Atonia-Related Regions in the Rodent Pons and Medulla

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Received 4 April 2000; accepted in final form 5 June 2000

Hajnik, T., Y. Y. Lai, and J. M. Siegel. Atonia-related regions in the rodent pons and medulla. J Neurophysiol 84: 1942–1948, 2000. Electrical stimulation of circumscribed areas of the pontine and medullary reticular formation inhibits muscle tone in cats. In this report, we present an analysis of the anatomical distribution of atonia-inducing stimulation sites in the brain stem of the rat. Muscle atonia could be elicited by electrical stimulation of the nuclei reticularis pontis oralis and caudalis in the pons as well as the nuclei gigantocellularis, gigantocellularis alpha, gigantocellularis ventralis, and paragigantocellularis dorsalis in the medulla of decerebrate rats. This inhibitory effect on muscle tone was a function of the intensity and frequency of the electrical stimulation. Average latencies of muscle-tone suppression elicited by electrical stimulation of the pontine reticular formation were 11.02 ± 2.54 and 20.49 ± 3.39 (SD) ms in the neck and in the hindlimb muscles, respectively. Following medullary stimulation, these latencies were 11.29 ± 2.44 ms in the neck and 18.87 ± 2.64 ms in the hindlimb muscles. Microinjection of N-methyl-D-aspartate (NMDA, 7 mM/0.1 μl) agonists into the pontine and medullary inhibitory sites produced muscle-tone facilitation, whereas quisqualate (10 mM/0.1 μl) injection induced an inhibition of muscle tone. NMDA-induced muscle tone change had a latency of 31.8 ± 35.3 s from the pons and 10.5 ± 0.7 s from the medulla and a duration of 146.7 ± 95.2 s from the pons and 55.5 ± 40.4 s from the medulla. The latency of quisquulate (QU)-induced reduction of neck muscle tone was 30.1 ± 37.9 s after pontine and 39.5 ± 21.8 s after medullary injection. The duration of muscle-tone suppression induced by QU injection into the pons and medulla was 111.5 ± 119.2 and 169.2 ± 145.3 s. Smaller rats (8 wk old) had a higher percentage of sites producing muscle-tone inhibition than larger rats (16 wk old), indicating an age-related change in the function of brain stem inhibitory systems. The anatomical distribution of atonia-related sites in the rat has both similarities and differences with the distribution found in the cat, which can be explained by the distinct anatomical organization of the brain stem in these two species.

METHODS
Surgical procedures
Experiments were performed on 54 male rats weighing between 170 and 510 g, corresponding to 8–16 wk of age. Animals were initially anesthetized with halothane (Fluothane) and ketamine HCl (Ketalar, 100 mg/kg im) for cannulation of the trachea. Both common carotid arteries were ligated to reduce bleeding. The animals’ heads were then fixed in a stereotaxic frame. After frontal and parietal craniotomy and immediately after removing the forebrain, anesthesia was discontinued. The brain stem was transected at the precollicular-postmammillary level with a spatula, and the forebrain was removed by aspiration. The rostral pole of the midbrain and the floor of the skull were covered with a small cotton ball soaked in hot saline solution. The exposed brain tissue was protected from dehydration with a thin layer of cotton gauze soaked with mineral oil. Multi-stranded stainless steel bipolar electrodes were implanted in the gastrocnemius muscle of the hindlimb, triceps brachii muscle of the forelimb and the splenius of the neck to record the electromyogram.
glutamate on muscle activity, 0.1 ml of the specific N-methyl-D-aspartate (NMDA) antagonist, DL-2-amino-5-phosphono- panoic acid (AP5) and non-NMDA receptor antagonist, 6-cyano-7-nitroquinonoxaline-2,3-dione (CNQX) were microinjected into the pontine site one minute before agonists were applied.

CHEMICALS. All chemicals for the microinjection studies were acquired from Tocris Neuramin, Bristol, UK. The concentration of chemicals was as follows: NMDA (7 mM), quisqualate (QU, 10 mM), AP5 (20 μM), and CNQX (10 μM).

Stimulation and recording

Recording began 3 h after decerebration, when muscle tone had stabilized. All animals received electrical stimulation that was administered through stainless steel concentric bipolar electrodes (Rhodes, SNEX-100) that were moved stereotaxically in 0.3-mm steps from H2.8 to 0.0 between A0.7–P0.3 and L0.2–1.5 mm in the pons and from H2.0 to −0.7 between P2.0–P3.3 and L0.0–0.6 in the medulla according to Paxinos and Watson (1986). These regions correspond to the approximate anatomical levels of the pontine and medullary atonia eliciting regions of the cat (Lai and Siegel 1988; Lai et al. 1987). In all experiments, except in latency studies, electrical stimuli consisted of 500-ms trains of 0.2-ms cathodal rectangular pulses at 10–250 Hz and 5–250 μA. EMG signals were amplified and recorded on a Grass polygraph (Model 78E). The amplitude of muscle activity was integrated with a time constant of 1 s using a Grass polygraph integrator (Model 7P10F). Integrated EMG signals were used to measure the magnitude of muscle-tone suppression. In four experiments, the latencies of EMG changes were measured by a stimulus triggered averaging technique. At each point, during 20 consecutive trials, EMG signals were digitized by an A/D converter (CED 1401) over a 250-ms peristimulus period with a binwidth of 100 μs, rectified, and averaged by a computer. In these experiments, 50- to 100-ms trains at 150 Hz (8–15 pulses) and at an intensity of three times threshold (40–50 μA) for muscle-tone suppression were used.

Histology

At the end of the experiments, iron was deposited at the dorsal and ventral limits of the stimulation tracks by passing a 50-μA positive DC current for 20 s through the electrode. Rats were perfused with saline, followed by a buffered 10% formalin solution, and the brains were removed. Serial coronal sections (60 μm) were cut by a cryostat, stained with Neutral red, and counterstained with a potassium-ferrocyanide solution to produce a Prussian-blue reaction with the deposited iron. Stimulation and injection sites were reconstructed according to the atlas of Paxinos and Watson (1986).

Data analysis

After perfusion of the four rats used for the measurement of response latencies, the length of motoneuron axons innervating splenius and gastrocnemius muscles was measured from the exit of ventral horn to the innervating muscles. Then, the brain stem with attached spinal cord was removed. The length of the spinal cord from C2 to L5 was measured to calculate the conduction velocity of the reticulospinal neurons. Student’s t-test was used for statistical analysis.

Results

Localization of areas producing muscle-tone suppression

Areas producing bilateral muscle-tone suppression by electrical stimulation in the rostral pons (A0.7–A0.28) were located between L0.6–1.2 and H2.5–0.8, while in the caudal pons (A0.28–P0.3) effective areas were located between L0.4–0.8 and H2.6–1.0. These areas corresponded to the nuclei reticularis pontis oralis and caudalis. In the medulla, responsive sites were located from

<table>
<thead>
<tr>
<th>Pons Medulla</th>
<th>&lt;300 g</th>
<th>&gt;300 g</th>
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<tr>
<td>Bilateral inhibition</td>
<td>50.6</td>
<td>28.1</td>
</tr>
<tr>
<td>Contralateral inhibition only</td>
<td>31.7</td>
<td>71.9</td>
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Data were collected from 34 rats.
P2.0 to P3.3, L0.2 to L1.1, and H1.7 to H-0.7 and included nuclei reticularis gigantocellularis, paragigantocellularis dorsalis, gigantocellularis alpha, and gigantocellularis ventralis.

The effect of electrical stimulation on muscle activity varied as a function of stimulation site (Fig. 1). Bilateral inhibition or inhibition followed by rebound facilitation was restricted to a compact region in the medial pons and medulla. In contrast, stimulation sites inducing contralateral inhibition without effects on the ipsilateral muscle tone were located in a more scattered area. The percentage of sites producing bilateral inhibition was higher in smaller rats (Table 1). Statistical analysis revealed that the correlation between body size and site-induced muscle-tone suppression was significant in both the pons and medulla (pons: $P < 0.01$, df = 45, 2-tailed $t$-test; medulla: $P < 0.05$, df = 22, 2-tailed $t$-test).

Inhibition of muscle tone by brain stem stimulation

Two types of bilateral muscle-tone suppressions with long and short duration resulted from electrical stimulation of the pontomedullary reticular formation (Fig. 2). Short responses terminated when stimulation was discontinued, while long responses outlasted stimulation, often by several seconds (11.4 ± 2.6 s). The inhibitory effect was consistently more profound in the contralateral as opposed to the ipsilateral side ($P < 0.001$, df = 67, paired $t$-test; Fig. 3) for both short and long responses.

Muscle-tone suppressions induced by pontomedullary stimulation were a function of the intensity (Figs. 3 and 4) and frequency of the stimulation (Fig. 3). Current intensity thresholds that produced muscle-tone suppression varied from 5 to 100 $\mu$A across the animals. Therefore, current values were normalized with reference to the individual threshold intensities to express quantitatively the magnitude of tone reduction as a function of stimulation intensity. The relationship between the stimulation intensity and the muscle-tone suppression was biphasic, with the maximal effect at 3 times threshold (Fig. 3). As with the intensity, the effect of stimulus frequency on the muscle-tone suppression was also biphasic with a maximal effect at 150 Hz.

Response latency and conduction velocity

Response latencies were measured in 28 trials carried out in four rats. Latency was defined as the time from the start of the stimulation to the point when the rectified and averaged EMG signal fell below baseline by two times of the standard deviation (Fig. 5). Base line mean and standard deviation were calculated from the 50-ms prestimulus period. To measure latencies, 150-Hz stimulus trains at three times threshold intensity were delivered to the pontine and medullary reticular formations. The latency of muscle-tone suppression was 11.02 ± 2.54 ms in the neck muscles and 20.49 ± 3.39 ms in
the hindlimb muscles when the pontine reticular formation was stimulated. Following medullary stimulation, latencies of 11.29 ± 6.2.44 ms in the neck and 18.87 ± 6.2.64 ms in the hindlimb were seen.

The conduction velocity of the reticulospinal inhibitory system was calculated from the latency differences of tone suppressions in ipsilateral neck and hindlimb muscles. Conduction velocity of rodent motoneurons was assumed to be 60 m/s (Bakels and Kernell 1993; Gardiner 1993). The length of motoneuron axons innervating neck and hindlimb muscles was taken as 5 and 67 mm, respectively. The time needed for action potentials to propagate from the axon of the motoneuron to the muscle (0.08 and 1.1 ms for the neck and hindlimb motoneurons, respectively) was deducted from the latencies. The remainder of the latency difference was caused by the greater distance the descending inhibitory impulses had to travel from C2 to L5. Thus the conduction velocity of the reticulospinal inhibitory system can be calculated by dividing the length of the spinal cord between C2 and L5 by the corrected latency difference between hindlimb and neck muscle-tone suppression. The length of the spinal cord between C2 and L5 was 47 ± 3 mm (range: 42–50 mm). The conduction velocity of the reticulospinal inhibitory system was calculated in this way as 6.79 ± 6.3.3.0 m/s (range: 3.12–26.40 m/s).

Changes in muscle activity produced by the microinjection of glutamate agonists

To determine if neuronal somas rather than axons of passage were responsible for the electrical stimulation effect, glutamate agonists and antagonists were microinjected into the brain stem. The concentration of glutamate agonists and antagonists used in this study was based on our previous work on the decerebrate cat (Lai and Siegel 1991). One-tenth microliter of quisqualate (10 mM) injected into the pons produced muscle-tone suppression, bilaterally (Fig. 6). The average latency of QU-induced muscle-tone suppression was 30.1 ± 37.9 and 39.5 ± 21.8 s after pontine and medullary injection, respectively. The duration of muscle-tone suppression was 111.5 ± 119.2 s when QU was injected into the pons and 169.2 ± 145.3 s when it was injected into the medulla. This QU-induced muscle-tone suppression could be blocked by prior injection of the specific non-NMDA antagonist, CNQX (10 μM/0.1 μl) into the sites (Fig. 6). In contrast, NMDA (7 mM/0.1 μl) injected into the same or nearby sites elicited muscle-tone facilitation (Fig. 7). The average latency of muscle-tone facilitation was 31.8 ± 35.3 s and duration was 146.7 ± 95.2 s when NMDA was microinjected into the pons. NMDA micro-

FIG. 5. Computer average of electromyogram (EMG) change produced by 20 trials of medullary stimulation. Fifty-millisecond trains with 150-Hz pulses and 50-μA intensity were applied into the right side of medial medulla. Baseline mean and SD were calculated from the 50-ms prestimulation period. A, the stimulation onset; and B, the time when the depression of the EMG activity exceeded twice the baseline SD. The latencies of medullary-induced muscle-tone suppression were 8.8 ms in the left neck muscle, 12.1 ms in the right neck muscle, and 17.3 ms in both left and right hindlimb muscles. Mean, baseline mean EMG activity; 2SD, 2 times baseline SD; LHM and RHM, left and right hindlimb muscles, respectively.

FIG. 6. Effect of quisqualate (QU) injection into the pons on muscle activity. A: microinjection of QU (10 mM/0.1 μl) into pons produced suppression of muscle tone in the neck (RNM and LNM) and hindlimb (RHM and LHM). B: the specific non-N-methyl-D-aspartate (non-NMDA) antagonist, 6-cyano-7-nitroquin- oxaline-2,3-dione (CNQX, 10 μM/0.1 μl) microinjected into the same site does not have an effect on muscle activity. However, prior CNQX injection blocked the QA effect on muscle activity (C). RFM, right forelimb muscle.
Muscle-tone suppression could be induced in the decerebrated rat by electrical and chemical stimulation of the medial pons and medulla. As in the decerebrate cat, quisqualate microinjected into the pons induced muscle-tone suppression, whereas NMDA agonist injected into the same or nearby sites elicited muscle-tone facilitation. The anatomical distribution of atonia-related sites in the rat has both similarities and differences from the distribution found in the cat. In the decerebrate cat, electrical stimulation of the nuclei magnocellularis and paramedianus of the medulla and the medial pontine reticular formation was found to be effective (Lai and Siegel 1990b, 1991; Lai et al. 1987). These sites are generally thought to correspond to the nuclei reticularis gigantocellularis, paragigantocellularis dorsalis, gigantocellularis alpha, gigantocellularis ventralis, and reticularis pontis oralis and caudalis stimulated in the rat. Our present study found that stimulation sites inducing bilateral inhibition were located in a restricted area of the medial pons and medulla, whereas sites where stimulation that caused contralateral inhibition only were encountered in a wider area that included the inhibitory regions that included the bilateral inhibitory area. In contrast, these two areas were found to be separated in the cat (Lai et al. 1987), in which stimulation in the medial medulla produced bilateral inhibition, while stimulation in more lateral medulla produced contralateral inhibition and facilitated ipsilateral neck muscles. One possible explanation for this species difference is a greater anatomical overlap of the atonia regulating area with other areas involved in the facilitation of muscle tone in the rat. Alternatively, it can be explained by the relatively small size of the rat brain causing electrical stimulation to spread to a relative wider range of structures (Ranck 1975; Rattay 1999) than in the cat, thus co-activating anatomically segregated inhibitory and facilitatory neurons.

Muscle-tone suppressions were either localized to the contralateral side or appeared bilaterally in the decerebrate rat. Those sites where stimulation induced bilateral muscle-tone suppression may be involved in the generalized regulation of muscle tone. In contrast, those sites where stimulation produced contralateral inhibition only may be involved in more specialized motor control, including acoustic startle response (Davis et al. 1982; Lee et al. 1996), lordosis (Cohen et al. 1987), and locomotion. Low-frequency (40 Hz), long-duration (10 s) stimulation in the ventromedial medulla has been shown to induce locomotion (Kinjo et al. 1990). Although high-frequency (>100 Hz) stimulation may cause depolarization block (Benazzouz et al. 1995, 1996), it is unlikely that the muscle-tone suppression seen in the present study is due to inactivation of neurons. We have found that muscle-tone suppression could be elicited by low-frequency (50 Hz) stimulation in the present study. Whereas, high-intensity stimulation is generally required for depolarization block (Rattay 1999). Our previous work in the decerebrate cat has also found that muscle-tone suppression could be elicited through a wide range of stimulation frequencies (10–200 Hz), and the inhibitory effect is the first alteration in muscle tone seen when stimulation intensity is gradually increased from subthreshold levels (Lai and Siegel 1990a). Furthermore, the QU-injection-induced muscle-tone suppression that we found in the present study at the effective electrical stimulation sites demonstrates that muscle-tone suppression is mediated by activation of pontine and medullary neurons rather than fibers of passage.

The percentage of sites where stimulation induced bilateral inhibition was higher in younger rats than in older ones as shown on Table 1. This suggests that aging is associated with a deterioration of the atonia-controlling system in humans. The reduction of stimulation sites with bilateral atonia induction seen in the older rats during our experiments may reflect a similar deterioration in the efficacy of the atonia systems. The rat may be a useful model for determining the neurological cause of this disorder.

The pontine sites where electrical stimulation induced bilat-
eral muscle-tone suppression overlaps with the sites where carbachol injection has been shown to induce atonia in the decerebrated rat (Taguchi et al. 1992) or paradoxical sleep in the freely moving rat (Bourgin et al. 1995; Gnegg and Pegram 1986). This part of the pontine reticular formation contains cholinocceptive cells (Jones 1990; Kimura et al. 1981) and receives innervation from the mesopontine cholinergic neurons (Jones 1990; Lai et al. 1993; Shiromani et al. 1988a,b), consistent with a cholinergic contribution to REM sleep atonia. In addition, glutamatergic fibers also participate in these phenomena, since microinjection of glutamate into the pontine and medial medullary reticular formation produces bilateral inhibition of muscle tone and blockade of medullary glutamatergic sites partially reversed pontine elicited atonia in cats (Lai and Siegel 1988). As in our previous studies in the cat (Lai and Siegel 1991), we found non-NMDA agonists microinjected into the medial pons and medulla of the decerebrate rat induced muscle-tone suppression, whereas NMDA injection elicited muscle-tone facilitation. There are NMDA and non-NMDA glutamatergic receptors in the pontomedullary reticular formation in the rat and cat (Lai et al. 1995, 1996; Petralia et al. 1994a,b; Winsky et al. 1996) at the sites where we obtained these effects. Glutamatergic neurons of the rostral brain stem project to the atonia induction sites in the pons (Lai at al 1993) and medulla (Lai et al. 1999).

Neuroanatomical studies using retrograde and anterograde transport tracers have demonstrated that the medioventral medulla, corresponding to the nuclei gigantocellularis alpha and ventralis, is a major source of reticulospinal projections. The medial pons also has a significant spinal projection (Kuyper and Maisky 1977; Matsuyama et al. 1988, 1993; Robbins et al. 1992; Suzuki 1985; Tohyama et al. 1979; Zemlan et al. 1984). Reticulospinal fibers from the pons and medulla descend both ipsi- and contralaterally. Anterograde and retrograde tracer studies have demonstrated that neurons in the feline pons (Matsuyama et al. 1999) and rodent medulla (Huismans et al. 1984; Marten et al. 1981) innervate both cervical and lumbar sacral spinal cord by way of axonal collaterals. We calculate that the average conduction velocity of the pontine inhibitory reticulospinal system is 6.8 m/s. The conduction velocity of descending neurons mediating atonia may be somewhat higher than the conduction velocity that we calculate for the inhibitory system. If one assumes a synaptic delay of 0.5 ms (Lorente de No 1935; Renshaw 1940) and two synapses in the lumbar spinal cord (Peterson 1978), the conduction velocity of reticulospinal neurons mediating atonia would be 8.0 m/s. Our previous study demonstrated that glutamatergic neurons in the medial pons project to the nucleus magnocellularis of the medioventral medulla in the cat (Lai et al. 1999). These results suggest that inhibitory stimulation in the medial pons induced bilateral muscle-tone suppression either directly or indirectly through the medullary reticulospinal neurons.

Cataplexy, a sudden loss of muscle tone in waking, appears to represent a triggering of the mechanism responsible for the suppression of muscle tone in REM sleep. Studies in the narcoleptic dog have indicated that the pontomedullary inhibitory system is responsible for cataplexy (Siegel et al. 1991, 1992). The decerebrate rat may be useful in the analysis of the neural physiology of the atonia system and its modification in pathological conditions.

This work was supported by the Medical Research Service of the Veterans Administration and National Institutes of Health Grants HL-41370, NS-14610, and NS-23724.

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This work was supported by the Medical Research Service of the Veterans Administration and National Institutes of Health Grants HL-41370, NS-14610, and NS-23724.


