I3vt infusion of 15.0 nmol orexin-A elevated body temperature by 1.8°C 3 hr after the infusion. In this and succeeding figures, difference of body temperature represents the body temperature expressed as difference from the corresponding value 1 day before the infusion. In this and succeeding figures, "i3vt infusion" represents that 15.0 nmol orexin-A or the same volume of PBS as controls was infused i3vt at 10:00 to 10:10 hr. Values are means ± SEM in this and succeeding figures. $P < 0.05$ vs PBS. $n = 5$ for each group.

perature was manifest during the light period when body temperature is normally low in rats. Taken together, it is fair to conclude that the orexin-induced changes in body temperature were not caused by normal daily fluctuations of food intake or ambulatory activity.

Central administration of orexins has been shown to induce feeding behavior in a relatively early phase after the infusion (1). Such results indicate that diet-induced thermogenesis resulting from orexin-A infusion and the concomitant elevation of ambulatory activity may cause an increase in body temperature. To exclude these possibilities, the present study was performed under food-deprived conditions during the light period when the rats tested were not allowed to eat. The current results reveal that orexin-A elevated body temperature even in a food-deprived condition. The present findings thus permit us to conclude that orexin-induced elevation of body temperature develops independently of the concomitant activation of feeding behavior, but not independently of ambulatory activity, as it was accelerated by orexin-A under even food-deprived conditions. A recent report notes that orexin-A increases arousal level in rats (27).

Here, a major question can be raised as to whether orexin-A-induced elevation of body temperature may simply be caused by increased ambulatory activity resulting from the increase in arousal level or by other central influence of thermoregulatory mechanisms. Exercise is one thermogenic factor that produces heat associated with energy metabolism in muscle (28). In the present study, the time courses of changes in body temperature after the infusion were different from those caused by ambulatory activity. In addition, time-course changes in orexin-induced elevation of body temperature in the anesthetized rats mimicked those in the unanesthetized rats. Taken together, the results indicate that orexin-A has a direct effect to elevate body temperature.

From the neuroanatomical point of view, one possible and perhaps the most likely explanation is that orexin-A may regulate body temperature through the MPOA, a major site of central thermoregulation. Supporting this assumption, projections of orexin neurons and a moderate distribution of orexin receptors have been identified in the region (2, 10). An additional possibility is that monoaminergic neurons in the brain such as noradrenalin, serotonin, and histamine neurons, all of which are found important in sleep-wakefulness regulation, may be involved in thermoregulation through neuronal projections to the MPOA (29–31). Noradrenergic neurons of the locus coeruleus, serotonergic neurons of the dorsal and median raphe nuclei, and histaminergic neurons of the tuberomammillary nuclei are densely innervated by orexin terminals (5). The final possibility is that orexin-A may directly control peripheral thermogenesis through the autonomic nervous system. Recently, a neuroanatomical study identified direct innervation

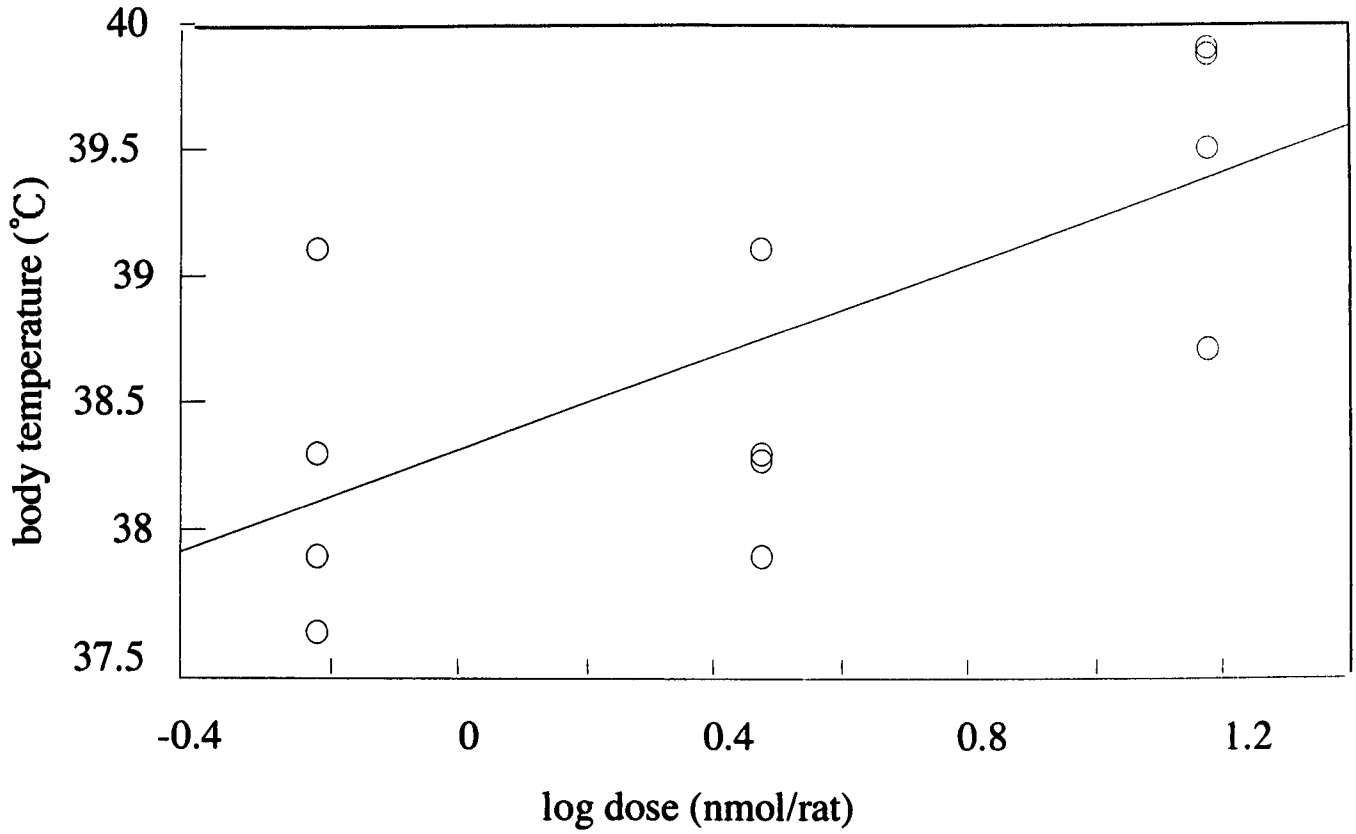

Figure 3. Dose-response relation between log dose of orexin-A and body temperature 6 hr after i3vt infusion of orexin-A in unanesthetized rats. Body temperature increased linearly with increase of molar orexin-A dose. $Y = 38.272 + 0.914X$, $r = 0.69$, $P < 0.05$. Mean body temperature in the PBS controls was 38.1° ± 0.1°C.

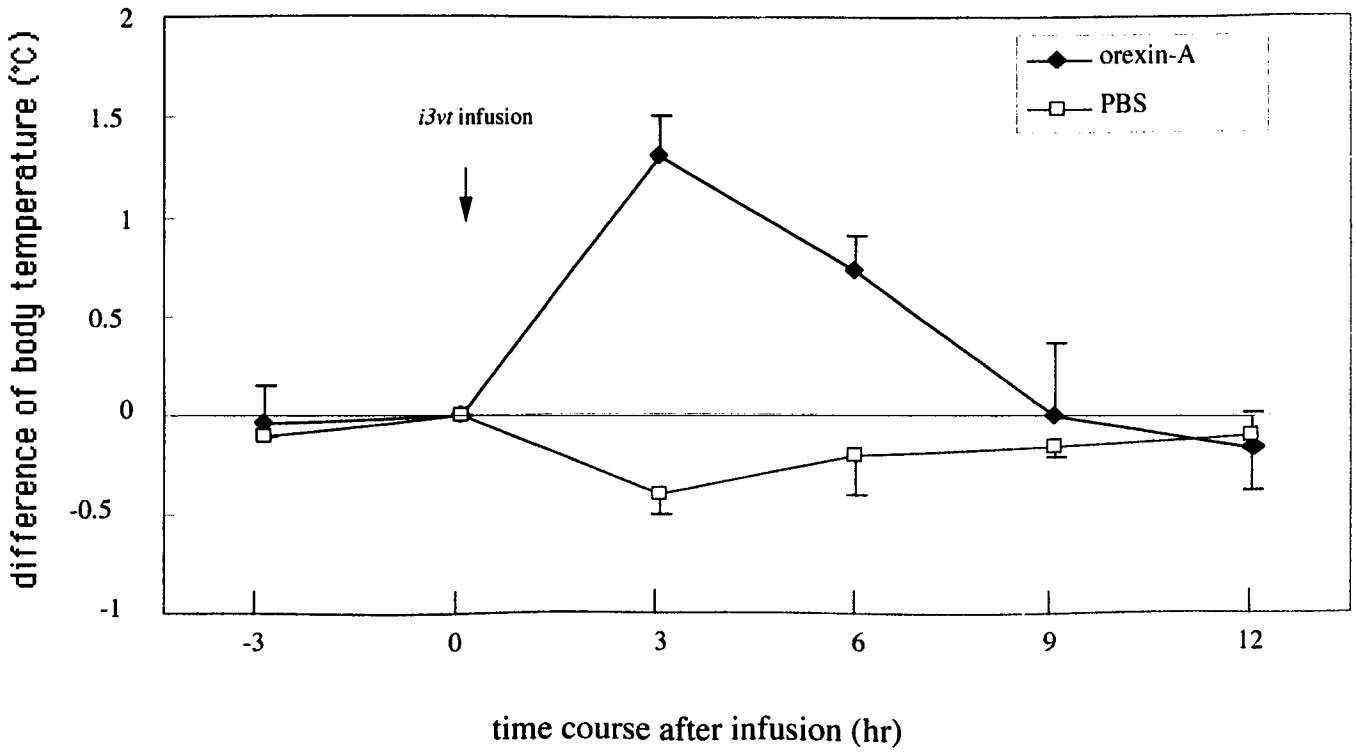

Figure 4. Time-course changes in difference of body temperature in anesthetized rats after i3vt infusion of orexin-A or PBS. Time-course changes in body temperature elevation in the anesthetized rats mimicked those in the unanesthetized rats. $P < 0.05$ vs PBS. $n = 5$ for each group.

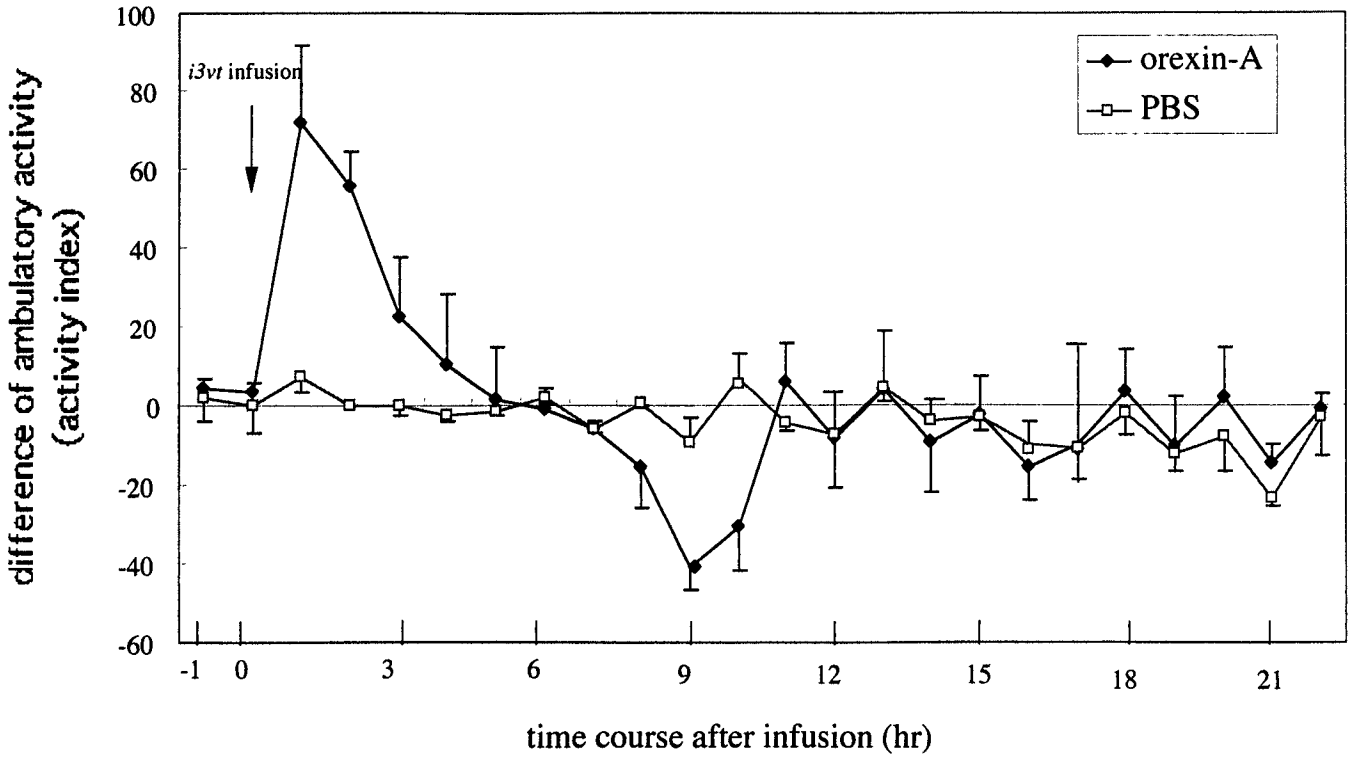

Figure 5. Time-course changes in difference of ambulatory activity after 15.0 nmol i3vt infusion of orexin-A or with PBS. Ambulatory activity increased markedly 1 hr after the infusion and then decreased along the time elapse. $P < 0.05$ vs PBS. $n = 5$ for each group.

by LHA orexin neurons of the sympathetic preganglionic neurons (32). Another neuroanatomical study using transsynaptic retrograde tracer demonstrated that the LHA was one of the origins of sympathetic influence upon BAT, a principle site for peripheral thermogenesis and energy expenditure (16). These findings support the notion that body temperature is, on the other hand, regulated by thermoregulatory efferent signal messages from orexin neurons in the LHA through the autonomic nervous system. To examine the possibility, the effect of orexin-A on gene expression of the UCP1 in BAT was examined. Unexpectedly, BAT UCP1 expression failed to respond to i3vt infusion of orexin-A. This result indicates that BAT UCP1 does not contribute to the elevation of body temperature induced by orexin-A. The present study showed UCP3 mRNA in MSL, unlike BAT UCP1, was up-regulated by i3vt infusion of 15.0 nmol orexin-A. The influence of sympathetic nerve activity upon muscle UCP3 is relatively small (33). Circulating FFAs, another candidate for up-regulation of muscle UCP3 (34), did not change significantly after the orexin-A infusion. It seems most probable that the increase in ambulatory activity *per se* may rather up-regulate UCP3 mRNA expression, when we consider the finding that exercise up-regulates such gene expression (35). Unlike BAT UCP1, however, the physiological role of muscle UCP3 in thermogenesis and energy expenditure is somewhat obscure. Previous findings suggest that thermogenic potency of UCP3 may not be entirely sufficient for the modulation of thermogenesis, even if its expression could influence resting energy expenditure (33). Taken together, it can be con-

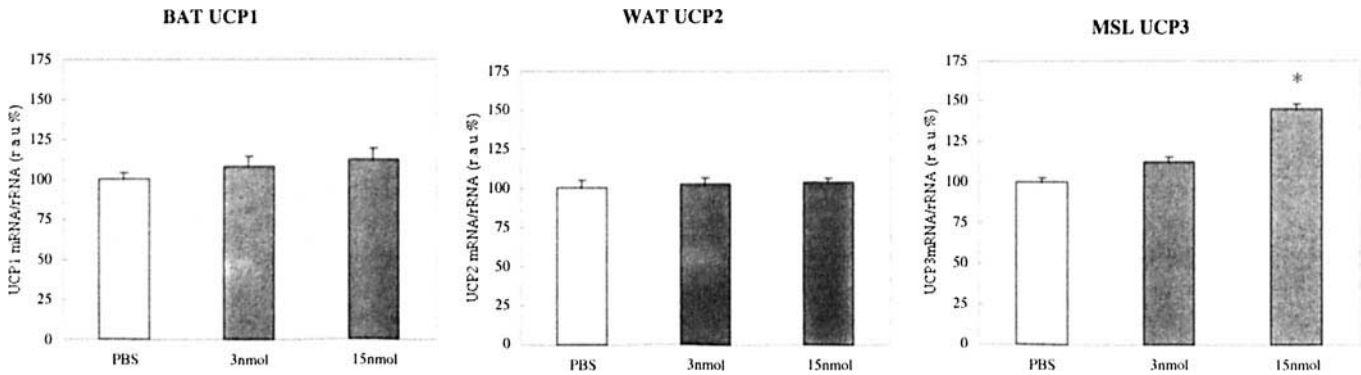

Figure 6. Changes in mRNA expression of the UCP family following infusion of orexin-A in rats. Columns and horizontal bars, means ± SEM. $n = 4$ for each group. * $P < 0.001$ vs the corresponding PBS control group. r.a.u.%, relative arbitrary unit.

Table I. Changes in Serum Glucose, Insulin, and Free Fatty Acid (FFA) After i3vt Infusion of 15.0 nmol Orexin-A

Item measured	Time course (hr)		
	0	6	12
Glucose (mg/dL)			
Orexin-A	121.2 ± 2.5	143.2 ± 6.2[a]	104.5 ± 2.5
PBS	123.0 ± 2.4	123.0 ± 4.5	114.0 ± 6.7
Insulin (μU/mL)			
Orexin-A	12.1 ± 0.7	16.3 ± 0.5[a]	7.2 ± 0.6
PBS	11.9 ± 0.5	12.8 ± 0.9	11.7 ± 2.3
FFA (mEQ/L)			
Orexin-A	0.66 ± 0.05	0.61 ± 0.06	0.68 ± 0.04
PBS	0.59 ± 0.03	0.51 ± 0.01	0.68 ± 0.03

Note. Values, means ± SEM. $n = 4$ for each group.
[a] $P < 0.05$ vs the corresponding phosphate-buffered saline (PBS) controls.

cluded that MSL UCP3 response to orexin-A is not a major factor for change in body temperature.

In addition to the increase in body temperature and ambulation, the present study revealed that i3vt infusion of orexin-A increased blood concentrations of glucose and insulin. In contrast to the effects on BAT sympathetic nerve activity, electrical stimulation or microinjection of 2-deoxy-D-glucose, a glucose analogue that produces intracellular glucoprivation, into the LHA accelerates adrenal sympathetic nerve activity and/or catecholamine secretion from the adrenal medulla (36). This result indicates that there exists definite regional differences in hypothalamic regulation of sympathetic nerve activity. The increase in circulating catecholamine secreted from the adrenal medulla stimulates hepatic glycolysis and elevates blood glucose, together with secondary increase in insulin secretion. This sympathetic pathway is essential in its contribution to energy supply from peripheral organs to the brain under energy-deficient emergency states. Taking into consideration the relationship between orexins and glucose-sensitive neurons in the LHA, the present i3vt infusion of orexin-A may increase serum glucose and insulin concentration through activation of the efferent pathway from the LHA to the adrenal medulla. It remains unclear to date how metabolic responses to orexin-A correlate with regulation of sleep/wakefulness. One possible explanation for the query is the increasing demand for energy supply in response to the orexin-induced increase in arousal level or the concomitant increase in ambulatory activity.

In summary, the present study demonstrates that i3vt infusion of orexin-A caused an increase in body temperature together with ambulatory activity, while leaving BAT UCP1 expression unaffected. The results implicate a contribution of orexins to centrally controlled thermogenesis in coordination with its regulation of arousal status.

We thank Dr. D.S. Knight, Department of Anatomy, Louisiana State University, for help in preparing the manuscript.

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