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Orexin Receptor Subtype Activation and Locomotor Behavior in the Rat

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Abstract

Aim—Orexin producing neurons, located primarily in the perifornical region of the lateral hypothalamus, project to a wide spectrum of brain sites where they influence numerous behaviors as well as modulating the neuroendocrine and autonomic responses to stress. While some of the actions of orexin appear to be mediated via the type 1 receptor, some are not, including its action on the release of one stress hormone, prolactin. We describe here the ability of orexin to increase locomotor behaviors and identify the importance of both receptor subtypes in these actions.

Methods—Rats were tested for their behavioral responses to the central activation of both the type 1 (OX₁R) and type 2 (OX₂R) receptor (ICV orexin A), as compared to OX₂R activation using a relatively selective OX₂R agonist in the absence or presence of an orexin receptor antagonist that possesses highest affinity for the OX₁R.

Results—Increases in locomotor activity were observed, effects which were expressed by not only orexin A, which binds to both the OX₁R and the OX₂R receptors, but also by the relatively selective OX₂R agonist, [(Ala¹¹, Leu¹⁵)-orexin B]. Furthermore the OX₁R selective antagonist only partially blocked the action of orexin A on most locomotor behaviors and did not block the actions of [(Ala¹¹, Leu¹⁵)-orexin B].

Conclusion—We conclude that orexin A exerts its effects on locomotor behavior via both the OX₁R and OX₂R and that agonism or antagonism of only one of these receptors for therapeutic purposes (i.e. sleep disorders) would not provide selectivity in terms of associated behavioral side effects.

Keywords

Orexin; Orexin Receptors; Locomotor Behavior; Stereotypy

In addition to the recognized importance of orexin-producing neurons in the perifornical region of lateral hypothalamus in the physiological regulation of sleep/wakefulness (Chemelli et al, 1999; Hara et al, 2001; Milekovsky et al, 2005; Taheri et al, 2002; Willie, et al 2001), several other potent actions of the peptide produced in those neurons have been chronicled. Indeed, the ability of exogenously administered orexin to stimulate arousal was reported by several groups (Hagan, et al 1999; Kiwaki, et al 2004) soon after the initial reports of the discovery of these peptides (De Lecea, et al 1998; Sakurai, et al 1998). Thus,

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in addition to exerting potent effects on sleep/wakefulness (Mileykovsky, et al 2005), the orexins have been reported to increase locomotor (Kiwaki, et al 2004) and feeding behaviors (Sakurai, et al 1998), activate the sympathetic nervous system thereby increasing blood pressure and heart rate (Chen, et al 2000; Machado, et al 2002; Samson, et al 1999; Shirasaka, et al 1999, Smith, et al 2002), and to stimulate the neuroendocrine pathways controlling stress hormone secretion (Al-Barzanji, et al 2001; Ida, et al 2000; Jaszberenyi, et al 2000; Russell, et al 2001; Sakamoto, et al. 2004; Samson, et al 2002). The actions of the orexins are mediated by two unique G-protein coupled receptors which bind both orexin A and orexin B with varying selectivity (Sakurai, et al. 1998). The orexin type 1 receptor (OX₁R) binds orexin A with higher affinity than orexin B in vitro. On the other hand the orexin type 2 receptor (OX₂R) binds both ligands with approximately equal affinity (Sakurai, et al. 1998). Both receptor subtypes are expressed throughout the central nervous system (CNS), although regional variations in relative expression densities have been reported (Cluderay, et al 2002; Hervieu, et al 2001, Lu, et al 2000, Marcus, et al 2004, Backberg et al 2001).

In previous studies (Samson, et al 2007) we have employed the relatively selective OX₁R antagonist, SB 408124 (Langmead, et al 2004), in an attempt to identify the receptor subtype responsible for the cardiovascular and neuroendocrine actions of the orexins. Indeed, pretreatment of rats with the OX₁R antagonist prior to intracerebroventricular (i.c.v.) orexin administration blocks the ability of the peptide to elevate blood pressure and heart rate (autonomic effects). In addition, the ability of orexin to activate the hypothalamo-pituitary-adrenal (HPA) axis via stimulation of corticotropin releasing factor (CRF) release was blocked by pretreatment with the OX₁R antagonist and importantly, immobilization stress-induced ACTH release was similarly blocked by that antagonist (Samson, et al 2007). However, this neuroendocrine effect of orexin was selective for the HPA axis, since blockade of the type 1 receptor did not prevent the ability of exogenous orexin to stimulate prolactin (PRL) release and did not alter the PRL response to immobilization stress (Samson, et al 2007). Those data suggest the importance of the type 2 receptor in the control of PRL secretion; however, since no OX₂R antagonist is available commercially, the importance of the type 2 receptor in PRL secretion can not be conclusively established.

In the original discovery paper, Sakurai and colleagues (1998) demonstrated an EC₅₀ for ORX-A on calcium mobilization from transfected CHO cells to be 30 nM for the OX₁R and 34 nM in cells transfected with the OX₂R. Thus ORX-A activates both receptor subtypes with approximately equal efficacy. On the other hand in those same transfected cells, ORX-B displayed an EC₅₀ for activation of the OX₁R of 4200 nM, but 60 nM for activation of the OX₂R. The authors concluded that the OX₂R was a nonselective receptor for the orexins, while the OX₁R was relatively selective for the ORX-A. In contrast, the relatively selective OX₂R receptor ligand employed in this manuscript, [Ala(11), D-Leu(15)]orexin-B, was demonstrated in transfected CHO cells to stimulate calcium mobilization with an EC₅₀ of 0.13 nM for the OX₂R and 52 nM for the OX₁R (Asahi, et al. 2003). Thus this ligand is considered relatively selective for the OX₂R. Finally the OX₁R receptor antagonist employed in our studies, SB-408124, was demonstrated by Langmead and colleagues (2004) to be a competitive antagonist with 50-fold higher selectivity for the OX₁R versus the OX₂R. Thus SB-408124 is a relatively selective OX₁R receptor antagonist.

Here we report that i.c.v. administration of orexin A causes dose related increases in several locomotor behaviors and that not all those effects can be ascribed to actions of orexin A on the type 1 receptors (i.e. they are not fully negated by pretreatment with OX₁R antagonist). Furthermore, we demonstrate that spontaneous behaviors also can be activated by a relatively selective type 2 receptor agonist and that the action of that OX₂R ligand is not blocked by the antagonist with highest affinity for the type 1 receptor. We conclude that

both the OX₁R and the OX₂R are important for the full expression of the several behavioral actions of the orexins, particularly those apparently accompanying the stress response.

Materials and Methods

Animals. All procedures have been approved by the animal care committee of Saint Louis University. Adult male rats (225-300 grams, Sprague Dawley, Harlan, Indianapolis, IN) were used in these protocols. They were maintained (12-12 light dark cycle, lights on 0600 hr, 23-25 C) with free access to food and water. Under ketamine (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA)/xylazine (TranquiVed, Vedco Inc. St. Joseph, MO) anesthesia (60mg/8mg mixture ml⁻¹, 1.0 ml kg⁻¹ body weight, intraperitoneal injection) rats were placed in a stereotaxic device and a 23 gauge, stainless steel cannula (17 mm) implanted into the right lateral cerebroventricle as previously described (Samson, et al 1999). Rats were allowed to recover to presurgery weights, minimally five days, before experimentation. Placement and patency of the lateral ventricular cannula was verified by the dipsogenic response to angiotensin II (50 picomole, Phoenix Pharmaceuticals, Burlingame, CA) at the end of all experiments.

Animals were moved to a quiet testing room (between 0600-0700 hr) and allowed to rest in their home cages for at least one hour. Individual rats were then placed in the recording chamber (OptoMax Activity monitor, Columbus Instruments, Columbus, OH) and physical activity monitored continuously for the next three hours. The activity monitor was configured as a two-dimensional array with rearing, with 16 beams/array separated by 1 inch intervals (beam diameter 0.125 inches). The calibration for "plane break delay" was set at 1 second and the ambulatory threshold was 1 inch/second. The margin was set at 1 inch. Data were calculated in 30 minute sampling intervals. The first two intervals served as the habituation phase during which time the animals initially explored the monitor cage and then resumed normal behavioral activity for the time of day. At the end of the habituation period, the lid was removed from the cage and a 28 gauge injection needle inserted via the implanted cannula, extending 1 mm past the cannula tip. Using a Hamilton syringe, two microliters of isotonic saline (vehicle control, sterile 0.9% NaCl) or saline containing 0.3, 1.0 or 3.0 nanomoles orexin A (Phoenix Pharmaceuticals, Burlingame, CA) or 3.0 nanomoles of the OX₂R agonist [(Ala¹¹, Leu¹⁵)-orexin B] (Tocris, Ellisville, MO) was injected over a 30 second interval and then the cage lid replaced. Activity was then monitored for the remaining 120 minutes of the protocol. For the antagonist study, the syringe was loaded so that the first one microliter of injectate contained either vehicle (1:20 solution of dimethylsulfoxide:saline) or vehicle containing the OX₁R antagonist (3.0 nanomole SB408124, Sigma Chemical, St. Louis, MO) (Samson, et al. 2007), and the second microliter contained either vehicle alone or vehicle containing 3 nanomole orexin A or the OX₂R agonist [(Ala¹¹, Leu¹⁵)-orexin B]. In all protocols the injections were made between the hours of 0800 and 1200. Food and water were not present in the activity chamber during the testing period. Individual animals were employed for only one injection protocol (no repeat testing) to avoid potential habituation to the protocol.

Data are expressed as means and standard errors of the means for each behavior, as cumulative behaviors for each 30 minute sampling interval. We present only the data from the final habituation period prior to i.c.v. injections, when exploratory behaviors had ceased. Data were examined for significant differences across treatment groups at each sampling interval (between groups ANOVA) and post hoc testing conducted (Scheffe's multiple comparisons). Any outcome with a probability of less than 5% was considered significant.

Results

There were no significant differences among treatments groups in any of the monitored behaviors during the habituation period prior to i.c.v. injections. Orexin A administration stimulated the following behaviors: total behavioral activity (defined as the sum of all beam breaks in both horizontal and vertical dimensions, Figure 1A), total distance traveled in the entire testing arena (inches traveled in the horizontal dimension, Figure 2A), ambulatory activity (number of horizontal beam breaks, Figure 3A), ambulatory time (total seconds in horizontal movement across at least one successive beam, Figure 4A), distance traveled in the margin of the testing arena (Horizontal distance in margin, Figure 5A), time spent in the margin of the testing arena (time spent in outer inch of arena, Figure 6A), rearing activity (number of vertical beam breaks in the rearing plane, Figure 7A), time spent rearing (Figure 8A) and vertical distance traveled (total of all vertical beam breaks, Figure 9A). Stereotypy burst activity (number of sessions that the animal engages in repetitive behavior, Figure 10A) and time spent in that activity (Figure 11A), also increased in a response to orexin A administration. On the other hand the total amount of time spent at rest was significantly decreased by orexin A administration (Figure 12A). The effects were dose dependent (i.e. significance attained at more than one dose and significant differences in the variable between orexin doses) in all behaviors with the exceptions of time spent in the margin (Figure 6A), rearing activity (Figure 7A), and vertical distance traveled (Figure 9A). In those behaviors, only the highest dose (3.0 nmole) of orexin A significantly stimulated the activity or time spent in that activity.

The relatively selective, OX₁R antagonist itself did not significantly alter behavioral activity; however, it did significantly inhibit the effect of orexin A on all behaviors studied. The OX₁R antagonist fully reversed (i.e. no significant differences in the animals pretreated with the antagonist before orexin A administration compared to saline vehicle controls) the action of orexin A to increase distance traveled in the margin of the testing arena (Figure 5B), time spent rearing (Figure 8B), vertical distance traveled (Figure 9B). The OX₁R antagonist significantly, but incompletely (i.e. effects were significantly attenuated by antagonist pretreatment compared to agonist only treated animals, however the behavior was still significantly different from that observed in vehicle treated controls), inhibited the remaining behaviors. Thus as with the neuroendocrine and autonomic effects of orexin A (Samson, et al 2007), this dose of the OX₁R antagonist was sufficient to significantly alter the behavioral activity of orexin A.

The preferentially selective OX₂R agonist, [(Ala¹¹, Leu¹⁵)-orexin B] at the doses tested significantly altered all behavioral activity. Dose related increases in total behavioral activity (Figure 1C), total distance traveled in the entire testing arena (Figure 2C), ambulatory activity (Figure 3C), ambulatory time (Figure 4C), rearing activity (Figure 7C), rearing time (Figure 8C), and time spent at rest (Figure 12C) were observed. The highest dose alone of the OX₂R agonist increased distance traveled (Figure 5C) and time spent (Figure 6C) in the margin, vertical distance traveled (Figure 9C), and stereotypy burst activity and time spent in that behavior (Figures 10C and 11C).

SB-408124 failed to significantly inhibit the effects of [(Ala¹¹, Leu¹⁵)-orexin B] on any behavioral activity studied with the exception of time spent at rest. In that case, only a partial reversal of the effect of [(Ala¹¹, Leu¹⁵)-orexin B] by the OX₁R antagonist was observed (Figure 12D).

Discussion

Loss or compromise of orexin-producing neurons is thought to be the underlying pathology that precipitates narcolepsy/cataplexy in humans (Thannickal, et al 2000). Indeed, laboratory animals genetically modified either to lack (Chemelli, et al 1999) the orexin gene (embryonic knockouts) or to express a toxic transgene in orexin neurons (the orexin-ataxin 3 transgenic mice or rats) that compromises those neurons in aging (Hara, et al 2001; Zhang, et al 2007) display similar behavioral characteristics. In addition, replacement of orexin in the orexin null mice reverses some, if not most, of the deficits observed in that model (Mieda, et al 2004). This has led to the suggestion that stable, bioavailable analogs of orexin might be a valuable therapeutic tools for the treatment of narcolepsy/cataplexy in humans (Willie, et al 2001). However, arousal is not the only physiological consequence of orexin actions in brain. Indeed, administration of exogenous orexin activates the autonomic nervous system resulting in increased arterial pressure and heart rate (Shirasaka, et al 2002), precipitates the neuroendocrine stress response (Ferguson and Samson, 2003), and stimulates physical behaviors often associated with stress or anxiety (Hagan, et al 1999; Ida, et al 2000; Kiwaki, et al 2004; Suzuki, et al 2005).

The two subtypes of orexin receptors have overlapping, yet distinct anatomic distributions in brain (Clauderay, et al 2002; Hervieu, et al 2001, Lu, et al 2000, Marcus, et al 2004) suggesting that some of the pharmacologic effects of the peptides might be separable on the basis of receptor subtype, and certainly brain site accessed by the peptide. This would be important in the design of orexin analogs that might be efficacious for the treatment of narcolepsy/cataplexy. We have embarked upon a series of experiments designed to identify the orexin receptor subtype responsible for the stress related responses to orexin administration in our animal models and recently reported that the ability of orexin A to activate the hypothalamo-pituitary-adrenal axis appears to be mediated via the OX₁R receptor (Samson, et al 2007). Similarly, the action of exogenous orexin A on sympathetic tone, and therefore cardiovascular function, appears to be transmitted via the OX₁R (Samson, et al 2007). However, the ability of orexin A to act in hypothalamus to stimulate another stress hormone, prolactin, was not abrogated by pretreatment with the OX₁R antagonist (Samson, et al 2007), suggesting important receptor subtype specificity may exist.

The observed increases in locomotor behavior, particularly total activity, ambulatory activity, and rearing may reflect seeking behavior (Wilson, et al 1976) because of the recognized action of orexin to stimulate appetite. However, the increased time spent and distance traveled in the margin of the open field suggests as well a possible anxiogenic action (Steimer and Driscoll, 2003). Increased stereotypy also argues in favor of an anxiogenic action of the peptide and our data suggests important roles for both receptor subtypes in those activities. Indeed, an anxiogenic behavioral effect complements the action of the peptide to act within brain to raise mean arterial pressure and heart rate (Samson, et al 1999, 2007) and to stimulate stress hormone secretion (Samson, et al 2007). The action of orexin A to raise MAP and HR and stimulate ACTH secretion was blocked by an OX₁R selective antagonist (Samson, et al 2007); however, the release of prolactin, another stress hormone, was not. Thus it remains possible that the neuroendocrine response to orexin A administration is mediated, like locomotor behavior, via both receptor subtypes.

The doses of agonists and antagonists chosen for this study reflect not only those employed previously by us to study their effects on food intake, neuroendocrine and cardiovascular function (Samson, et al 1999, 2002, 2007) but also those employed by many others including Kiwaki and colleagues (Kiwaki, et al 2004) for, in their case, in situ tissue injections of orexin A. Indeed, those authors reported a significant increase in spontaneous

locomotor activity, defined as the total of all beam breaks per 2 hour interval, with doses ranging from 0.125 to 2.0 nmol injected directly into the hypothalamic paraventricular nucleus. Those effects could be blocked by pretreatment in situ with 20 microgram of another relatively selective OX₁R antagonist, SB-334867 (Kiwaki, et al 2004). Thus using intracerebroventricular administration of orexin A and SB-408124 antagonist, we obtained data in agreement with theirs, while examining not only the subsets of locomotor activity, but also the potential role of the type 2 receptor. How do these in vivo doses compare to reported levels of immunoreactive orexin A in cerebrospinal fluid (CSF)? Fujiki and colleagues (Fujiki, et al 2001) reported CSF levels in the rat to be 1.0 – 1.5 pg/ml. Our highest dose would result in CSF levels immediately following injection into the lateral cerebral ventricle well in excess of those in vivo levels; however, dilution into the CSF in the ventricle and again when entering the third and fourth ventricle, together with enzymatic degradation and the necessity for the peptide to diffuse out of the CSF to tissue sites of action, may possibly reduce concentrations finally reaching the sites of action to those that approximate physiologic levels. To be sure, while CSF levels reflect levels that experienced similar dilution and degradation, it is possible that synaptic cleft levels of orexin A are in fact much higher than those measured in the ventricular cavity. Thus the physiologic relevance of the doses of peptide employed in most in vivo studies cannot be speculated upon. Similarly, these in vivo doses of peptide cannot be compared to published binding affinities of the agonists and antagonist since those variables were calculated ex vivo and the presence of extracellular factors affecting in vivo binding characteristics, including temperature, were not accommodated when those calculations were made. The present study does not claim the dose of agonists to be physiologically relevant, instead our data demonstrate the potential relevance of the OX₂R in these behavioral effects of orexin A, as we have demonstrated for the neuroendocrine and central autonomic actions of the peptide (Samson, et al 2007).

We report here that a wide variety of behavioral responses to orexin A are effectively, but only partially, compromised by a relatively selective, OX₁R antagonist demonstrating the importance of the type 1 receptor in the motor behavior associated with arousal. However, the ability of orexin A to alter in most cases in a dose related manner many of these characteristic behaviors may also to be expressed at least in part by actions on the type 2 receptors since pretreatment with an OX₁R antagonist did not completely reverse many of the actions of orexin A and did not prevent the stimulatory actions of the OX₂R preferring agonist. Indeed the failure of the OX₁R antagonist to completely reverse the effect of orexin A or to significantly alter the action of the OX₂R agonist on the time spent in the margin, generally considered to be a measure of anxiety in rodents (Steamer and Driscoll, 2003), is a good example of the possible importance of both receptor subtypes.

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Figure 1.

The effect of orexin A alone (Panel A), orexin A plus the OX₁R antagonist (Panel B), the OX₂R agonist alone (Panel C), or the OX₂R agonist plus the OX₁R antagonist (Panel D) on total behavioral activity. In this and all subsequent figures significance is indicated as follows: * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$, versus vehicle controls (ANOVA with Scheffe's multiple comparisons). Data collection intervals were 30 min in duration and intracerebroventricular (i.c.v.) injections were made following the second interval.

Figure 2.

The effect of orexin A alone (Panel A), orexin A plus the OX₁R antagonist (Panel B), the OX₂R agonist alone (Panel C), or the OX₂R agonist plus the OX₁R antagonist (Panel D) on total distance traveled.

Figure 3.

The effect of orexin A alone (Panel A), orexin A plus the OX₁R antagonist (Panel B), the OX₂R agonist alone (Panel C), or the OX₂R agonist plus the OX₁R antagonist (Panel D) on ambulatory activity.

Figure 4. The effect of orexin A alone (Panel A), orexin A plus the OX₁R antagonist (Panel B), the OX₂R agonist alone (Panel C), or the OX₂R agonist plus the OX₁R antagonist (Panel D) on time spent in ambulatory activity.

Figure 5.

The effect of orexin A alone (Panel A), orexin A plus the OX₁R antagonist (Panel B), the OX₂R agonist alone (Panel C), or the OX₂R agonist plus the OX₁R antagonist (Panel D) on distance traveled in the margin of the testing arena.

Figure 6.

The effect of orexin A alone (Panel A), orexin A plus the OX₁R antagonist (Panel B), the OX₂R agonist alone (Panel C), or the OX₂R agonist plus the OX₁R antagonist (Panel D) on time spent in the margin of the testing arena.

Figure 7.

The effect of orexin A alone (Panel A), orexin A plus the OX₁R antagonist (Panel B), the OX₂R agonist alone (Panel C), or the OX₂R agonist plus the OX₁R antagonist (Panel D) on rearing activity.

Figure 8.

The effect of orexin A alone (Panel A), orexin A plus the OX₁R antagonist (Panel B), the OX₂R agonist alone (Panel C), or the OX₂R agonist plus the OX₁R antagonist (Panel D) on time spent rearing.

Figure 9.

The effect of orexin A alone (Panel A), orexin A plus the OX₁R antagonist (Panel B), the OX₂R agonist alone (Panel C), or the OX₂R agonist plus the OX₁R antagonist (Panel D) on vertical distance traveled.

Figure 10.

The effect of orexin A alone (Panel A), orexin A plus the OX₁R antagonist (Panel B), the OX₂R agonist alone (Panel C), or the OX₂R agonist plus the OX₁R antagonist (Panel D) on stereotypy behavior.

Figure 11.

The effect of orexin A alone (Panel A), orexin A plus the OX₁R antagonist (Panel B), the OX₂R agonist alone (Panel C), or the OX₂R agonist plus the OX₁R antagonist (Panel D) on time spent in stereotypy behavior.

Figure 12.

The effect of orexin A alone (Panel A), orexin A plus the OX₁R antagonist (Panel B), the OX₂R s agonist alone (Panel C), or the OX₂R agonist plus the OX₁R antagonist (Panel D) on time spent resting.