Orexin A (hypocretin 1) injected into hypothalamic paraventricular nucleus and spontaneous physical activity in rats

Kohji Kiwaki,1 Catherine M. Kotz,2,3,4 Chuanfeng Wang,2 Lorraine Lanningham-Foster,1 and James A. Levine1,3

1Endocrine Research Unit, Mayo Clinic and Mayo Foundation, Rochester 55905; 2Veterans Affairs Medical Center and 3Minnesota Obesity Center, Minneapolis 55417; and 4Departments of Food Science and Nutrition, University of Minnesota, Saint Paul, Minnesota 55108

Submitted 24 March 2003; accepted in final form 21 November 2003

Orexin A (hypocretin 1) injected into hypothalamic paraventricular nucleus and spontaneous physical activity in rats. Am J Physiol Endocrinol Metab 286: E551–E559, 2004. First published December 2, 2003; 10.1152/ajpendo.00126.2003.—In humans, nonexercise activity thermogenesis (NEAT) increases with positive energy balance. The mediator of the interaction between positive energy balance and physical activity is unknown. In this study, we address the hypothesis that orexin A acts in the hypothalamic paraventricular nucleus (PVN) to increase nonfeeding-associated physical activity. PVN-cannulated rats were injected with either orexin A or vehicle during the light and dark cycle. Spontaneous physical activity (SPA) was measured using arrays of infrared activity sensors and night vision videotaped recording (VTR). O2 consumption and CO2 production were measured by indirect calorimetry. Feeding behavior was assessed by VTR. Regardless of the time point of injection, orexin A (1 nmol) was associated with dramatic increases in SPA for 2 h after injection (orexin A: 6.27 ± 1.95 × 103 beam break count, n = 24; vehicle: 1.85 ± 1.13 × 103, n = 38). This increase in SPA was accompanied by compatible increase in O2 consumption. Duration of feeding was increased only when orexin A was injected in the early light phase and accounted for only 3.5 ± 2.5% of the increased physical activity. In a dose-response experiment, increases in SPA were correlated with dose of orexin A linearly up to 2 nmol. PVN injections of orexin receptor antagonist SB-334867 were associated with decreases in SPA and attenuated the effects of PVN-injected orexin A. Thus orexin A can act in PVN to increase nonfeeding-associated physical activity, suggesting that this neuropeptide might be a mediator of NEAT.

OBESEITY AFFECTS ONE-THIRD of the American population and is the second leading cause of death in the United States after smoking (1). Treatment of obesity has proven difficult, and this intractability may be due to the fact that energy balance is regulated through multiple and complex mechanisms that are not fully understood (4). Maintenance of body weight is achieved by an intricate balance between energy intake and expenditure. We found that changes in nonexercise activity thermogenesis (NEAT) mediate resistance to weight gain with overfeeding in sedentary adults (32). There is evidence that “spontaneous” physical activity (SPA) is familial (63) and shows marked interindividual differences in its contribution to daily energy expenditure (49); however, the mediator of the interaction between overfeeding and physical activity is unknown.

Orexins (A and B, also known as hypocretin 1 and 2) are recently identified neuropeptides synthesized exclusively in the lateral hypothalamus, an area classically believed to be a crucial “feeding” center (25). Initial interest in these neuropeptides concentrated on their orexigenic actions, since central injection of orexins increased food intake, and prepro-orexin mRNA was shown to be upregulated with fasting (50). Apart from appetite regulation, orexins have been implicated in the central nervous system (CNS) regulation of arousal and sleep, cardiovascular function, temperature, metabolic rate, locomotor activity, pituitary secretion, glucose homeostasis, and gastric acid secretion (17, 37, 48, 51, 53, 58). Edwards et al. (12) reported that, following intracerebroventricular administration of orexin A, hypothalamic c-fos, a marker of neuronal activation, was highly expressed in the hypothalamic paraventricular nucleus (PVN) and orexin A injected directly into the PVN stimulated food intake, although this effect was not powerful. These authors suggested that the PVN might not be the primary site of action of the orexins in their orexigenic role; rather, the orexins might perform some other role via the PVN. The PVN is demonstrated to be one of the sites innervated by orexin-containing neurons (39, 44) and to express orexin receptor 2 (36, 62).

These observations intrigued us, and we wondered whether spontaneous physical activity (SPA) is not random but rather intricately regulated in the CNS. Furthermore, we would propose that the interaction between overfeeding and physical activity undergoes processing within the hypothalamus due to its role in energy homeostasis.

In this study, we address the hypothesis that orexin A acts on the PVN to increase SPA independently of feeding behavior. Our approach was to inject orexin A into the PVN and measure SPA, thermogenesis, and feeding behavior by use of arrays of infrared activity sensors, indirect calorimetry, and infrared 24-h videotaping. Additionally, we observed effects of the orexin receptor antagonist (selective for orexin 1 receptor, OX1R) SB-334867 injected into the PVN.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Charles River, Wilmington, MA; 250–300 g at time of surgery) were housed individually in cages with a 12:12-h light-dark photocycle (lights on at 7:00 AM) in a room at 22 ± 2°C. Food (Laboratory Rodents Diet 5001; PMI Nutrition

Address for reprint requests and other correspondence: J. A. Levine, Endocrine Research Unit, Mayo Clinic, Rochester, MN 55905 (E-mail: levinjames@mayo.edu).

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International, St. Louis, MO) and water were allowed ad libitum. The protocol was approved by the Mayo Foundation Institutional Animal Care and Use Committee.

Cannulation and Verification of Placement

Rats were anesthetized with ketamine-xylazine mixture (100:10 mg/kg body wt ip) and fitted with a 28-gauge stainless steel cannula placed just above the right PVN. Stereotaxic coordinates were determined from the rat brain atlas of Paxinos and Watson (42) and are as follows: –0.5 mm lateral and –1.9 mm posterior to bregma and 7.3 mm below the skull surface. The injector extended 1 mm beyond the end of the guide cannula. For all cannulations, the incisor bar was set at 3.3 mm below the ear bars. At least 10 days elapsed following surgery before experimental trials. After the experiments, brains were dissected out and stored in a 10% formaldehyde solution for placement verification by histology. This verification was performed in a blinded fashion whereby the physical activity data were unknown to the histologist. A cannula was deemed incorrect if the actual injection site was farther than 0.25 mm away from the targeted site. This rationale is based on diffusion coefficients of injection volume delivered (40) and our previous data (28, 60). Rats with misplaced cannulas were excluded from data analysis in studies 1–3. The number of rats listed in the experimental design represents the number of rats in the final analysis (all cannulas correctly placed).

Chemicals

Orexin A (Phoenix Pharmaceuticals, Mountain View, CA) was dissolved in artificial cerebrospinal fluid (aCSF). The dose of orexin A (1 nmol) used in this study was based on previous studies that observed feeding after PVN injection of orexin A (10, 12, 56).

SB-334867 [1-(2-methylbenzoxazol-6-y1)-3-[1,5]naphthyridin-4-y1 urea hydrochloride] was synthesized by GlaxoSmithKline (11, 19) and was dissolved in 5% DMSO in aCSF. SB-334867 has a 50-fold higher affinity for OX1R than that for the orexin 2 receptor (OX2R) (11).

Injections

Injection cannulas (33 gauge) were purchased from Plastics One (Roanoke, VA). They are fabricated such that, when inserted to their maximum depth, they protrude 1.0 mm beyond the tip of the guide cannula. They are fabricated such that, when inserted to their maximum depth, they protrude 1.0 mm beyond the tip of the guide cannula placed just above the right PVN. Stereotaxic coordinates were determined from the rat brain atlas of Paxinos and Watson (42) and are as follows: –0.5 mm lateral and –1.9 mm posterior to bregma and 7.3 mm below the skull surface. The injector extended 1 mm beyond the end of the guide cannula. For all cannulations, the incisor bar was set at 3.3 mm below the ear bars. At least 10 days elapsed following surgery before experimental trials. After the experiments, brains were dissected out and stored in a 10% formaldehyde solution for placement verification by histology. This verification was performed in a blinded fashion whereby the physical activity data were unknown to the histologist. A cannula was deemed incorrect if the actual injection site was farther than 0.25 mm away from the targeted site. This rationale is based on diffusion coefficients of injection volume delivered (40) and our previous data (28, 60). Rats with misplaced cannulas were excluded from data analysis in studies 1–3. The number of rats listed in the experimental design represents the number of rats in the final analysis (all cannulas correctly placed).

Measurement of O2 Consumption, CO2 Production, SPA, and Duration of Feeding Behavior

O2 consumption and CO2 production were measured using a customized, high-precision, single-chamber indirect calorimeter (Columbus Instruments, Columbus, OH) as we have reported previously (29, 33). Thermogenesis was calculated from O2 consumption and CO2 production. Calibration of the calorimeter was performed before each measurement. The animal was placed inside the cylindrical calorimeter chamber (acrylic; diameter 30 cm, height 20 cm, volume 15 liters) along with the food and water bowls in study 1 or without food and water in studies 2 and 3. The chamber lid was attached and sealed, and room air was pumped at atmospheric pressure through the chamber at 0.802 l/min. Data on O2 consumption and CO2 production were then collected every minute for 24 h and stored on a personal computer. Each data point was identified by a time stamp.

SPA was measured simultaneously with the O2 consumption and CO2 production measurements. Measurements were performed using customized, high-precision racks of collimated infrared activity sensors (Columbus Instruments) placed around the acrylic chamber. There were 45 collimated beams of infrared light crossing the 30-cm-diameter cage, allowing the detection of 1 cm of movement in three orthogonal axes. Photosensors registered an activity unit each time a beam was interrupted. In this fashion, activity was detected simultaneously on all three axes: forward and backward, side to side, and up and down. Data for SPA were summed for every minute and stored on the PC with use of the time stamp for identification. Data were thereby derived simultaneously for O2 consumption and SPA, for each animal, minute by minute, over the 24-h measurement period. Duration of SPA was defined as duration when the activity sensors read greater than zero.

An 8-mm charge-coupled device video camera with infrared and digital time recording capabilities was used for the measuring of feeding behavior. The experiments were videotaped from overhead, and duration of feeding behavior was measured by investigators blinded to whether the animal received orexin A or aCSF.

Experimental Design

On test days, rats were first acclimated to the experimental 15-liter cylindrical cages for >24 h. Food and water pots were placed on the floor of the cylindrical cage, and access to them was ad libitum during the acclimation period. Pots were prevented from tipping by using a purposely built acrylic plate that we designed. The unmanned animal room was sound plus light proof and locked throughout the experiment.

Study 1: effect of orexin A injected at different time points on SPA, O2 consumption, and feeding behavior. In three separate experiments, rats were injected with 1 nmol of orexin A (n = 15) or same volume (0.5 μl) of aCSF (n = 22) at different times of the day: experiment 1, injection at 8:00 AM and 4:00 PM, n = 5 (orexin A) and 12:00 (aCSF); experiment 2, injection at 2:00 PM, n = 6 (orexin A and aCSF); experiment 3, injection at 12:00 PM and 7:00 PM, n = 4 (orexin A and aCSF). O2 consumption was measured only in experiment 1. Food and water pots were placed on the floor of the cage, and access to them was ad libitum during the observation. Twenty-four-hour food consumption was measured by weighing food at 8:00 AM in experiment 1, at 2:00 PM in experiment 2, and at 12:00 noon in experiment 3.

Study 2: relationship between dose of orexin A and its effect on SPA and thermogenesis. Each rat (n = 5) was injected at intervals of 24 h with different doses of orexin A (0.0625, 0.125, 0.25, 0.5, 1, and 2 nmol) and vehicle during the light cycle. The order of injections with different doses of orexin A or vehicle was randomized. After injections, SPA and thermogenesis were measured for 2 h. Food and water were not provided for 2 h before and for the 2 h of observation.

Study 3: effect of SB-334867 on SPA and thermogenesis. Two injections spaced 5 min apart: 1) vehicle plus vehicle, 2) 20 μg of SB-334867 plus vehicle, 3) vehicle plus 1 nmol of orexin A, and 4) 20 μg of SB-334867 plus 1 nmol of orexin A, were given to each rat (n = 5) at intervals of 24 h during the light cycle. The order of injections 1–4 was randomized. After injections, SPA and thermogenesis were measured for 2 h. Food and water were not provided for 2 h before and for the 2 h of observation.
Statistical Analyses

The results are expressed as means ± SE in Figs. 1–6. SPA, O₂ consumption, thermogenesis, and feeding behavior were analyzed using Mann-Whitney’s U-test. Comparison of SPA induced by orexin A between different time points was made by the Kruskal-Wallis rank test. Correlation between orexin A-induced SPA and feeding behavior was analyzed by Spearman’s rank correlation test. Correlation between ΔSPA and Δthermogenesis in the dose-response experiment was analyzed by Pearson’s correlation test. P < 0.05 was considered statistically significant.

RESULTS

Effect of Orexin A on SPA and O₂ Consumption

Adult male Sprague-Dawley rats were microinjected into the right lateral PVN with 1 nmol of orexin A or vehicle (aCSF) at different times of day (experiment 1, injections at 8:00 AM and 4:00 PM; experiment 2, at 2:00 PM; experiment 3, at 12:00 noon and 7:00 PM, light on at 7:00 AM and light off at 7:00 PM). Figure 1 represents the mean 24-h time course of SPA (Fig. 1A) and oxygen consumption (Fig. 1B) in experiment 1. Orexin A was associated with an increase in SPA for 1–2 h after injection followed by a decrease in SPA for 1–3 h afterward. The same pattern in SPA was observed after orexin A injections in experiments 2 and 3 (data not shown). Transition of O₂ consumption in response to orexin A was very similar to that of SPA; orexin A was associated with an increase in O₂ consumption for 1–2 h after injection. Regardless of the time point of injection, orexin A was associated with dramatic increases in SPA for 2 h after injection (Fig. 2, A and B). With all experiments combined, the mean ± SD of SPA for 2 h after injection was as follows: orexin A, 6.27 ± 1.95 × 10³ beam break count; and vehicle, 1.85 ± 1.13 × 10³ (no. of injections was 24 for orexin A-treated animals and 38 for aCSF-treated animals). Interestingly, there was no difference in total SPA for 24 h between orexin A and vehicle in all three experiments (orexin A, 3.80 ± 0.86 × 10⁴ beam break count, n = 15; vehicle, 3.60 ± 0.52 × 10⁴ beam break count, n = 22).

Types of orexin A-induced physical activity were primarily intense grooming and face washing behavior and secondarily rearing and searching behavior. These behaviors appeared immediately (≤5 min) after the injections. After review of the videotaped recording, it was apparent that the intensity of

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**Fig. 1.** Twenty-four-hour time course of effect of orexin A on spontaneous physical activity (SPA; A) and O₂ consumption (B). Rats received paraventricular nucleus (PVN) injection of artificial cerebrospinal fluid (aCSF) or 1 nmol of orexin A at 8:00 AM and 4:00 PM. Arrows indicate injections. SPA is shown as mean total beam breaks (±SE) for 1 h in each group (A, left) and as Δ (difference of mean SPA between orexin A group and aCSF group; A, right). O₂ consumption is shown as mean ml/kg body wt (±SE) for 1 h in each group (B, left) and as Δ (difference of mean O₂ consumption between orexin A group and aCSF group; B, right). * # Significant difference from respective aCSF control group: * orexin A group > aCSF group; and # orexin A group < aCSF group. Open horizontal bars, lights-on period; filled horizontal bars, lights-off period.
orexin A-induced behavior was greater than for similar types of spontaneous activities (grooming and rearing) observed in the absence of experimental manipulations.

**Effect of Orexin A on Feeding Behavior**

It is known that orexin A is associated with increased feeding behavior. Duration of feeding behavior for 2 h after injection was measured in a blinded fashion, using videotaped recording. For five time points throughout the day, only at one time point, 8:00 AM, was duration of feeding behavior longer for orexin A-injected animals than for aCSF (Fig. 2C). The effect of orexin A on feeding behavior accounted for 3.5 ± 2.5% (n = 5) of the increase in SPA at this time point. Figure 3 represents a relationship between SPA and duration of feeding behavior for 2 h after injection in all experiments. There was no correlation between SPA and duration of feeding behavior induced by orexin A. In all three experiments, there was no difference in 24-h food consumption between aCSF and orexin A (orexin A, 24.1 ± 4.8 g, n = 15; vehicle, 25.7 ± 2.9 g, n = 22).

**Relationship Between Dose of Orexin A and Its Effect on SPA and Thermogenesis**

To establish a dose-response relationship, different doses of orexin A, from 0.0625 to 2 nmol, were injected into the PVN. For 2 h after the injection, SPA and thermogenesis were measured without food and water. Both SPA (Fig. 4A) and thermogenesis (Fig. 4B) were well correlated with a dose of orexin A in the examined range. Orexin A at 0.125 nmol was associated with a significant increase in SPA, and the lower

**Fig. 2.** Effect of orexin A injection at different time points on SPA (A and B) and feeding behavior (C). Rats received PVN injection of aCSF or 1 nmol of orexin A at different times (8:00 AM and 4:00 PM in experiment 1; 2:00 PM in experiment 2; 12:00 noon and 7:00 PM in experiment 3). Data (means ± SE) are shown as total beam breaks for 2 h after each injection (A), duration of SPA for 2 h after each injection (B), and total duration of feeding behavior for 2 h after each injection (C). *Significant difference from respective aCSF control group.

**Fig. 3.** Effect of orexin A on SPA and duration of feeding behavior. SPA and duration of feeding for 2 h after PVN injection of orexin A in each animal across 3 experiments was plotted. SPA was represented by beam break count in A and by duration (min) in B.
dose, 0.0625 nmol, was associated with a significant increase in thermogenesis. There was a strong correlation between ΔSPA (difference of SPA between orexin A and vehicle) and Δthermogenesis (difference of thermogenesis between orexin A and vehicle) (r = 0.774, P < 0.0001, n = 30).

Effect of SB-334867 on SPA and Thermogenesis

To establish specificity of the effect of orexin A at the PVN, orexin receptor antagonist SB-334867 was injected into the PVN. SB-334867 (20 μg) was associated with decreases in SPA and partially attenuated the effect of orexin A on both SPA (Fig. 5A) and thermogenesis (Fig. 5B).

Verification of Injection Sites

Figure 6A illustrates an example of histological verification of correct location of injections into the PVN. Figure 6B illustrates a map of actual injection sites and demonstrates those injections deemed correctly and incorrectly located. All animals underwent histological confirmation of cannula placement. Investigators were blinded to the orexin response. In nine incorrectly cannulated rats, the mean ± SD of SPA for 2 h after injection was as follows: 1 nmol of orexin A, 2.28 ± 1.17 × 10³ beam break count; and vehicle, 2.17 ± 1.39 × 10³ (no. of injections was 15 for orexin A and 8 for vehicle). There was no significant difference in SPA between orexin A and vehicle in these animals.

**DISCUSSION**

The energy cost associated with SPA may be carefully modulated rather than spontaneous. We proposed that a potential mediator of SPA is orexin A. Furthermore, we thought that orexin A might mediate this effect via the PVN. To address the hypothesis that orexin A in the PVN mediates SPA, we injected rats with orexin A or vehicle control and monitored SPA by use of infrared beams and videotape recording. The data demonstrated that orexin A, in all the animals where the cannula was sited in the PVN, was associated with dramatic increases in SPA for 2 h after the injection. After these 2 h, SPA did not return to baseline, but the animals appeared to compensate and become more sedentary than controls for the next 1–3 h. Increases in SPA for 2 h after injection were dose dependent. In incorrectly cannulated rats, no increase in SPA was observed after the orexin A injection. These data demonstrate a potential role for orexin A at the PVN in SPA and moreover demonstrate that SPA may be intricately modulated through neurohumoral factors in the hypothalamus.

To establish the specificity of the effect of orexin A at the PVN, we observed the effect of orexin receptor antagonist SB-334867 injected into the PVN. Decreases in SPA followed the antagonist injection, and it partially blocked the effect of orexin A on both SPA and thermogenesis. SB-334867 has a
50-fold higher affinity for the OX₁R than for the OX₂R (11). In the rat brain, differential expression of OX₁R and OX₂R has been reported, and OX₂R was predominantly expressed in the PVN (36, 62). OX₁R is considered to be a selective receptor for orexin A, and OX₂R is considered to be a nonselective receptor for both orexin A and orexin B. Despite lower affinity at OX₂R, high concentration of SB-334867 at the site, the PVN, might attenuate the effect of orexin A via block of OX₂R; however, because it was also reported that intraperitoneal injection of this compound attenuated orexin A-evoked grooming (11), selective OX₂R antagonist is needed to verify the role of the receptor subtype in the effect of orexin A at the PVN.

Our data are consistent with other observations regarding the neurohumoral role of orexin A. Central orexin systems may be critical regulators of sleep/wake states based on neuroanatomic and genetic data (7, 8, 13, 34, 41, 44). Specifically, orexin A may activate arousal centers (17). Our observations that PVN-injected orexin A induces SPA are compatible with the fact that orexin A acts to maintain the waking state (47). The behaviors that we observed in response to orexin A injection were consistent with wakefulness, namely, intense grooming, face-washing behavior, and rearing and searching activity. Several reports also describe an immediate increase in grooming, face-washing activity, and locomotor activity after intracerebroventricular injection of orexin A (17, 21, 22, 24, 38). Additionally, there are reports showing the relationship between endogenous orexin and locomotor activity. Kiyashchenko et al. (27) showed that orexin A release into cerebrospinal fluid was higher during active waking than in quiet waking in cats. Estabrooke et al. (15) reported that Fos expression in orexin neurons correlated with locomotor activity in rats. Moreover, Hara et al. (18) showed that genetic ablation of orexin neurons in mice resulted in a decrease in spontaneous motor activity and that these transgenic mice became obese despite hypophagia. These reports also strongly suggest that orexin plays a role in regulation of SPA.

Corticotropin-releasing factor (CRF) is suggested to be involved in orexin A-induced behaviors (21), because it is known that grooming behavior is closely associated with environmental stress (16), and intracerebroventricular administration of orexin A increases plasma corticosterone (17, 31) and adrenocorticotropic levels (23, 52). Intracerebroventricular administration of CRF increased grooming behavior (30) and intracerebroventricular administration of α-helical CRF, a CRF antagonist, blocked the orexin-induced behaviors, specifically grooming and face washing (22). In terms of brain concentration, CRF is synthesized mainly in the PVN (55), and this peptide is also thought to be integral to energy balance. The PVN is one of the sites densely innervated by orexin-containing neurons and expresses OX₂R (8, 36, 62). Thus our results are consistent with the thesis that orexin A stimulates these specific behaviors via CRF. The dopaminergic system is also
suggested to mediate the orexin A-induced activity and grooming behavior. Involvement of the ventral tegmental area dopaminergic system in intraventricularly administered orexin-induced hyperactivity in rats has been demonstrated (38). Further study is needed to determine the downstream pathways of orexin A-induced hyperactivity.

It is known that orexin A injected into the PVN induces feeding. However, our data demonstrate that PVN-injected orexin A induced feeding behavior only when it was injected early in the light cycle when feeding behavior was minimal in control animals. There was no relation between the magnitude of orexin A-induced SPA and duration of feeding behavior. In other words, PVN-injected orexin A can increase SPA without induction of feeding behavior. This elevation in activity without concomitant feeding behavior is consistent with our previous studies examining the effect of orexin A injected into the lateral hypothalamus, another brain site integral to energy homeostasis (20). The early-light cycle feeding induced by PVN-injected orexin A in this study is in agreement with data from others indicating that orexin A injected into the ventricles and into the lateral hypothalamus stimulates feeding only during the light cycle (20, 67). The reason why stimulation of feeding after orexin A injection occurs only during the light cycle remains unclear. It has been demonstrated that for orexin A injected into the lateral hypothalamus there is a twofold greater feeding response to orexin A in food-restricted rats, which have a high level and rate of feeding (60). Thus level and/or rate of feeding are unlikely to be related to the lack of feeding after orexin during the dark cycle. Espana et al. (14) reported that orexin A-induced (icv administration) feeding was largely circadian dependent and that orexin A-induced increase in grooming was moderately circadian dependent. These authors suggested the possibility that the increase in feeding appeared to largely reflect the wake-increase actions. Other reports regarding orexin A injection into the PVN have shown variable results (10, 12, 56). Edwards et al. (12) reported that PVN-injected orexin A (0.03–0.3 nmol) stimulated food intake, although this effect was not powerful. Dube et al. (10) showed that 1 nmol of orexin A injected into the PVN stimulated food intake, but they reported no other behavioral effects than feeding and drinking. Differences in dose of orexin A (0.03–1 nmol, injected under light cycle in all reports) and the generally moderate effect of orexin A on feeding may explain the discrepant results in feeding response between these three reports. In the present study, we injected 1 nmol of orexin A and observed increased feeding when it was injected in the early light cycle, and we also observed remarkably increased physical activity. This observation contrasts somewhat with that of Dube et al.; however, they did not examine SPA. We recorded animals’ behavior by using videotaped recording and quantified their activity by using arrays of infrared activity sensors. Our results suggest that orexin A in the PVN acts predominantly to increase nonfeeding physical activity.

Levels of SPA in humans cluster in families (63) and for mice within strains (5). These observations suggest that identifiable genetic components may exist to account for variance in NEAT. Additionally, we observed NEAT to increase with positive energy balance (32). Recently, decreased plasma orexin A levels in obese individuals was demonstrated (2). Because orexin A can pass the blood-brain barrier (26), peripheral orexin A levels might reflect its CNS levels. In the mouse hypothalamus, age-related decline in OX2R messenger RNA levels has been shown (59), and it is known that old rats have decreased activity (43). The decline in orexin with obesity and aging may support the hypothesis that orexin A in the PVN is a mediator of NEAT. Lubkin and Stricker-Krongrad (35) reported that intracerebroventricular injection of orexin A resulted in an increase in metabolic rate that was not dependent on an increase in activity in mice. We measured O2 consumption and CO2 production by indirect calorimetry. Increased O2 consumption accompanied increased activity and the thermogenic response to orexin A, which clearly tracks the orexin A effect on SPA. Thus we consider the increase in O2 consumption and thermogenesis to be a result of the increase in SPA associated with orexin A. The reason for this contrast may be due to differences in injection site between studies; site-specific injections reach a much more limited receptor population than ventricular injections. With a ventricular injection, it is unknown where injectate ultimately and predominantly reaches; thus effects observed could result from stimulation of receptors at multiple locations.

Unlike neuropeptide Y, a potent orexigenic and hypothermic signal with a clearly defined pattern in energy balance states, orexin A activity in energy balance states appears to be more complicated. Some studies show no difference in orexin A activity after fasting, (3, 45, 65), whereas others show elevated orexin expression or activity after fasting (50, 66, 69). Orexin A activity during states of positive energy balance has not been thoroughly tested. One study showed that high-fat feeding increased gene expression of orexin A (64), which is consistent with our hypothesis of increased orexin activity with positive energy balance. However, studies of obese animal models show either decreases in orexin A expression or activity (6, 54, 68) or no change (57, 61). These conflicting data likely reflect the complicated role of orexin A in energy balance: although orexin administration stimulates feeding, it also elevates activity, perhaps predominantly, which enhances thermogenesis. Therefore, one cannot simply place orexin A in the class of obesity-promoting neuropeptides on the basis of its feeding-stimulatory properties. This is clearly illustrated by the data from Hara et al. (18), who demonstrated that loss of orexin A activity resulted in an obese animal model. Although these animals ate less, they were also much less active. The obese state of the animals in this study indicates that the decreased energy expenditure brought about by the loss of orexin A function was clearly more potent than the hypophagia in affecting energy balance. Thus, although the present data and those of others (18, 64) indicate that positive energy balance may stimulate orexin A, further studies examining endogenous orexin A activity in lean and obese animal models during overfeeding paradigms will help to determine what role orexin A plays in modulating NEAT.

In summary, injection of orexin A into the PVN dramatically increases SPA. The increase in SPA for 2 h occurs regardless of the time of injection. The effect of orexin A on SPA appears to be predominantly through an increase in general activity. However, we concur with others that orexin A via the PVN also stimulates feeding behavior. Orexin A therefore appears to be a neurohumoral mediator of SPA, NEAT, and feeding. Its role in the physiology of energy balance requires further investigation.
ACKNOWLEDGMENTS

We thank Mary Mullet for expert technical assistance with stereotactic surgery and Shelly K. McCrady and Lana Nyisse for technical help.

GRANTS

This work was supported by the National Institute of Diabetes and Digestive and Kidney Diseases (Grants DK-56650, DK-63226, and DK-57573), the Department of Veterans Affairs, and Novo Nordisk ( Bagsvaerd, Denmark).

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