Increased and Decreased Muscle Tone With Orexin (Hypocretin) Microinjections in the Locus Coeruleus and Pontine Inhibitory Area

LYUDMILA I. KIYASHCHENKO,1,2 BORIS Y. MILEYKOVSKII,1,2 YUAN-Y. LAI,2 AND JEROME M. SIEGEL2

1Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg 194223, Russia; and 2Veterans Administration Medical Center Sepulveda and Department of Psychiatry and Biobehavioral Sciences, UCLA School of Medicine, North Hills, California 91343

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Kiyashchenko, Lyudmila I., Boris Y. Mileykovskiy, Yuan-Y. Lai, and Jerome M. Siegel. Increased and decreased muscle tone with orexin (hypocretin) microinjections in the locus coeruleus and pontine inhibitory area. J Neurophysiol 85: 2008–2016, 2001. Orexin-A (OX-A) and orexin-B (OX-B) (hypocretin 1 and hypocretin 2) are synthesized in neurons of the perifornical, dorsomedial, lateral, and posterior hypothalamus. The locus coeruleus (LC) receives the densest extrahypothalamic projections of the orexin (OX) system. Recent evidence suggests that descending projections of the LC have a facilitatory role in the regulation of muscle tone. The pontine inhibitory area (PIA), located ventral to LC, receives a moderate OX projection and participates in the suppression of muscle tone in rapid-eye-movement sleep. We have examined the role of OX-A and -B in muscle-tone control using microinjections (0.1 μM to 1 μM, 0.2 μl) into the LC and PIA in decerebrate rats. OX-A and -B microinjections into the LC produced ipsi- or bilateral hindlimb muscle-tone facilitation. The activity of LC units was correlated with the extent of hindlimb muscle-tone facilitation after OX microinjections (100 μM, 1 μl) into fourth ventricle. Microinjections of OX-A and -B into the PIA produced muscle-tone inhibition. We did not observe any significant difference in the effect of OX-A and -B on muscle tone at either site. Our data suggest that OX release activates LC units and increases noradrenergic tonus in the CNS. Moreover, OX-A and -B may also regulate the activity of pontine cholinoleptic and cholinergic neurons participating in muscle-tone suppression. Loss of OX function may therefore disturb both facilitatory and inhibitory motor processes.

INTRODUCTION

Sakurai and co-authors (1998) identified two novel neuropeptides, the orexins (OXs), which are synthesized in neurons of the lateral and posterior hypothalamus. These neuropeptides are identical to the hypocretins previously described by De Lecea and collaborators (1998). Axons of OX cells have a widespread distribution throughout the adult brain in areas outside the hypothalamus, including the cerebral cortex, medial portions of the thalamus, subfornical organ, area postrema, hippocampus, amygdala, indusium griseum, locus coeruleus (LC), and raphe nuclei (Date et al. 1999; De Lecea et al. 1998; Horvath et al. 1999; Lai and Siegel 1990a). Initial projections excite LC units in the in vitro slice preparation (Hagan et al. 1999; Kilduff and Peyron 2000; Lubkin and Stricker-Krongrad 1998). Stricker-Krongrad 1998; Sweet et al. 1999; Takahashi et al. 1999), but this function has been questioned (Edwards et al. 1999; Ida et al. 1999; Taheri et al. 1999). Current evidence indicates that OX-A and -B are involved in the CNS regulation of endocrine and autonomic functions, glucose and energy homeostasis, reproduction, and the sleep-wakefulness cycle (Hagan et al. 1999; Kilduff and Peyron 2000; Lubkin and Stricker-Krongrad 1998; Moriguchi et al. 1999; Pu et al. 1998; Samson et al. 1999; Siegel 1999; Takahashi et al. 1999). The pontine inhibitory area (PIA) elicits muscle-tone facilitation in studies in decerebrate cats (D’Ascanio et al. 1989; Stampacchia et al. 1987). The LC receives the densest extrahypothalamic projections of the OX system (Hagan et al. 1999; Horvath et al. 1999; Nambu et al. 1999; Peyron et al. 1998). OX-A and -B applications excite LC units in the in vitro slice preparation (Hagan et al. 1999; Horvath et al. 1999). These findings suggest that OX release in the LC could have a role in muscle-tone control. The pontine inhibitory area (PIA) elicits muscle-tone suppression with cholinergic (George et al. 1964; Takakusaki et al. 1994) and glutamatergic (Lai and Siegel 1990a) stimulation. Lesions of this region produce rapid-eye-movement (REM) sleep without muscle atonia (Henley and Morrison 1974; Jouvet and Delorme 1965). The middle portion of the pontine reticular nucleus, oral part, and subcoeruleus region from which these effects were elicited, termed the PIA, has a low to moderate concentration of OX axons (Peyron et al. 1998). Thus OX release in the PIA might also affect muscle tone. The current study was undertaken to determine the effects of OX microinjections into the LC and PIA on the regulation of muscle tone and motor activity.

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Address for reprint requests: J. M. Siegel, Dept. of Psychiatry, UCLA, Neurobiology Res. 151A3, VAMC, 16111 Plummer St., North Hills, CA 91343 (E-mail: JSiegel@ucla.edu).
METHODS

All procedures were approved by the Animal Studies Committee of the Sepulveda Veterans Affairs Medical Center/UCLA in accordance with U.S. Public Health Service guidelines. Seventy-two Wistar rats (250–300 g) were operated on, and 48 of them showed muscle rigidity and were used in our experiments. We used decerebrate rats with postdecerebrate electromyographic (EMG) levels from 20 to 300 μV in our studies (Lai and Siegel 1988, 1990a,b, 1991). Animals with EMG level less than 20 μV or showing unilateral muscle rigidity were excluded from this study.

Surgery

Animals were anesthetized with halothane (Fluothane) followed by ketamine HCL (Ketalar, 70 mg/kg ip) for cannulation of the trachea and decerebration. Two holes (diameter, 2.0 mm) were symmetrically drilled over the skull above the LC nuclei and PIA (Hajnik et al. 2000) at 9.2 mm posterior to bregma and 1.3 mm lateral to midline for microinjections and LC unit recordings. Then two rectangular holes were cut in each parietal bone in preparation for decerebration. The transverse anterior and posterior borders of these holes were located 1 and 5 mm posterior to bregma with longitudinal sagittal borders 0.5 mm from midline. Precollicular-postmammillary decerebration was carried out 4.0–5.0 mm posterior to bregma using a stainless steel spatula, taking care not to injure the sagittal vein. Excess fluid was aspirated by syringe and absorbed with Gelfoam (Upjohn). Guide cannulae, 0.6-mm diam, were fixed on the skull bone for OX-A, OX-B, saline, and clonidine microinjections into the fourth ventricle. Coordinates of all structures were based on the rat brain atlas (Paxinos and Watson 1997).

Rectal temperature was continuously monitored with an electrical thermometer (Model BAT-12, Physitemp) and maintained between 37 and 38°C with a Heat Therapy Pump (Model NO. TP-500, Gaymar Industries). Experiments were begun when bilateral postdecerebrate muscle rigidity appeared (0.5–3 h).

Microinjections

The animals were divided into four groups: the first group (n = 17) was used for OX-A microinjections into the LC and PIA, the second group (n = 19) for OX-B microinjections into these structures, the third group (n = 4) for control saline microinjections into the LC and PIA, and the fourth group (n = 8) for saline, OX-A, OX-B, and clonidine microinjections into fourth ventricle during LC unit recordings. In each experiment, four injection sites (2 in the LC and 2 in the PIA) were sequentially tested each with one microinjection. Sites were tested in random order. Subsequent microinjections were performed when muscle tone returned to background. The microinjections into the LC and PIA consisted of 0.2 μl administered at a rate 10 nl/s. OX-A and -B (Peptide Institute, Osaka, Japan) were dissolved in 0.9% saline to get 0.1, 0.2, 1, and 100 mM. Clonidine microinjections into fourth ventricle during LC unit recording followed by inhibition in response to a pinch applied to hindlimb paw, and histological location within the LC tyrosine hydroxylase-positive cell cluster.

Histology

Cathodal current (0.1–0.2 mA, 4–6 s) was passed through the microelectrodes at the end of each track. The location of recorded neurons was determined by using the track made by the microelectrode, depth of the marking lesion, and depth measurements on the microdrive. Rats were deeply anesthetized with pentobarbital (70 mg/kg ip) and perfused transcardially with 0.1 M phosphate-buffered saline (PBS, pH = 7.4) followed by 4% paraformaldehyde in 0.1 M PBS. Brains were removed, cut into 60-μm-thick sections, and immunostained for tyrosine hydroxylase (TH). Sections were rinsed three times in 0.05 M Trizma-buffered saline (pH = 7.4) followed by a 24-h incubation in a 1/400 dilution of primary mouse antiserum to TH (Chemicon, Temecula, CA) with a solution of 2% normal goat serum in 0.05 M Trizma-buffered saline (pH 7.4) at 4°C. This was followed by a 2-h incubation in a 1/200 dilution of biotinylated goat anti-mouse IgG (Vector, Burlingame, CA). The tissue was then incubated for 2 h with the avidin-biotin complex (Vector). Each incubation was followed by three rinses. Dilutions and rinses were prepared with 2% normal goat serum in 0.05 M Trizma-buffered saline. The sections were then treated with 0.05% solution of 3,3′ diaminobenzidine and 0.01% hydrogen peroxide. The electrode tracks and the TH-positive cells were identified on a Nikon microscope and plotted with a NeuroLucida interface according to the rat brain atlas (Paxinos and Watson 1997).

Data analysis

The latency of muscle-tone changes was measured from the start of the microinjection to either a 30% increase or a 30% decrease in nonintegrated EMG amplitude compared with baseline. The duration

Stimulation and recording

Constant-current square-wave pulses (0.2 ms, 30 Hz, 50–100 μA, continuous stimulation for 5–10 s) were delivered using an S88 stimulator (Grass Instruments) coupled to a Grass SIU5 stimulus isolation unit. The areas of the pontine reticular formation producing hindlimb muscle-tone inhibition were located by stereotaxic coordinates (Hajnik et al. 2000) and confirmed with electrical stimulation via a tungsten monopolar microelectrode (A-M Systems). The LC was located by stereotaxic coordinates. OX-A, OX-B, or saline was microinjected into the identified areas.

EMGs were recorded from the neck (splenius muscle) and hindlimbs (gastrocnemius and tibialis anterior muscles) bilaterally with stainless steel wires and were amplified using a Grass polygraph (Model 78D). We chose these ankle extensors and flexors because LC electrical stimulation has been shown to excite spinal motoneurons innervating these muscles (Fung et al. 1987) and facilitate their muscle tone (Lai et al. 1989). Extracellular unit recordings were performed using tungsten monopolar microelectrodes (A-M Systems). Spikes were amplified with a Model 1700 A-M Systems amplifier. Unit pulses and EMGs were recorded on a PC using the 1401plus interface and Spike2 program (Cambridge Electronic Design, Cambridge, UK). The rate of digitization was 372 Hz for EMG and 21 kHz for unit activity.
was calculated from the time of onset of a sustained increase or decrease in muscle tone until the return to baseline levels. The latencies and durations were averaged for right and left hindlimbs for microinjections that produced bilateral muscle-tone variations. The latencies and durations of muscle-tone changes were analyzed by ANOVA. Unit firing rates were analyzed by Wilcoxon matched-pairs test. The average unit firing rates were calculated for 10 s before and after OX microinjections when sustained muscle-tone facilitation was observed.

Regression analysis was used for estimating the correlation between changes of LC unit firing rate and integrated ipsilateral gastrocnemius EMG. LC unit excitation and inhibition predominantly affect extensor tonus in the ipsilateral limbs (Andre et al. 1995; Pompeiano 1998). The changes of integrated EMG and firing rate were calculated in percent of baseline level. Digital EMG integration was performed with 10-s epochs. All average values are means ± SE.

RESULTS

OX-A and -B microinjections in the vicinity of LC

Thirty-four OX-A microinjections (0.2 μM to 1 mM, 0.2 μl) were performed in the vicinity of LC (Fig. 1) in 17 decerebrate rats. Seventeen microinjection sites located in the LC produced bilateral muscle-tone facilitation (Fig. 2A), and 12 sites located similarly evoked ipsilateral muscle-tone facilitation (Fig. 2B). Four OX-A microinjections into the subcoeruleus nucleus, alpha part (SubCA) inhibited muscle tone bilaterally (Fig. 2B). Similarly, evoked ipsilateral muscle-tone facilitation (Fig. 2B) similarly evoked ipsilateral muscle-tone facilitation (Fig. 2B). Similar doses of OX-B were injected into 38 sites in the vicinity of LC in 19 decerebrate rats. The location of OX-B microinjection sites was similar to those represented in Fig. 1. Eighteen microinjections into the LC produced bilateral muscle-tone facilitation, and 11 sites evoked ipsilateral muscle-tone facilitation. Five OX-B microinjections into the SubCA decreased muscle tone bilaterally, two produced ipsilateral muscle-tone inhibition, and two microinjections were ineffective.

Latency and duration of muscle-tone facilitation

OX-A and -B microinjections in the vicinity of LC increased hindlimb muscle rigidity in a dose-dependent manner. The response thresholds were between 0.1 and 0.2 μM for OX-A and -B. The latency of muscle-tone facilitation (Fig. 3A) changed from 200 ± 13 s (n = 7, 0.2 μM) to 66 ± 8 s (n = 7, 1 mM) for OX-A and from 182 ± 17 s (n = 7, 0.2 μM) to 63 ± 7 s (n = 7, 1 mM) for OX-B. The duration of muscle-tone facilitation also had a dose-dependent time course (Fig. 3B) and ranged from 530 ± 67 s (n = 7, 0.2 μM) to 2211 ± 208 s (n = 7, 1 mM) for OX-A and from 491 ± 40 s (n = 7, 0.2 μM) to 2116 ± 285 s (n = 7, 1 mM) for OX-B. We did not observe significant differences between the action of equimolar concentrations of OX-A and -B on hindlimb muscle tone (for latency: Fh0.2 μM = 0.72, P > 0.5; Fh0.1 μM = 0.36, P > 0.75; F100 μM = 0.08, P > 0.85; F1 mM = 0.13, P > 0.75; df = 1, 12; for duration: Fh0.2 μM = 0.29, P > 0.75; Fh0.1 μM = 0.46, P > 0.75; F100 μM = 0.08, P > 0.8; F1 mM = 0.07, P > 0.8; df = 1, 12). Saline microinjections (n = 6) into the vicinity of LC in four control rats did not produce muscle-tone facilitation within the 1-h postinjection observation period.

OX-A and -B microinjections into the PIA

Thirty-four OX-A microinjections (0.2 μM to 1 mM, 0.2 μl) were performed into the PIA sites (Fig. 1) identified previously by electrical stimulation (50–100 μA) in 17 rats. Twenty microinjection sites produced bilateral muscle-tone inhibition (Fig. 4A). Eight sites evoked ipsilateral hindlimb muscle-tone inhibition with contralateral muscle-tone facilitation (Fig. 4B), and six sites induced contralateral muscle-tone inhibition (Fig. 4C). OX-B microinjections were performed into 38 identified inhibitory sites of the PIA in 19 rats. Twenty-two microinjection sites produced bilateral muscle-tone inhibition, 10 sites induced ipsilateral muscle-tone inhibition with contralateral muscle-tone facilitation, and 6 sites evoked contralateral muscle-tone inhibition.

FIG. 1. Location of orexin-A (OX-A) microinjection zones in the vicinity of locus coeruleus (LC) and pontine reticular formation inducing ipsilateral muscle-tone facilitation (*), bilateral muscle-tone facilitation (○), ipsilateral muscle-tone inhibition (□), bilateral muscle-tone inhibition (■), and contralateral muscle-tone inhibition (▲) in decerebrate rats. PnO, pontine reticular nucleus, oral. Location of orexin-B (OX-B) microinjection zones in the vicinity of LC and pontine reticular formation was similar.
Latency and duration of muscle-tone inhibition

The latency of bilateral muscle-tone inhibition produced by OX-A microinjections had a dose-dependent time course and changed from $225 \pm 6\ s$ ($n = 5, 0.2\, \mu M$) to $52 \pm 8\ s$ ($n = 5, 1\, \mu M$) for OX-A and from $221 \pm 3\ s$ ($n = 5, 0.2\, \mu M$) to $57 \pm 8\ s$ ($n = 5, 1\, \mu M$) for OX-B (Fig. 5A). The response thresholds for OX-A and -B were between 0.1 and 0.2 $\mu M$. The duration of muscle-tone inhibition (Fig. 5B) changed from $208 \pm 5\ s$ ($n = 5, 0.2\, \mu M$) to $965 \pm 8\ s$ ($n = 5, 1\, \mu M$) for OX-A and from $179 \pm 1\ s$ ($n = 5, 0.2\, \mu M$) to $810 \pm 54\ s$ ($n = 5, 1\, \mu M$) for OX-B. We did not observe significant differences between the action of equimolar concentrations of OX-A and -B in the PIA (for latency: $F_{0.2\, \mu M} = 0.01, P > 0.9; F_{1\, \mu M} = 0.01, P > 0.9; F_{100\, \mu M} = 0.90, P > 0.5; F_{1000\, \mu M} = 0.52, P > 0.5; df = 1, 8$ for duration: $F_{0.2\, \mu M} = 1.50, P > 0.5; F_{1\, \mu M} = 0.69, P > 0.5; F_{100\, \mu M} = 0.20, P > 0.75; F_{1000\, \mu M} = 2.26, P > 0.25; df = 1, 8$). Control microinjections of saline at the same sites ($n = 6$) did not produce hindlimb muscle-tone inhibition in three control rats.

**FIG. 2.** Bilateral muscle-tone facilitation (A), ipsilateral muscle-tone facilitation (B), and bilateral muscle-tone inhibition (C) after OX-A microinjections (A: 0.2 $\mu M$, B: 0.2 $\mu M$, C: 1.0 $\mu M$) in the vicinity of LC and subcoeruleus nucleus, alpha part (SubCA). SpR, SpL, EMG of muscle splenius (right and left side); TaR, TaL, EMG of tibialis anterior muscle (right and left side); GcR, GcL, EMG of gastrocnemius muscle (right and left side). Top horizontal left bars indicate the time and duration of injections. Muscle-tone changes during OX-B microinjections in the vicinity of LC were similar.

**FIG. 3.** Latency (A) and duration (B) of muscle-tone facilitation after OX-A (■) and OX-B (●) microinjections in the vicinity of LC. The vertical bars represent SE ($n = 7$) for each point of the curve.

**FIG. 4.** Bilateral hindlimb muscle-tone inhibition (A), ipsilateral hindlimb muscle-tone inhibition with contralateral muscle-tone facilitation (B), and contralateral muscle-tone inhibition (C) after OX-A microinjections (A: 1 $\mu M$, B: 0.2 $\mu M$, C: 1 $\mu M$) into pontine reticular formation. See abbreviations in Fig. 2. Top horizontal bars at the left indicate the time and duration of injections. Muscle-tone changes during OX-B microinjections in the pontine reticular formation were similar.

**FIG. 5.** Latency (A) and duration (B) of muscle-tone inhibition after OX-A (■) and OX-B (●) microinjections into the pontine inhibitory area (PIA). The vertical bars represent the SE ($n = 5$) for each point of the curve.
LC unit activity after OX-A and -B microinjections into the fourth ventricle

Microinjections of OX-A and -B into the fourth ventricle between the LC nuclei produced muscle-tone facilitation in eight rats with postdecerebrate muscle-tone rigidity. We used these microinjections to determine if LC units participate in the muscle-tone facilitation induced by OX-A and -B.

Eighteen recorded neurons met the criteria for noradrenergic cells (Fig. 6). These neurons showed a regular (4.7 ± 2.0 spike/s, n = 18) firing rate when hindlimb rigidity was observed and had an average spike duration of 2.4 ± 0.1 ms (n = 18). Pinching of the hindlimb paw on either side evoked an initial (0.3–0.8 s) burst of activity following by inhibition (lasting 1–3 s). Immunohistochemistry confirmed that these cells were located in TH-positive regions. Clonidine injections in the fourth ventricle performed after OX-A and -B microinjections inhibited activity in all putative noradrenergic neurons and suppressed muscle tone. Complete inhibition of LC unit activity and muscle-tone suppression after clonidine microinjection occurred at average latencies 28.0 ± 1.7 s (n = 18) and 42.2 ± 3.9 s (n = 18), respectively. Unit activity and muscle tone returned in parallel to the baseline level by 52.9 ± 2.0 min (n = 18) after clonidine microinjections.

Ten LC units were observed during OX-A microinjections (100 µM, 1 µl) into the fourth ventricle. The discharge frequency of nine of these cells was increased by an average 187 ± 24% (n = 9, T = 0, P < 0.01) compared with the baseline and the magnitude of the increase was correlated with the size of EMG facilitation (Fig. 7A). Excitation of LC cells after OX-A microinjections preceded muscle-tone facilitation by an average 5.8 ± 0.9 s (n = 9). We used a regression analysis for estimating the numerical relationship between changes of integrated gastrocnemius EMG and ipsilateral LC unit firing rate after OX-A microinjections (Fig. 8A). The regression equation of EMG (%) on rate (%) after OX-A microinjections was EMG = 48.34 + 0.46Rate (SE = 26.09, 0.16; t = 2.80; P < 0.05; df = 14). The correlation coefficient between EMG and ipsilateral LC unit firing rate was 0.59 (n = 16, P < 0.05). One LC cell did not change firing frequency after OX-A microinjection.

FIG. 6. Electrophysiological and histological identification of noradrenergic LC neuron. A: slow and regular spontaneous firing during muscle rigidity. B: long duration of spike. C: fast activation followed by inhibition in response to a pinch stimulus applied to hindlimb paw. D: time course of LC unit and muscle-tone changes after clonidine microinjection in the 4th ventricle. E: histological location of tyrosine hydroxylase-positive cells near the recording track in the LC. LC(R), LC unit (right side). See other abbreviations in Fig. 2.
The discharge rate of eight LC units was analyzed during OX-B microinjections into the fourth ventricle. All cells were excited by OX-B microinjections (177.61%, n = 8, T = 0, P < 0.01), and their activity correlated with hindlimb muscle-tone facilitation (Figs. 7B and 8B). The increased firing frequency of LC units preceded the muscle-tone increase after OX-B microinjections by an average 6.2 ± 1.0 s (n = 8). The regression equation of EMG (%) on rate (%) after OX-B microinjections was EMG = 45.41 + 0.52Rate (SE = 26.59, 0.19; t = 2.83; P < 0.05; df = 14) and the correlation coefficient between these variables was 0.60 (n = 16, P < 0.05).

**DISCUSSION**

We found that microinjections of OX-A and -B in the vicinity of LC increase LC unit activity and produce a correlated facilitation of muscle tone in decerebrate rats. No difference was observed in the effect of OX-A and -B on muscle tone and LC unit firing rate. Our data concerning excitatory OX influences on LC neurons are consistent with results obtained in rat brain slices. Intra- and extracellular recordings revealed that OX-A applications evoked depolarization of LC units and increased their firing frequency (Hagan et al. 1999). In a similar manner, hypocretin-2 (OX-B) applications depolarized all tested LC cells and increased their spontaneous rate. Membrane depolarization persisted in the presence of tetrodotoxin, indicating that the response to OX-B was synaptically mediated (Horvath et al. 1999; Ivanov and Aston-Jones 2000).

Trivedi and co-authors (1998) using in situ hybridization with receptor subtype-specific oligonucleotide probes reported that only OX1R were located in the LC. These receptors are relatively selective for OX-A, whereas OX1R is nonselective for OX-A and -B (Sakurai et al. 1998). OX-A fibers project widely in the brain stem, and the LC receives the densest OX-A innervation (Cutler et al. 1999; Hagan et al. 1999). These data were complemented by studies that revealed a widespread distribution of both OX-A and -B immunoreactive fibers in the rat brain and in the LC (Date et al. 1999). Horvath and co-authors (1999) using electron microscopy showed that all TH-positive cells in the LC receive asymmetrical (excitatory) synaptic contacts from multiple axons containing hypocretin-2. A specific radioimmunoassay performed for OX-A and -B showed that OX-B concentration was double OX-A level in the rat brain stem (Mitsuma et al. 1999, 2000).

Electrophysiological and pharmacological studies indicate that LC units with descending projections have a facilitatory action on spinal motoneurons (Andre et al. 1995; D’Ascanio et al. 1989; Fung and Barnes 1981; Fung et al. 1991, 1994; Rispoli et al. 1994). Moreover, noradrenergic LC neurons also may produce an indirect excitatory effect on spinal motoneurons by inhibition of pontine cholinergic and cholinocceptive neurons participating in descending muscle-tone suppression (Horn et al. 1987; Pompeiano et al. 1987). LC units reduce their activity during non-REM sleep and cease discharge for an extended period during REM sleep with its associated muscle atonia (Hobson et al. 1975; Jacobs 1986). It has been shown recently that a significant decrease of REM sleep occurred after OX-A microinjections into the LC in freely moving rats (Bourgin et al. 2000).

Wu et al. (1999) reported that LC neurons cease discharge immediately before and throughout cataplexy periods in canine narcoleptics. These results combined with our recent data showing a correlation between LC activity and muscle tone...

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**FIG. 7.** LC unit excitation and muscle-tone facilitation after OX-A (A) and OX-B (B) microinjections (100 µM) into the 4th ventricle. Top horizontal bars at the left indicate the time and duration of injections. LC(L), LC unit (left side). See other abbreviations in Fig. 2.

**FIG. 8.** Regression diagrams showing relationship between changes of gastrocnemius EMG (EMG %) and ipsilateral LC unit firing rate (Rate %) after OX-A (A) and OX-B (B) microinjections into the 4th ventricle. ◦, experimental points (n = 16). For OX-A microinjections: EMG = 48.34 + 0.46Rate (SE = 26.09, 0.16; t = 2.80; P < 0.05; df = 14), R = 0.59 (n = 16, P < 0.05). For OX-B microinjections: EMG = 45.41 + 0.52Rate (SE = 26.59, 0.19; t = 2.83; P < 0.05; df = 14), R = 0.60 (n = 16, P < 0.05).
These receptors may not be sufficient to discriminate between OX-A and -B could be responsible for the similar response not yet been ruled out. Other receptors with a similar response have indicated that glutamate may locally modulate activity in noradrenergic LC neurons through \( N \)-methyl-\( D \)-aspartate (NMDA) and non-NMDA receptors (Luque et al. 1995; Sen-goku et al. 1999; Van Bockstaele and Colago 1996). The PIA receives dense glutamatergic projections from the mesencephalic reticular nucleus, the retrorubral nucleus, and the ventral part of the paralemniscal tegmental field (Lai et al. 1993). Glutamate microinjections into the PIA evoked muscle-tone facilitation (Garcia-Rill and Skinner 1988) and even a low level of OX innervation of the PIA may be sufficient to trigger potent reticulospinal inhibition.

We also propose that OXs may act on their presynaptic receptors located on glutamatergic axons and modulate brain stem neuron activity by presynaptic glutamatergic release as it was described for hypothalamic cells in addition to their direct postsynaptic action (Van den Pol et al. 1998). The LC receives glutamate inputs from nucleus paragigantocellularis (Ennis and Aston-Jones 1988). Ultrastructural and intracellular studies have indicated that glutamate may locally modulate activity in noradrenergic LC neurons through \( N \)-methyl-\( D \)-aspartate (NMDA) and non-NMDA receptors (Luque et al. 1995; Sengoku et al. 1999; Van Bockstaele and Colago 1996). The PIA receives dense glutamatergic projections from the mesencephalic reticular nucleus, the retorubral nucleus, and the ventral portion of the paralemniscal tegmental field (Lai et al. 1993). Glutamate microinjections into the PIA evoked muscle-tone suppression in decerebrate cats (Lai and Siegel 1991). Ultrastructural studies of pontine nuclei revealed that asymmetric glutamatergic synaptic contacts are located on dendrites, neuronal somata and axons (Border and Mihailoff 1991). Our hypothesis that OXs may modulate brain stem neuron activity by presynaptic glutamatergic release is supported by data about the similar latency of muscle-tone facilitation and inhibition (50–120 s) after injections of small doses of glutamate agonists into the pontomedullary region (Lai and Siegel 1991). The latency of muscle-tone facilitation and LC unit firing rate increase (2–3 min) after OX microinjections into fourth ventricle is presumably related to the diffusion of drugs from the ventricle into the LC.

At present only two OX receptor types have been identified. However, the possibility that other OX receptor types exist has not yet been ruled out. Other receptors with a similar response to OX-A and -B could be responsible for the similar response profile to these ligands. Alternatively, the relative selectivity of these receptors may not be sufficient to discriminate between OX-A and -B when applied by microinjection.

We have recently shown that a massive loss of OX neurons is linked to narcolepsy (Thannical et al. 2000). The loss of OX input to the LC would reduce a major facilitatory drive to the LC. We hypothesize that a loss of this facilitatory input allows phasic motor inhibition elicited by emotion to cause a loss of muscle tone (cataplexy). In neurologically normal individuals, phasic OX release prevents these losses of tone.

The role of OX in the control of both LC and PIA suggests that a malfunction of this system can affect both motor facilitation and motor inhibition. In this regard, it is interesting to note that many narcoleptic patients not only experience sudden losses of muscle tone in waking (cataplexy) but also have a pathological absence of muscle atonia during REM sleep, producing the REM sleep behavior disorder (Schenck and Mahowald 1992). Malfunction of the OX system may be responsible for both of these symptoms of narcolepsy.

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REFERENCES


D’Ascanio P, Horn E, Pompeiano O, and Stampacchia J. Injections of \( N \)-methyl-\( D \)-aspartate (NMDA) and non-NMDA receptors (Luque et al. 1995; Sen-goku et al. 1999; Van Bockstaele and Colago 1996). The PIA receives dense glutamatergic projections from the mesencephalic reticular nucleus, the retorubral nucleus, and the ventral portion of the paralemniscal tegmental field (Lai et al. 1993). Glutamate microinjections into the PIA evoked muscle-tone suppression in decerebrate cats (Lai and Siegel 1991). Ultrastructural studies of pontine nuclei revealed that asymmetric glutamatergic synaptic contacts are located on dendrites, neuronal somata and axons (Border and Mihailoff 1991). Our hypothesis that OXs may modulate brain stem neuron activity by presynaptic glutamatergic release is supported by data about the similar latency of muscle-tone facilitation and inhibition (50–120 s) after injections of small doses of glutamate agonists into the pontomedullary region (Lai and Siegel 1991). The latency of muscle-tone facilitation and LC unit firing rate increase (2–3 min) after OX microinjections into fourth ventricle is presumably related to the diffusion of drugs from the ventricle into the LC.

At present only two OX receptor types have been identified. However, the possibility that other OX receptor types exist has not yet been ruled out. Other receptors with a similar response to OX-A and -B could be responsible for the similar response profile to these ligands. Alternatively, the relative selectivity of these receptors may not be sufficient to discriminate between OX-A and -B when applied by microinjection.

We have recently shown that a massive loss of OX neurons is linked to narcolepsy (Thannical et al. 2000). The loss of OX input to the LC would reduce a major facilitatory drive to the LC. We hypothesize that a loss of this facilitatory input allows phasic motor inhibition elicited by emotion to cause a loss of muscle tone (cataplexy). In neurologically normal individuals, phasic OX release prevents these losses of tone.

The role of OX in the control of both LC and PIA suggests that a malfunction of this system can affect both motor facilitation and motor inhibition. In this regard, it is interesting to note that many narcoleptic patients not only experience sudden losses of muscle tone in waking (cataplexy) but also have a pathological absence of muscle atonia during REM sleep, producing the REM sleep behavior disorder (Schenck and Mahowald 1992). Malfunction of the OX system may be responsible for both of these symptoms of narcolepsy.
OREXIN AND MUSCLE TONE


