

# Muscle Tone Facilitation and Inhibition After Orexin-A (Hypocretin-1) Microinjections Into the Medial Medulla

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**Mileykovskiy, Boris Y., Lyudmila I. Kiyashchenko, and Jerome M. Siegel.** Muscle tone facilitation and inhibition after orexin-A (hypocretin-1) microinjections into the medial medulla. *J Neurophysiol* 87: 2480–2489, 2002; 10.1152/jn.00810.2001. Orexins/hypocretins are synthesized in neurons of the perifornical, dorsomedial, lateral, and posterior hypothalamus. A loss of hypocretin neurons has been found in human narcolepsy, which is characterized by sudden loss of muscle tone, called cataplexy, and sleepiness. The normal functional role of these neurons, however, is unclear. The medioventral medullary region, including gigantocellular reticular nucleus, alpha (GiA) and ventral (GiV) parts, participates in the induction of locomotion and muscle tone facilitation in decerebrate animals and receives moderate orexinergic innervation. In the present study, we have examined the role of orexin-A (OX-A) in muscle tone control using microinjections (50  $\mu$ M, 0.3  $\mu$ l) into the GiA and GiV sites in decerebrate rats. OX-A microinjections into GiA sites, previously identified by electrical stimulation as facilitating hindlimb muscle tone bilaterally, produced a bilateral increase of muscle tone in the same muscles. Bilateral lidocaine microinjections (4%, 0.3  $\mu$ l) into the dorso-lateral mesopontine reticular formation decreased muscle rigidity and blocked muscle tone facilitation produced by OX-A microinjections into the GiA sites. The activity of cells related to muscle rigidity, located in the pedunculopontine tegmental nucleus and adjacent reticular formation, was correlated positively with the extent of hindlimb muscle tone facilitation after medullary OX-A microinjections. OX-A microinjections into GiV sites were less effective in muscle tone facilitation, although these sites produced a muscle tone increase during electrical stimulation. In contrast, OX-A microinjections into the gigantocellular nucleus (Gi) sites and dorsal paragigantocellular nucleus (DPGi) sites, previously identified by electrical stimulation as inhibitory points, produced bilateral hindlimb muscle atonia. We propose that the medioventral medullary region is one of the brain stem target for OX-A modulation of muscle tone. Facilitation of muscle tone after OX-A microinjections into this region is linked to activation of intrinsic reticular cells, causing excitation of midbrain and pontine neurons participating in muscle tone facilitation through an ascending pathway. Moreover, our results suggest that OX-A may also regulate the activity of medullary neurons participating in muscle tone suppression. Loss of OX function may, therefore, disturb both muscle tone facilitatory and inhibitory processes at the medullary level.

## INTRODUCTION

The orexins (OXs) or hypocretins are synthesized in neurons of the perifornical, dorsomedial, lateral, and posterior hypothalamus (de Lecea et al. 1998; Sakurai et al. 1998). OXs have

been implicated in the regulation of various brain and body functions, such as feeding (Lubkin and Stricker-Krongrad 1998; Sakurai et al. 1998; Sweet et al. 1999; Takahashi et al. 1999), energy homeostasis (Moriguchi et al. 1999; Williams et al. 2000), neuroendocrine (Nowak et al. 2000; Pu et al. 1998) and cardiovascular processes (Samson et al. 1999), sleep (Bourgin et al. 2000; John et al. 2000; Xi et al. 2001), locomotion (Hagan et al. 1999; Nakamura et al. 2000), and muscle tone control (Kiyashchenko et al. 2001; Siegel 1999). It has been shown that most human narcoleptics have reduced levels of hypocretin-1 (OX-A) in their cerebrospinal fluid and brain (Nishino et al. 2000; Peyron et al. 2000). In addition, a massive loss of OX-synthesizing hypothalamic cells is linked to human narcolepsy (Thannical et al. 2000). It has been reported that mutation of the hypocretin receptor-2 gene is the genetic cause of canine narcolepsy (Lin et al. 1999). Mice with a null mutation of the prepro-orexin gene show symptoms of narcolepsy (Chemelli et al. 1999), including sudden loss of muscle tone.

We have recently examined the role of orexin-A (OX-A) and orexin-B (OX-B) in muscle tone control using microinjections into the locus coeruleus (LC) and pontine inhibitory area (PIA), including middle portions of the oral and caudal pontine reticular nuclei and subcoeruleus region in decerebrate rats. OX-A and OX-B microinjections into the LC produced ipsilateral or bilateral hindlimb muscle tone facilitation, and microinjections into the PIA evoked bilateral muscle atonia (Kiyashchenko et al. 2001). LC neurons receive the densest extrahypothalamic OX projections (Hagan et al. 1999; Horvath et al. 1999; Nambu et al. 1999; Peyron et al. 1998), facilitate spinal motoneurons (Holstege and Kuypers 1987; White and Neuman 1980, 1983), and abruptly cease discharge at cataplexy onset (Wu et al. 1999). Peyron et al. (1998) reported that medioventral medullary region, including the alpha (GiA) and ventral (GiV) parts of the gigantocellular nucleus (Gi), receives moderate OX innervation. Electrical and chemical stimulation of this region produces controlled locomotion and muscle tone facilitation in decerebrate cats and rats (Garcia-Rill and Skinner 1987a; Kinjo et al. 1990). Garcia-Rill et al. (1983, 1986) reported that the medioventral medullary region inducing locomotion receives the majority of descending projections from

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the midbrain locomotor region (MLR), including the pedunculo-pontine tegmental nucleus (PPTg) and caudal half of the cuneiform nucleus (CnF). Electrical and chemical stimulation of this midbrain region also induces locomotion on a treadmill (Garcia-Rill et al. 1983; Skinner and Garcia-Rill 1984) and facilitates muscle tone (Juch et al. 1992; Lai and Siegel 1990a) in decerebrate cats and rats.

The present study was undertaken to determine whether muscle tone changes could be produced by OX-A microinjections into the medioventral medullary region and which neuronal mechanisms may be involved.

## METHODS

All procedures were approved by the Animal Studies Committee of the Sepulveda V.A. Medical Center/UCLA in accordance with U.S. Public Health Service guidelines. Forty-two Wistar rats (250–300 g) were operated on, and 33 of them showed muscle rigidity within 0.5–3 h after decerebration with hindlimb electromyogram (EMG) amplitudes ranging from 30 to 400  $\mu$ V. Nine animals, with EMG level less than 30  $\mu$ V, were used to investigate the role of rigidity in OX-A and lidocaine effects on muscle tone.

### Surgery

Animals were anesthetized with halothane, followed by ketamine HCL (Ketalar; 70 mg/kg ip) for cannulation of the trachea and decerebration. Two holes (2 mm diam) were drilled over the skull above the MLR and PIA, 8.5 mm posterior to bregma and 1.6 mm lateral to the midline, for the insertion of cannulae, stimulating and unit recording electrodes (Hajnik et al. 2000; Milner and Mogenson 1988). One hole (2.0 mm diam) was drilled above the GiA, GiV, Gi, and dorsal paragigantocellular nucleus (DPGi), 11.0 mm posterior to bregma, for the insertion of stimulating electrodes and cannulae. Coordinates of all structures were based on the atlas of Paxinos and Watson (1997).

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 the longitudinal sagittal border 0.5 mm from the midline. Precollicular-postmamillary decerebration was performed 4.0–5.0 mm posterior to bregma with a stainless steel spatula, taking care not to injure the sagittal vein. Excess fluid was aspirated by syringe and absorbed with Gelfoam (Pharmacia, Piscataway, NJ/Upjohn, Kalamazo, MI). Then anesthesia was discontinued. Rectal temperature was continuously monitored with an electrical thermometer (model BAT-12; Physitemp Instruments, Clifton, NJ) and maintained between 37 and 38°C with a Heat Therapy Pump (model TP-500; Gaymar Industries, Orchard Park, NY). Experiments were begun when postdecerebrate muscle rigidity appeared (0.5–3 h after decerebration).

### Stimulation and recording

Electrical stimulation (0.2 ms; 50 Hz; 30–200  $\mu$ A, continuous stimulation 10–15 s) via tungsten monopolar microelectrodes (A-M Systems, Carlborg, WA) was used to identify the midbrain, pontine, and medullary sites producing muscle tone facilitation and muscle tone inhibition. Stimulation was performed using electrode insertion in increments of 0.3 mm. Cathodal stimuli were delivered using a S88 stimulator (Grass Instrument) and Grass SIU5 stimulus isolation unit. The anode, a screw electrode, was placed in the frontal cranial bone. Bipolar stimulating electrodes (stainless steel, 100  $\mu$ m, tip separation 80–100  $\mu$ m) were used for reduction of electrical artifact during unit recording. Stimulation artifact was determined to have a duration of less than 1.3 ms. Bipolar stimulating electrodes were inserted in the pontine reticular nucleus, oral part (PnO), 8.3–8.8 mm posterior to

bregma, 0.6–1.6 mm lateral to the midline, and 8.5–9.5 mm below the skull. This region was identified previously as rostral part of the PIA, producing bilateral muscle tone suppression in decerebrate rats (Hajnik et al. 2000).

Extracellular unit recordings were performed using monopolar tungsten microelectrodes (A-M Systems, Carlborg, WA). Spikes were amplified with a model 1700 A-M Systems amplifier. EMG was recorded from the gastrocnemius and tibialis anterior muscles bilaterally with stainless steel wires (100  $\mu$ m) and amplified using a Grass polygraph (model 78D).

Unit pulses and EMG were recorded on a personal computer, using the 1401 plus interface and Spike 2 program (Cambridge Electronic Design, Cambridge, UK). The rate of digitization was 372 Hz for EMG and 21 kHz for unit activity. Digital EMG integration was performed over 10-s epochs.

### Microinjections

In each experiment, two to five injection sites in the medial medulla were sequentially tested, each with one OX-A microinjection. Sites were tested in random order. Subsequent microinjections were performed when muscle tone returned to background. OX-A (Peptide Institute, Osaka, Japan) was dissolved in 0.9% saline to obtain 50- $\mu$ M concentration of OX-A. The solution was stored at 4°C for a maximum of 2 wk. Control saline microinjections ( $n = 16$ ) into the medial medulla were performed in four rats. The PPTg and adjacent reticular formation were reversibly inactivated by unilateral or bilateral microinjections of lidocaine (4%). The OX-A and lidocaine injections consisted of 0.3  $\mu$ l administered at a rate of 10 nl/s. The effective radial spread of lidocaine was approximately 0.4 mm (Tehovnik and Sommer 1997). All injections were performed using a 1- $\mu$ l Hamilton microsyringe and injecting cannulae with outside tip diameter of 0.2 mm.

### Identification of mesopontine neurons related to muscle rigidity

Mesopontine reticular neurons related to rigid muscle tone were identified using the following criteria: 1) location in the dorsolateral mesopontine region where stimulation produces bilateral muscle tone facilitation (Lai and Siegel 1990a); 2) regular firing rate during postdecerebrate rigidity (Hoshino and Pompeiano 1976); 3) long duration (>1 ms) action potentials (Garcia-Rill et al. 1983); 4) positive correlation of firing rate with muscle tone during stimulation of inhibitory PnO sites that produced muscle tone suppression (Mileykovskiy et al. 2000); 5) firing rate resumption preceding muscle tone recovery; and 6) absence of firing rate change with passive manipulation of the hindlimbs (Garcia-Rill and Skinner 1988).

### Histology

Cathodal current (0.1–0.2 mA, 4–6 s) was passed through the microelectrodes at the end of each recording track. Rats were deeply anesthetized by pentobarbital sodium (70 mg/kg ip) and perfused transcardially with 0.01 M PBS, pH 7.4, followed by 4% paraformaldehyde in 0.1 M PBS. Brains were removed and cut into 60- $\mu$ m sections. The location of recorded neurons was determined by using the track made by the microelectrode, the depth of the marking lesion, and the depth measurements on the microdrive. The electrode tracks and marking lesions were visualized on a Nikon microscope and plotted with a NeuroLucida interface according to the rat brain atlas (Paxinos and Watson 1997). Cannula tracks were visualized on a Leica MZ6.

### Data analysis

The latency of muscle tone changes was measured from the start of the microinjection to either a 50% increase or a 50% decrease in

nonintegrated EMG amplitude compared with baseline. The duration was calculated from the time of onset of sustained increase or decrease in muscle tone to the return of tone to baseline level. The latencies and durations were averaged for right and left hindlimbs for microinjections that produced bilateral muscle tone variations.

Unit firing rates were analyzed by Wilcoxon matched-pairs test. The average unit firing rates were calculated for 10 s before and during electrical stimulation, or after OX-A microinjections when sustained muscle tone facilitation was observed. Regression analysis was used for estimating the correlation between changes of mesopontine unit firing rate and integrated ipsilateral gastrocnemius EMG. We selected this hindlimb muscle because mesopontine units related to motor activity send mainly ipsilateral descending projections (Garcia-Rill and Skinner 1987b). The changes of integrated EMG and firing rate were calculated in percent of baseline level. All average values are means  $\pm$  SE.

## RESULTS

### Identification of medullary sites producing muscle tone changes

Eighteen rats with postdecerebrate hindlimb EMG were used for identification of medullary sites producing muscle tone changes during electrical stimulation (30–100  $\mu$ A, 10–15 s). Figure 1A shows the distribution of muscle tone facilitating sites, muscle tone inhibitory sites, and sites producing locomotion on frontal sections of the rat brain. Twenty-six sites located in the GiA and GiV produced bilateral facilitation of hindlimb muscle tone during electrical stimulation (Fig. 2A). Twenty-five medullary sites induced a contralateral hindlimb muscle tone increase, and 11 sites evoked ipsilateral muscle tone facilitation. Seven medial medullary sites produced only bilateral tibialis anterior muscle tone facilitation. Controlled

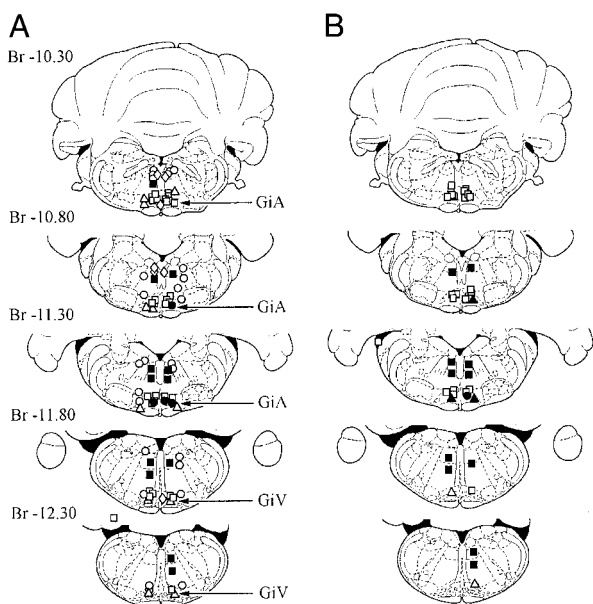


FIG. 1. Coronal views of the rat medulla showing the location of sites producing changes of rigid hindlimb muscle tone in decerebrate rats during electrical stimulation (A) and after orexin-A (OX-A) microinjection (B). Stimulating currents,  $<100 \mu$ A, OX-A concentration, 50  $\mu$ M.  $\square$ , bilateral muscle tone facilitation;  $\circ$ , contralateral muscle tone facilitation;  $\triangle$ , ipsilateral muscle tone facilitation;  $\diamond$ , bilateral facilitation of only tibialis anterior muscles;  $\blacksquare$ , bilateral muscle tone suppression;  $\bullet$ , hindlimb locomotion;  $\blacktriangle$ , hindlimbs jerks. GiA and GiV, gigantocellular reticular nucleus, alpha and ventral parts, respectively; Br, bregma.

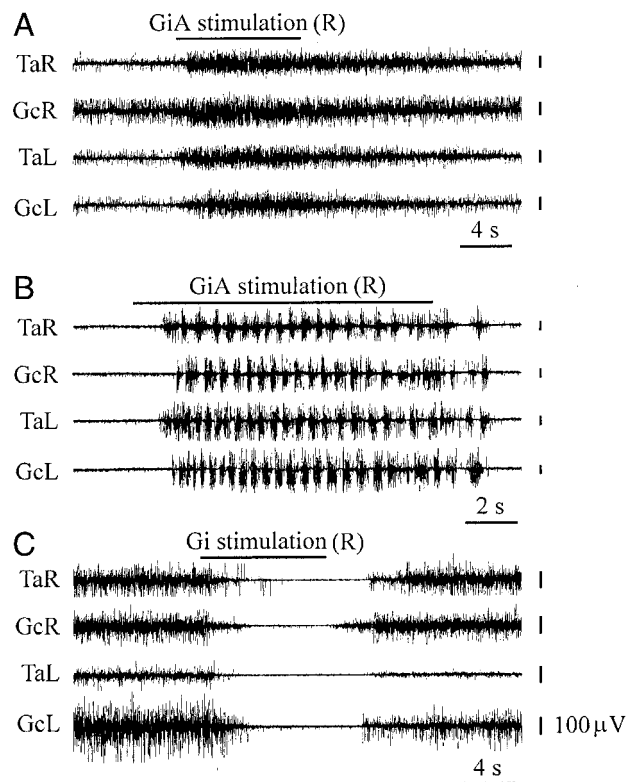


FIG. 2. Bilateral hindlimb muscle tone facilitation (A) and locomotion (B) during GiA electrical stimulation (R, right side). C: bilateral hindlimb muscle tone suppression during the Gi stimulation (R, right side). TaR, TaL, electromyogram (EMG) of tibialis anterior muscle (right and left side, respectively); GcR, GcL, EMG of gastrocnemius muscle (right and left side, respectively). Stimulating currents,  $<100 \mu$ A.

hindlimb locomotion in rats (Fig. 2B) was recorded during electrical stimulation of four sites located in caudal GiA near the raphe magnus nucleus (Fig. 1A). On the other hand, electrical stimulation of the DPGi sites ( $n = 7$ ) and Gi sites ( $n = 5$ ) produced bilateral hindlimb muscle tone suppression in rats with postdecerebrate muscle rigidity (Fig. 2C).

### OX-A microinjections into the identified medullary sites

OX-A microinjections into 23 GiA sites, identified previously as bilateral facilitatory sites, produced a bilateral increase of hindlimb muscle tone (Figs. 1B and 3A). The latency of muscle tone facilitation ranged from 68 to 337 s, with a mean latency of  $188 \pm 14$  s (mean  $\pm$  SE,  $n = 23$ ). The duration of muscle tone facilitation after OX-A microinjections into the GiA ranged from 232 to 1,180 s, with a mean duration of  $697 \pm 59$  s ( $n = 23$ ). OX-A microinjections into four GiA locomotor-inducing sites evoked bilateral hindlimb jerks in three cases (Fig. 3B) and ipsilateral hindlimb stepping ( $n = 1$ ). The latency and duration of hindlimb jerks and stepping ranged from 74 to 162 s and from 491 to 1,612 s, respectively.

On the other hand, OX-A microinjections into the DPGi and Gi inhibitory sites ( $n = 11$ ) produced bilateral muscle tone suppression (Fig. 3C). The latency of muscle tone suppression ranged from 16 to 140 s, with a mean of  $73 \pm 13$  s ( $n = 11$ ). The duration of muscle tone suppression varied from 156 to 682 s, with an average of  $348 \pm 62$  s ( $n = 11$ ). OX-A microinjections into previously selected GiV sites ( $n = 6$ ),

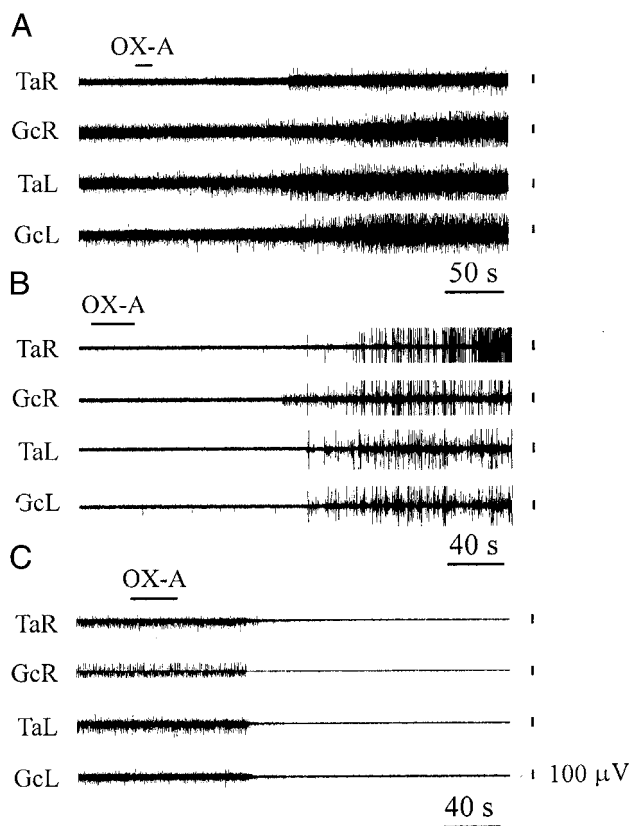


FIG. 3. Bilateral hindlimb muscle tone facilitation (A) and bilateral jerks (B) after OX-A microinjections into the GiA. C: bilateral hindlimb muscle tone inhibition after OX-A microinjections into the Gi. Top horizontal left bars indicate the time and duration of injections. See abbreviations in Fig. 2.

inducing bilateral muscle tone facilitation during electrical stimulation (Fig. 1A), produced weak (<50%) ipsilateral ( $n = 2$ ) and bilateral ( $n = 1$ ) increase of hindlimb EMG or were ineffective ( $n = 3$ ). Control saline microinjections ( $n = 16$ ) into the muscle tone facilitatory and inhibitory sites located in the GiA, GiV, DPGi, and Gi did not produce hindlimb muscle tone changes within the 1-h postinjection observation period.

*Lidocaine microinjections into the dorsolateral mesopontine reticular formation*

We hypothesized that the long-latency muscle tone facilitation after OX-A microinjections into the GiA and the low effectiveness of GiV microinjections could be related to the interaction of GiA intrinsic reticular neurons with pontine and mesencephalic structures, producing muscle tone facilitation. Our previous results showed that neurons located in the anatomic equivalent of the MLR are related to muscle tone facilitation in decerebrate rats (Mileykovskiy et al. 2000). Lidocaine microinjections into the PPTg and surrounding reticular formation were, therefore, performed in nine rats to determine the role of this region in muscle tone facilitation produced by OX-A microinjections into the GiA.

Unilateral lidocaine microinjections ( $n = 17$ ) into the sites located in the PPTg and central part of the lateral parabrachial nucleus (LPBC) produced ipsilateral ( $n = 10$ ), contralateral ( $n = 3$ ) or bilateral ( $n = 4$ ) hindlimb muscle tone suppression (Figs. 4 and 5, A and B). Ten lidocaine microinjections inhib-

ited unilateral or bilateral muscle tone completely, and seven microinjections evoked a decrease of EMG amplitude by an average of  $67 \pm 3\%$  (min = 59, max = 78,  $n = 7$ ) compared with the baseline. The latency of muscle tone inhibition after lidocaine microinjections ranged from 92 to 294 s, with a mean of  $172 \pm 14$  s ( $n = 17$ ). The duration of muscle tone suppression ranged from 1,228 to 2,567 s, with a mean of  $1,792 \pm 85$  s ( $n = 17$ ). In contrast, unilateral lidocaine microinjections ( $n = 10$ ) into the deep mesencephalic nucleus (DpMe) evoked ipsilateral or bilateral muscle tone facilitation (Fig. 5C). The latency and duration of muscle tone facilitation after injection into the DpMe ranged from 157 to 269 s, with a mean of  $189 \pm 10$  s ( $n = 10$ ), and from 926 to 2,135 s, with a mean of  $1,607 \pm 116$  s ( $n = 10$ ), respectively.

Fifteen sites located in the GiA and 4 sites located in the ventral part of the Gi and facilitating muscle tone during electrical stimulation were tested with OX-A microinjections to increase hindlimb muscle tone before and after 19 bilateral lidocaine microinjections into the PPTg and LPBC. In 12 cases, bilateral lidocaine microinjections decreased muscle tone bilaterally and prevented muscle tone facilitation after OX-A microinjections into the GiA (Fig. 6, A and B). Forty-five minutes after lidocaine administration, OX-A microinjections into the same GiA sites facilitated bilateral hindlimb muscle tone again (Fig. 6C). In four cases, bilateral lidocaine microinjections did not prevent hindlimb jerks with weak muscle tone facilitation after OX-A microinjections into the GiA. OX-A microinjections ( $n = 3$ ) into the ventral part of the Gi, performed after lidocaine injections, suppressed hindlimb muscle tone if complete muscle atonia did not occur after bilateral lidocaine injections.

To determine whether the postdecerebrate muscle rigidity

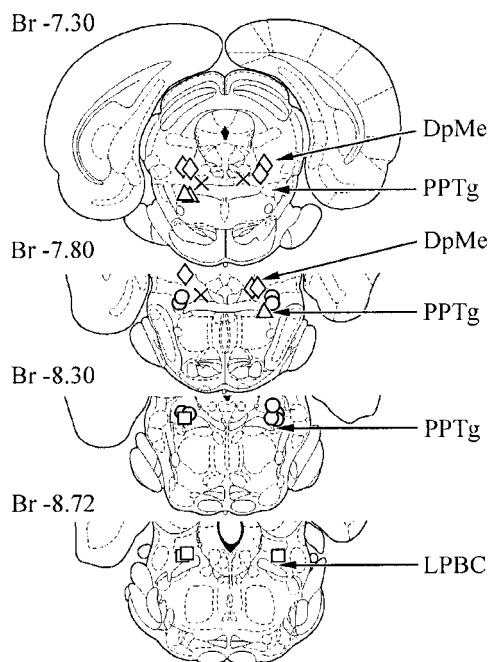


FIG. 4. Location of mesopontine sites suppressing and facilitating hindlimb muscle tone after lidocaine microinjections. □ and ○, ipsilateral and bilateral muscle tone suppression, respectively; △, contralateral muscle tone suppression; × and ◇, ipsilateral and bilateral muscle tone facilitation. DpMe, deep mesencephalic nucleus; LPBC, lateral parabrachial nucleus, central part; PPTg, pedunculopontine tegmental nucleus; Br, bregma.

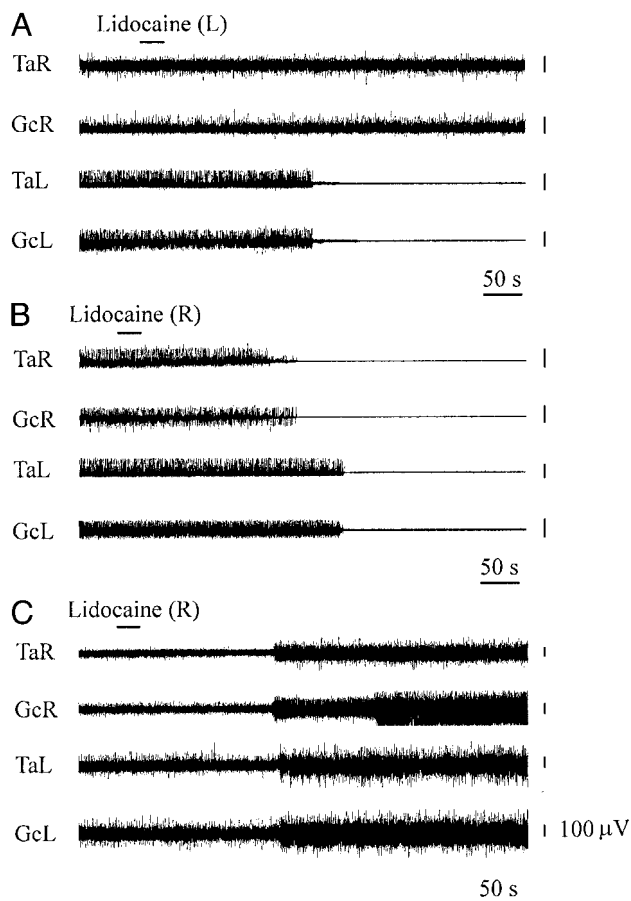


FIG. 5. Ipsilateral (A) and bilateral (B) muscle tone suppression and bilateral muscle tone facilitation (C) after unilateral lidocaine microinjections into the PPTg and DpMe, respectively. Top horizontal left bars indicate the time and duration of injections. See abbreviations in Fig. 2.

level and lidocaine-induced muscle tone decrease have a role in the OX-A facilitatory effect on muscle tone, we used a control group of decerebrate rats ( $n = 9$ ) without muscle rigidity for experiments similar to those described above. We observed muscle tone facilitation after electrical stimulation and following OX-A microinjections into the GiA sites in five animals (Fig. 7A). The latency and duration of muscle tone facilitation after OX-A microinjections ranged from 122 to 193 s, with a mean of  $159 \pm 13$  s ( $n = 5$ ) and from 215 to 603 s, with a mean of  $331 \pm 71$  s ( $n = 5$ ), respectively. Bilateral lidocaine microinjections into dorsolateral mesopontine region prevented muscle tone facilitation after OX-A microinjections into these GiA sites for 30–40 min (Fig. 7B). However, OX-A microinjections performed at the same sites after this period evoked muscle tone facilitation (Fig. 7C).

#### Influence of OX-A microinjections into the GiA on mesopontine neurons

To obtain additional evidence of participation of dorsolateral mesopontine region in muscle tone facilitation produced by OX-A microinjections into the GiA, discharges of 72 spontaneously active units were recorded in the PPTg and adjacent reticular formation in 6 rats. Eighteen neurons satisfied the criteria for cells related to rigid muscle tone. These neurons had an average firing rate of  $13.0 \pm 1.1$  spike/s ( $n = 18$ , min =

4, max = 22) and a spike duration of  $2.18 \pm 0.05$  ms (min = 1.9, max = 2.3) during muscle tone rigidity. Neurons were located mainly in the mesopontine region, producing muscle tone facilitation (Fig. 8, A and B), and were completely inhibited by PnO stimulation, suppressing muscle tone (Fig. 9, A and B). The firing rate resumption in these units preceded the muscle tone recovery after PnO stimulation by an average of  $3.9 \pm 0.2$  s ( $n = 18$ , min = 2.8, max = 5.7). Passive manipulation of hindlimbs did not affect the activity of these mesopontine cells.

The firing rate of nine neurons related to rigid muscle tone was analyzed during OX-A microinjections into the nine GiA sites facilitating muscle tone. Excitation of these cells after OX-A microinjections preceded muscle tone facilitation by an average of  $7.8 \pm 1.1$  s ( $n = 9$ , min = 3, max = 14). The discharge frequency of all cells was increased by an average of  $185 \pm 13\%$  ( $n = 9$ , min = 115, max = 322,  $P < 0.01$ ) compared with the baseline and correlated with muscle tone facilitation after OX-A microinjections (Fig. 10, A and B). The regression equation of gastrocnemius EMG on ipsilateral me-

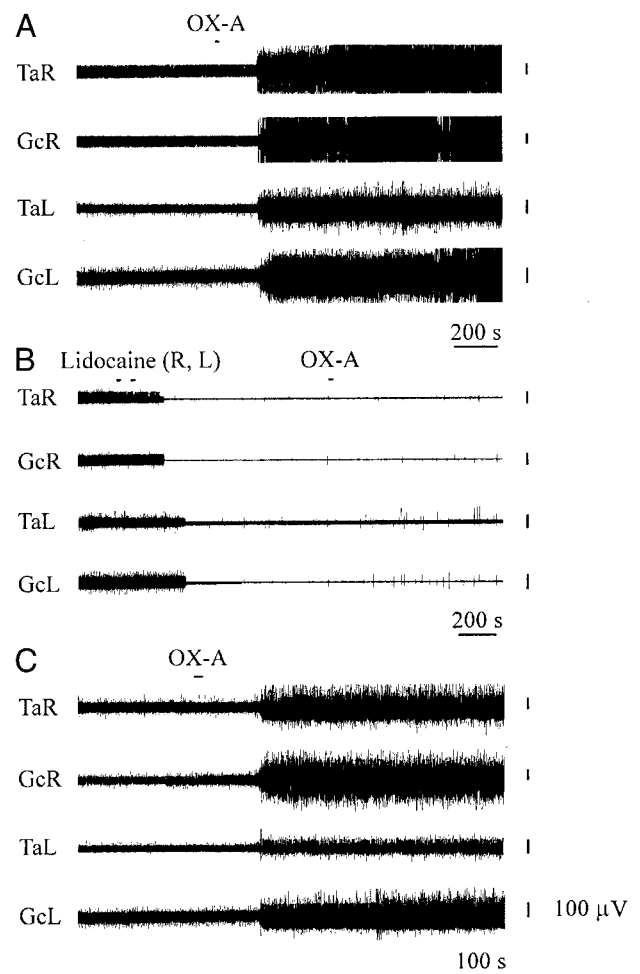


FIG. 6. Changes of hindlimb muscle tone after OX-A microinjections into the GiA site, facilitating muscle tone, performed before and after bilateral lidocaine microinjections into the PPTg. A: muscle tone facilitation after OX-A microinjection preceding lidocaine administration. B: bilateral lidocaine microinjections prevent muscle tone facilitation after OX-A microinjection. C: OX-A microinjection into the same GiA site again facilitates muscle tone in 45 min after bilateral lidocaine microinjections. Top horizontal bars indicate the time and duration of injections. See abbreviations in Fig. 2.

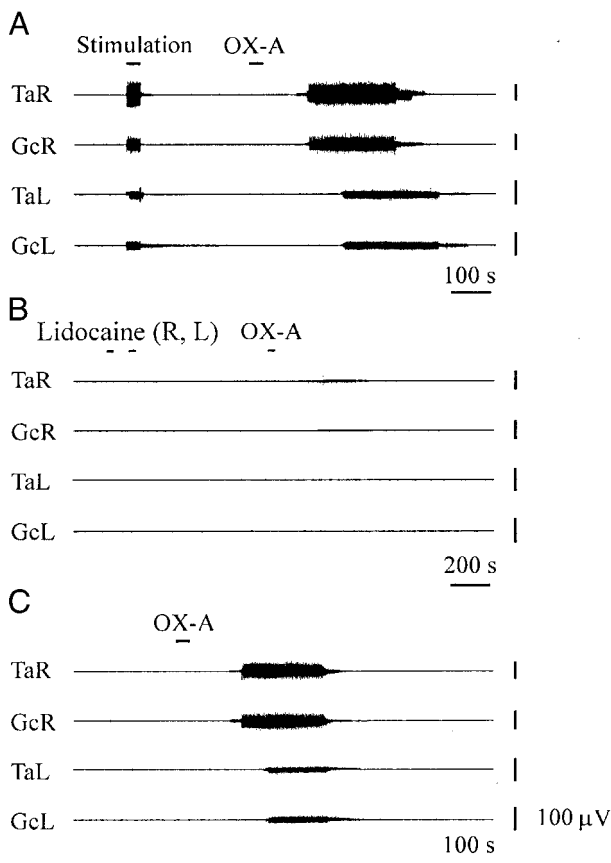


FIG. 7. Changes of hindlimb muscle tone in rats with zero postdecerebrate rigidity after OX-A microinjections into the GiA site, facilitating muscle tone, performed before and after bilateral lidocaine microinjections into the PPTg. *A*: muscle tone facilitation during electrical stimulation and after OX-A microinjection preceding lidocaine administration. *B*: OX-A injected into the GiA does not evoke muscle tone facilitation after bilateral lidocaine microinjections. *C*: OX-A microinjection into the same GiA site again facilitates muscle tone in 45 min after lidocaine bilateral microinjections. Top horizontal bars indicate the time of electrical stimulation and duration of injections. See abbreviations in Fig. 2.

sopontine unit firing rate after microinjections was  $EMG\% = 94.6 \pm 0.33 \text{ Rate}\%$  ( $SE = 27.3, 0.15; t = 3.46, 2.26; df = 1, 16; P < 0.05$ ). The correlation coefficient between gastrocnemius EMG and ipsilateral mesopontine unit firing rate was 0.49 ( $n = 18, P < 0.05$ ).

DISCUSSION

The current study shows that medullary sites producing muscle tone facilitation after OX-A microinjections were mainly located in the GiA. Microinjections into the GiV did not produce significant muscle tone facilitation, although the neurons located in this nucleus receive a moderate orexinergic innervation (Peyron et al. 1998) and send axons to spinal cord as do the GiA neurons (Kinjo et al. 1990; Shen et al. 1990). Bilateral lidocaine microinjections into the PPTg and adjacent dorsolateral mesopontine reticular formation blocked muscle tone facilitation produced by OX-A microinjections into the GiA. Moreover, OX-A microinjections into the GiA produced excitation of neurons located in the dorsolateral mesopontine region and related to muscle tone facilitation. These findings suggest that muscle tone facilitation after OX-A microinjec-

tions into the GiA is related to ascending excitation by GiA neurons of pontine and mesencephalic structures facilitating muscle tone. There are several sites in the midbrain and pons where low-threshold electrical stimulation or chemical excitation can elicit stepping and muscle tone facilitation in decerebrate and intact animals. These sites coincide the PPTg, CnF, medial part of the periaqueductal gray, superior colliculus, lateral lemniscus, parabrachial nucleus, and pontomedullary locomotor strip (Bandler et al. 1991; Coles et al. 1989; Garcia-Rill and Skinner 1988; Parker and Sinnamon 1983; Sinnamon et al. 1987; Zhang et al. 1990). Some of these sites correspond with locomotor systems partly independent of MLR. In particular, stepping produced by hypothalamic stimulation requires an intact anterior dorsal tegmentum, anterior ventromedial midbrain, oral pontine reticular nucleus, and PPTg (Levy and Sinnamon 1990; Sinnamon and Benaur 1997). There are data that selective excitotoxic lesion of the CnF or PPTg does not evoke a locomotor deficit in freely behaving animals (Allen et al. 1996; Inglis et al. 1994a,b; Olmstead and Franklin 1994). In contrast, results presented by other authors do not support this point. Microinjections of cobalt chloride, which can block synaptic transmission, and application of toxic doses of excitatory amino acid into subregions of the MLR significantly decrease locomotion induced by dopamine injections into the nucleus accumbens or picrotoxin injections into the subpallidal region (Brudzynski et al. 1993). Locomotor activity elicited by injections of picrotoxin into the subpallidal region and amphetamine into the nucleus accumbens is reduced by administration of procaine into the PPTg (Brudzynski and Mogenson 1985; Mogenson and Wu 1988). Suppression of induced and spontaneous locomotion was obtained in the precollicular-postmammillary decerebrate and decorticate animals after injections

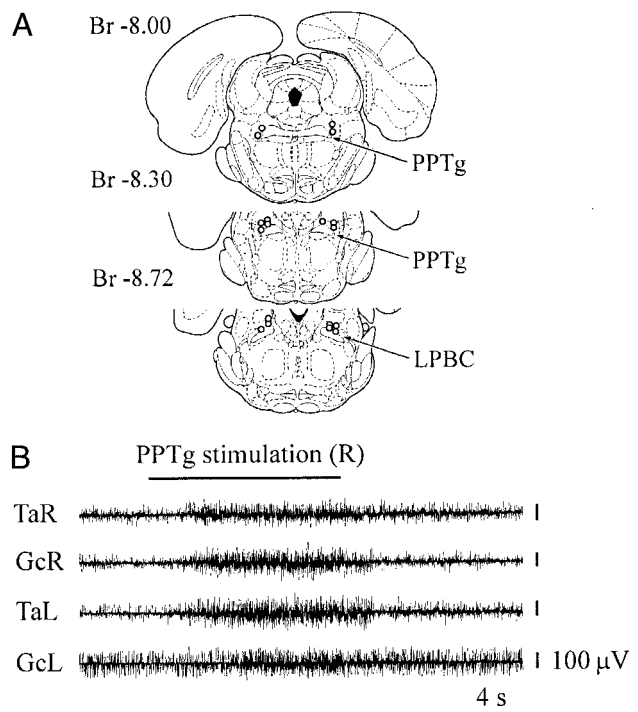


FIG. 8. Location of neurons related to rigid muscle tone (*A*) and muscle tone facilitation (*B*) during electrical stimulation of the PPTg and surrounding mesopontine reticular formation. Stimulating currents,  $<100 \mu A$ . PPTg, pedunculopontine tegmental nucleus; see other abbreviations in Fig. 2.

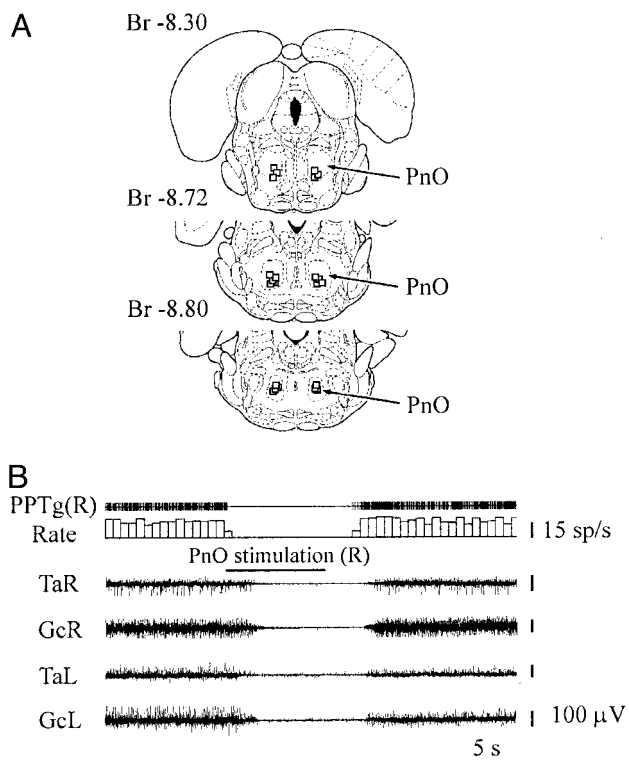


FIG. 9. Location of PnO sites suppressing hindlimb muscle tone bilaterally (A) and inhibition of PPTg neuron related to rigid muscle tone during electrical stimulation of the PnO inhibitory site. Stimulating currents, <100 μA. PnO, pontine reticular nucleus, oral part; PPTg(R), tonically active PPTg unit (right side); see other abbreviations in Fig. 2.

of muscimol, diazepam, and GABA into the MLR (Garcia-Rill et al. 1985; Pointis and Borenstein 1985). Locomotor activity induced by amphetamine applications into the nucleus accumbens is reduced by GABA injections into the MLR in freely moving animals (Brudzynski et al. 1986). Symmetric lesions of the LPBC also reduced motor activity in open field and labyrinth tests (Knupfer et al. 1988). The immunocytochemical staining of c-Fos protein in rats with chemically induced locomotion shows of activation of cells belonging to the PPTg, CnF, ventrolateral periaqueductal gray, and region of the dorsal tegmental bundle (Brudzynski and Wang 1996). These data indicate that neurons related to locomotion are widespread in the dorsolateral mesopontine region and explain how partial inactivation or destruction of MLR elements could be ineffective in blocking locomotion. In parallel with this, it is possible to conclude that the dorsolateral mesopontine region is a part of a distributed brain stem system controlling locomotor activity and, therefore, selective destruction of MLR subregions is not accompanied by lack of locomotion (Allen et al. 1996). We propose that muscle tone decrease after lidocaine microinjections into the dorsolateral mesopontine region is related to elimination of numerous inputs to this distributed brain stem system in decerebrate animals. We found that the most effective sites for lidocaine blockade of muscle tone are in the caudal part of the PPTg and rostral division of the LPBC. The high effectiveness of these sites may be linked with lidocaine spreading in several areas participating in locomotion and muscle tone facilitation, in particular, in the PPTg, CnF, dorsal tegmental bundle, LPBC, and region under superior cerebellar peduncle.

In a recent study, we have reported that neurons located in the anatomic equivalent of the MLR participate in muscle tone facilitation in decerebrate rats. These cells are inhibited by electrical stimulation of PnO and Gi inhibitory sites, as well as by lumbar perivertebral pressure producing bilateral hindlimb muscle atonia (Mileykovskiy et al. 2000). We propose that PPTg, CnF, and dorsal PnO units participating in muscle tone facilitation (Juch et al. 1992; Lai and Siegel 1990a; Mileykovskiy et al. 2000) are excited by medioventral medullary neurons sending rostral projections (Steininger et al. 1992) and receiving orexinergic innervation. In turn, these midbrain and pontine muscle facilitating structures directly (Rye et al. 1988; Skinner et al. 1990a; Spann and Grofova 1989) or through medullary reticular neurons (Bayev et al. 1988; Garcia-Rill and Skinner 1987a,b; Hermann et al. 1997; Nakamura et al. 1990; Rye et al. 1988) excite spinal motor systems. The PPTg descending fibers terminate in the PnO, caudal pontine reticular nucleus, and in the GiA and GiV (Grofova and Keane 1991). Skinner et al. (1990b) reported that about 10% of cholinergic and a large number of noncholinergic PPTg neurons project to the medioventral medulla region related to locomotion. Descending projections from CnF sites producing locomotion were found in the ipsilateral Gi and magnocellular reticular nucleus (Steeves and Jordan 1984).

We also propose that noradrenergic neurons of the LC are activated during OX-A microinjections into the GiA. In our recent study, we reported excitation of 30% LC neurons during

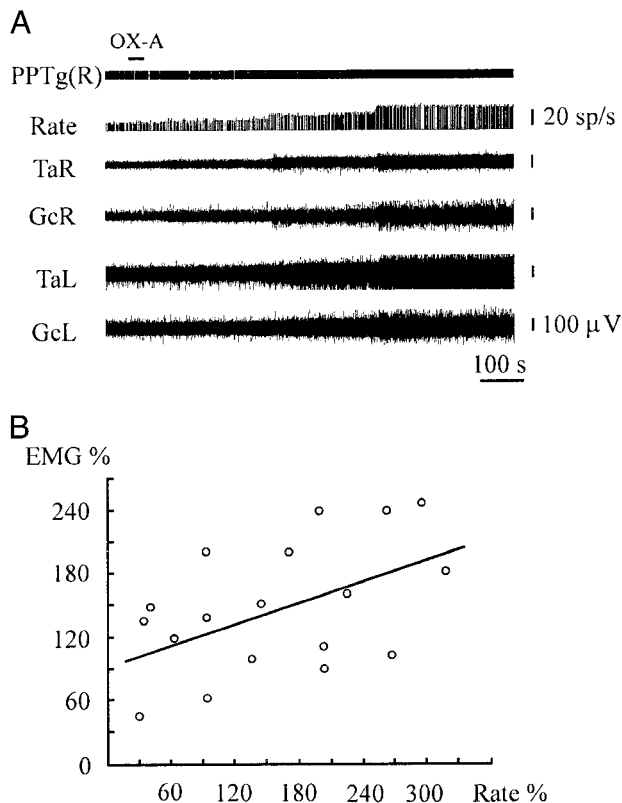


FIG. 10. Excitation of PPTg neuron and muscle tone facilitation after OX-A microinjections into the GiA (A). Regression diagram (B) showing relationship between changes of gastrocnemius EMG (EMG%) and unit firing rate (Rate%) located in the dorsolateral mesopontine region. Top horizontal bar at the left indicates the time and duration of injection. See abbreviations in Fig. 2.

electrical stimulation of the medial medulla, especially of the medioventral medullary region (Mileykovskiy et al. 2000). Pharmacological, electrophysiological, and behavioral studies indicate an overall facilitatory action of norepinephrine on spinal motor systems (Holstege and Kuypers 1987; White and Neuman 1980, 1983). We have recently shown that microinjections of OX-A and OX-B in the vicinity of LC increase the activity of noradrenergic neurons and produce a correlated facilitation of muscle tone in decerebrate rats (Kiyashchenko et al. 2001).

Electrical stimulation and chemical excitation of the medioventral medulla produce locomotor activity in decerebrate cats and rats (Garcia-Rill and Skinner 1987a; Kinjo et al. 1990). However, we did not observe significant hindlimb stepping as a result of OX-A microinjections into this region. We propose that the excitatory effect of OX-A microinjections into the GiA is not sufficient for induction of locomotion in decerebrate rats. This result suggests that this peptide has a modulating rather than a triggering role in phasic motor regulation.

We have observed hindlimb muscle tone suppression as a result of OX-A microinjections into the medial division of the DPGi and Gi. We recently reported similar inhibitory motor effects after microinjections of OX-A and OX-B in PnO sites, producing muscle tone suppression during electrical stimulation (Kiyashchenko et al. 2001). These pontine and medullary structures receive a relatively low level of orexinergic innervation (Peyron et al. 1998). However, they are very effective inhibitors of muscle tone. The PnO, DPGi, and Gi are the main parts of a brain stem–reticulospinal inhibitory system hyperpolarizing spinal motoneurons (Jankowska et al. 1968; Magoun and Rhines 1946; Takakusaki et al. 1989, 1993, 1994). The DPGi and Gi receive excitatory inputs from the medial and dorsolateral pontine inhibitory regions (Mileykovskii 1994; Takakusaki et al. 1989, 1994) and participate in induction of REM sleep atonia (Chase and Morales 1990; Lai and Siegel 1990a,b; Sakai et al. 1979; Schenkel and Siegel 1989). Electrical stimulation of the DPGi and Gi sites inhibits the discharge in 70% of noradrenergic LC cells and suppresses neuron activity linked to muscle tone facilitation in the anatomical equivalent of the MLR (Mileykovskiy et al. 2000).

Muscle tone facilitation after lidocaine inactivation of the DpMe suggests that neuronal populations modulating activity of the pontine and medullary inhibitory structures are located in the rostral brain stem and forebrain. Electrical and chemical stimulation (kainic acid) of the medial part of the midbrain reticular formation, lateral division of the parafascicular thalamic nucleus, and deep layers of the frontoparietal cortex block the motor activity in freely moving rats and suppress the hindlimb muscle tone induced by hypothalamic and red nucleus stimulation in anesthetized animals (Mileykovskii et al. 1991; Mileikovsky et al. 1994).

Thus, our results, combined with our recent data showing muscle tone facilitation after OX microinjections in the LC (Kiyashchenko et al. 2001), support the hypothesis that orexins participate in muscle tone regulation at the brain stem level. We propose that the GiA is an important brain stem target for OX influences on spinal muscle tone facilitatory systems. The loss of OX function in human narcoleptics (Peyron et al. 2000; Thannical et al. 2000) may decrease the activity of these brain stem structures facilitating muscle tone and allow the phasic

motor inhibition elicited by emotion to cause a loss of muscle tone (Siegel 1999).

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