The Motor Inhibitory System Operating During Active Sleep Is Tonia
cally Suppressed by GABAergic Mechanisms During Other States

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Xi, Ming-Chu, Francisco R. Morales, and Michael H. Chase. The motor inhibitory system operating during active sleep is tonically suppressed by GABAergic mechanisms during other states. J Neurophysiol 86: 1908–1915. 2001. The present study was undertaken to explore the neuronal mechanisms responsible for muscle atonia that occurs after the microinjection of bicuculline into the nucleus pontis oralis (NPO). Specifically, we wished to test the hypothesis that motoneurons are postsynaptically inhibited after the microinjection of bicuculline into the NPO and determine whether the inhibitory mechanisms are the same as those that are utilized during naturally occurring active (rapid eye movement) sleep. Accordingly, intracellular records were obtained from lumbar motoneurons in cats anesthetized with α-chloralose before and during bicuculline-induced motor inhibition. The microinjection of bicuculline into the NPO resulted in a sustained reduction in the amplitude of the spinal cord Ia-monosynaptic reflex. In addition, lumbar motoneurons exhibited significant changes in their electrophysiological properties [i.e., a decrease in input resistance and membrane time constant, a reduction in the amplitude of the action potential’s afterhyperpolarization (AHP) and an increase in rheobase]. Discrete, large-amplitude inhibitory postsynaptic potentials (IPSPs) were also observed in high-gain recordings from lumbar motoneurons. These potentials were comparable to those that are only present during the state of naturally occurring active sleep. Furthermore, stimulation of the medullary nucleus reticularis gigantocellularis evoked a large-amplitude IPSP in lumbar motoneurons after, but never prior to, the injection of bicuculline; this reflects the pattern of motor responses that occur in conjunction with the phenomenon of "reticular response-reversal." The preceding changes in the electrophysiological properties of motoneurons, as well as the development of active sleep-specific IPSPs, indicate that lumbar motoneurons are postsynaptically inhibited following the intrapontine administration of bicuculline in a manner that is comparable to that which occurs spontaneously during the atonia of active sleep. The present results support the conclusion that the brain stem-spinal cord inhibitory system, which is responsible for motor inhibition during active sleep, can be activated by the injection of bicuculline into the NPO. These data suggest that the active sleep-dependent motor inhibitory system is under constant GABAergic inhibitory control, which is centered in the NPO. Thus during wakefulness and quiet sleep, the glycinegically mediated postsynaptic inhibition of motoneurons is prevented from occurring due to GABAergic mechanisms.

INTRODUCTION

We have recently reported that a behavioral state that resembles naturally occurring active sleep arises after the microinjection of bicuculline, a GABA_A receptor antagonist, into the nucleus pontis oralis (NPO) of the pontine reticular formation. Conversely, microinjections of either GABA or a GABA agonist into the NPO results in prolonged episodes of wakefulness and increased motor activity (Xi et al. 1999, 2001). However, it remained to be determined if the atonia induced by the injection of bicuculline into the NPO was produced by processes of postsynaptic inhibition or disinhibition. If a postsynaptic inhibitory mechanism was responsible, was this the same system utilized during naturally occurring active sleep (also referred to as rapid eye movement sleep) as well as during carbachol-induced active sleep-like state (for reviews, see Chase and Morales 1990, 2000)?

In previous studies we have shown that the neuronal mechanisms of postsynaptic inhibitions of motoneurons are responsible for motor inhibition during active sleep. For examples, motoneurons are tonically hyperpolarized during active sleep (Morales and Chase 1978, 1982; Morales et al. 1987a). Superimposed on the hyperpolarized membrane potential of motoneurons are discrete, large-amplitude postsynaptic inhibitory potentials (active sleep IPSPs) (Morales and Chase 1982; Morales et al. 1987a; Soja et al. 1991). In addition, there are significant changes in the electrophysiological properties of motoneurons during active sleep such as a decrease in excitability, input resistance and membrane time constant (Morales and Chase 1981; Soja et al. 1991). The postsynaptic inhibition of motoneurons during active sleep is glycinegically mediated (Chase and Morales 1990; Chase et al. 1989; Soja et al. 1991). Furthermore, electrical stimulation of brain stem reticular sites evokes characteristic, large-amplitude IPSPs in motoneurons during active sleep but not during quiet sleep or wakefulness (Chandler et al. 1980; Chirwa et al. 1991; Fung et al. 1982). These evoked potentials have been shown to be an essential aspect of the phenomenon of "reticular response-reversal," in which responses to a physiological stimulus are reversed from excitation during wakefulness to inhibition during active sleep (Chase and Babb 1973).

We hypothesize that lumbar motoneurons are postsynaptically inhibited following the microinjection of bicuculline into the NPO, and the inhibitory mechanisms are the same as those that are utilized during naturally occurring active sleep as well as during carbachol-induced active sleep-like state (Chase and Morales 1990, 2000). To test this hypothesis, we examined the basic electrophysiological properties and state-dependent syn-
aptic activity impinging on spinal cord motoneurons after the microinjection of bicuculline into the NPO.

METHODS

Surgical procedures

The present experiments were performed on six adult cats (3.0–5.0 kg). All experimental procedures were conducted in accord with the “Guide for the Care and Use of Laboratory Animals” (7th edition, National Academy Press, Washington, DC, 1996). Surgical procedures have been described, in detail, in previous papers (Morales et al. 1987b; Xi et al. 1997). Briefly, all surgical procedures were carried out under halothane anesthesia. The left hindlimb nerves of the hamstring (including posterior biceps and semitendinosus, and anterior biceps and semimembranosus) and sciatic were excised at their distal ends and positioned on stimulating electrodes made of silver wire. The lumbosacral spinal cord was exposed by laminectomy (L₄–S₁). The dura was retracted, and the right L₇ dorsal and ventral roots were cut distally. The left dorsal roots L₅, L₆, L₇, S₁, and S₂ were excised to eliminate the possibility of disfacilitation of α motoneurons via the γ-loop. Spinal and leg pools were constructed with skin flaps and filled with warm mineral oil (37°C).

After completion of all surgical procedures, α-chloralose (60 mg/kg iv) was administered slowly over a period of 1 min while halothane was discontinued. The chloralose solution was filtered prior to use to generate a more stable anesthetized preparation (Kohlmeier et al. 1996). Supplementary doses of α-chloralose (30 mg/kg, iv) were administered periodically throughout the remainder of the experiment, to maintain the animal under a deep anesthesia. Previous studies from this laboratory (Kohlmeier et al. 1996; López-Rodríguez et al. 1995; Xi et al. 1997) have shown that the brain stem-spinal cord inhibitory system that mediates atonia during active sleep can be activated in the α-chloralose-anesthetized preparation following the injection of carbachol into the NPO. We therefore utilized the α-chloralose-anesthetized preparation to determine if the same inhibitory system could also be activated following the injection of bicuculline into the NPO.

During recording sessions, the cats were immobilized with gallamine triethiodide (Flaxedil, 1.0 mg/kg) and artificially ventilated. The level of anesthesia was ensured by checking that the pupils were constricted and that the blood pressure and heart rate were stable and did not alter in response to a paw pinch. The blood pressure and end tidal CO₂ were continuously monitored and maintained within the range of normal physiological values (100–140 mmHg for mean blood pressure and 3–5% for end tidal CO₂).

Stimulation and recording

To examine the effect of microinjections of bicuculline on the amplitude of the Iₐ-monosynaptic reflex, both right L₇ dorsal and ventral roots were excised at their exit from the dural sac and placed on bipolar silver wire electrodes. The reflex was evoked by the electrical stimulation of the right L₇ dorsal root at an intensity just suprathreshold for group I afferents; the reflex response was recorded from the right L₇ ventral root.

In experiments in which the phenomenon of reticular response-reversal (Chandler et al. 1980; Chase and Babb 1973; Chirwa et al. 1987a; Fung et al. 1982) was studied, a stainless-steel electrode was lowered into the medullary nucleus reticularis gigantocellularis (NRGc; P 9, L 1, H –8, Berman 1968) for monopolar electrical stimulation (4 pulses at 400 Hz; pulse duration, 0.8 ms; intensity: 20–120 μA). The recording sessions commenced 2 h after the cessation of halothane administration to ensure the systemic clearance of halothane (Cowles et al. 1968; Yanagida et al. 1975). Intracellular recordings were obtained from antidromically identified motoneurons using glass micropipettes filled with either 2 M K-citrate or 3 M KCl (tip resistances: 10–20 and 5–10 MΩ, respectively). The electrodes were connected to a high-input impedance preamplifier (Axoclamp 2A).

High-gain (×100) DC and low-gain (×10) DC intracellular activity as well as extracellular AC records of the Iₐ-monosynaptic reflex response, recorded from the ventral root, were displayed on an oscilloscope and stored on a video cassette recorder by means of a PCM recording adapter (Vetter, Model 4000). The data were digitized off-line at 20 kHz and analyzed with a microcomputer (Apple Power Macintosh) using specially designed software.

Drug administration

Bicuculline (bicuculline methiodide, a GABAₐ antagonist, 0.25 μl, 10 mM in saline), muscimol (muscimol hydrobromide, a GABAₐ agonist, 0.25 μl, 10 mM in saline), and carbachol (carbamylcholine chloride, 0.25 μl, 22 mM in saline) were individually injected into the NPO using a 1.0-μl Hamilton syringe with its tip positioned at the stereotaxic coordinates P 3.0, L 2.0, H-3.5 (Berman 1968). Motor inhibition following the injection of bicuculline or carbachol was determined on the basis of a decrease in the amplitude of the Iₐ-monosynaptic reflex (Morales et al. 1987b; Pereda et al. 1990).

Data analysis

The following electrophysiological properties of motoneurons were measured: resting membrane potential, amplitude of action potential, input resistance, membrane time constant, rheobase, and the amplitude and time course of the action potential’s afterhyperpolarization (AHP).

The methods used to analyze the preceding basic electrophysiological properties of motoneurons are standard procedures that we have employed and described in other studies in full detail (Engelhardt et al. 1995; Morales et al. 1987b; Soja et al. 1991; Xi et al. 1997). The following is a brief summary of these methods:

RESTING MEMBRANE POTENTIAL. The membrane potential was determined by measuring the difference between the DC potential recorded intracellularly and that recorded immediately after withdrawing the microelectrode from its intracellular position.

ACTION POTENTIAL. The amplitude of the antidromically evoked action potentials was determined by measuring the difference between the DC potential recorded at the base and the peak of the action potential.

INPUT RESISTANCE. Input resistance was calculated by the “direct” method using computer-averaged voltage responses (100 trials) to the injection of low-intensity (1–3 nA) depolarizing and/or hyperpolarizing current pulses of 50-ms duration.

MEMBRANE TIME CONSTANT. A determination of the membrane time constant was based on an analysis of the decay phase of the averaged cell membrane voltage change following a 50-ms current pulse. For cells in which the membrane potential exhibited the nonlinear behavior described by Ito and Oshima (1965), the raw voltage data were corrected to avoid underestimating the membrane time constant. This procedure involved successively “peeling” exponential terms with the longest time constant from semilog plots of V or dV/dt versus t.

RHEOBASE. Rheobase was determined as the minimum stimulus intensity of a 50-ms duration intracellular depolarizing current pulse that constantly elicited an action potential.

ACTION POTENTIAL’S AHP. The AHP was examined following action potentials elicited by passing a short (500 μs) suprathreshold current pulse through the intracellular electrode. The duration of the AHP was measured from the beginning of the current pulse to the return of the membrane potential to baseline. The amplitude of the AHP was calculated by subtracting the membrane potential value immediately preceding the initiation of the current pulse from the
value at the peak of the AHP. The half-width of the AHP was measured by determining its duration at half its amplitude. The half-decay width was defined as the time between the AHP peak and the data point on its decay phase corresponding to half its amplitude.

Experimental data values are expressed as means ± SE of measurements. The statistical level of significance of the difference between sample means was evaluated using the two-tailed, unpaired or paired Student’s t-test \( (P < 0.05) \).

**Histological procedures**

At the end of each experiment, the site of drug injection was marked with 0.5 \( \mu l \) of a 2% solution of Chicago sky blue dye in 0.5 M Na-acetate. The animal was then killed with a lethal dose of pentobarbital sodium (Nembutal) and perfused with saline followed by a solution of 10% formaldehyde. Coronal serial sections of brain stem tissue were examined to verify the site of drug injection (Fig. 1A).

**RESULTS**

**Monosynaptic reflex**

The microinjection of bicuculline into the NPO resulted in a sustained reduction in the amplitude of the Ia-monosynaptic reflex within 2–5 min (Fig. 1B). The latency to the decrease in reflex amplitude was similar to that of muscle atonia observed after the injection of bicuculline in intact, unanesthetized, chronically prepared cats \( (Xi et al. 1999, 2001) \). The suppression of the reflex usually lasted ≤ 2 h; afterward it could be re-induced by another injection of bicuculline. The mean reflex amplitude was reduced from 0.89 ± 0.09 mV during control conditions (prior to the injection of bicuculline) to 0.49 ± 0.05 mV after bicuculline administration. This 44.9% reduction in mean reflex amplitude, which was statistically significant \( (7 \text{ injections}, P < 0.05) \), was used as the criterion for the effectiveness of a bicuculline injection; for ease of communication in the present manuscript, the suppression of reflex activity will be referred to as bicuculline-induced motor inhibition.

**Basic electrophysiological properties**

Data were obtained from 93 lumbar motoneurons with stable resting membrane potentials and antidromic action potentials ≥ 65 mV. Thirty-four motoneurons were recorded only during control conditions; 37 motoneurons were recorded during bicuculline-induced motor inhibition; and 3 were recorded both before and during bicuculline-induced motor inhibition. Another 19 motoneurons were recorded in the experiments in which a combination of injections of carbachol into the NPO and the subsequent injection of muscimol into the same region of the NPO was carried out.

The action potential amplitude of motoneurons ranged from 65.4 to 90.7 mV before and from 65.0 to 90.8 mV during bicuculline-induced motor inhibition. The mean action potential amplitude of motoneurons during control conditions was -67.8 ± 1.0 mV, the corresponding value for motoneurons during bicuculline-induced motor inhibition was -70.7 ± 0.9 mV (Table 1). This hyperpolarization of 2.9 mV in mean membrane potential was statistically significant \( (P < 0.05) \) (Table 1).

Microinjections of bicuculline resulted in a sustained reduction in input resistance (Table 1). The reduction of 29.4% in the mean input resistance was statistically significant \( (P < 0.01) \). There was also a statistically significant decrease of 31.7% in the mean membrane time constant during bicuculline-induced motor inhibition \( (P < 0.01) \), Table 1).

The excitability of motoneurons was significantly reduced during bicuculline-induced motor inhibition, which is reflected by an increase in rheobase. There was a statistically significant increase (35.1%) in mean rheobase during bicuculline-induced motor inhibition \( (P < 0.01) \), Table 1).

### Table 1. Electrophysiological properties of lumbar motoneurons

<table>
<thead>
<tr>
<th>Membrane Property</th>
<th>Control</th>
<th>Bicuculline*</th>
</tr>
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<tbody>
<tr>
<td>Spike amplitude, mV</td>
<td>78.1 ± 0.8 (37)</td>
<td>78.5 ± 0.7 (39)</td>
</tr>
<tr>
<td>Membrane potential, mV†</td>
<td>-67.8 ± 1.0 (35)</td>
<td>-70.7 ± 0.8 (37)</td>
</tr>
<tr>
<td>Input resistance, MΩ‡</td>
<td>1.7 ± 0.1 (34)</td>
<td>1.2 ± 0.2 (35)</td>
</tr>
<tr>
<td>Time constant, ms†</td>
<td>6.3 ± 0.3 (34)</td>
<td>4.3 ± 0.3 (35)</td>
</tr>
<tr>
<td>Rheobase, nA‡</td>
<td>13.4 ± 1.2 (35)</td>
<td>18.1 ± 0.9 (37)</td>
</tr>
<tr>
<td>AHP amplitude, mV†</td>
<td>4.0 ± 0.4 (31)</td>
<td>3.2 ± 0.3 (34)</td>
</tr>
<tr>
<td>AHP duration, ms</td>
<td>72.1 ± 4.0 (31)</td>
<td>69.0 ± 3.2 (34)</td>
</tr>
<tr>
<td>AHP half-width, ms</td>
<td>29.0 ± 2.5 (31)</td>
<td>25.9 ± 1.9 (34)</td>
</tr>
<tr>
<td>AHP half-decay-width, ms</td>
<td>19.7 ± 1.8 (31)</td>
<td>17.2 ± 2.0 (34)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Numbers in parentheses indicate the number of motoneurons sampled. AHP, afterhyperpolarization. * These measurements were made during atonia following the injection of 0.25 μl of a solution of bicuculline (10 mM in saline) into the nucleus pontis oralis; † \( P < 0.05 \); ‡ \( P < 0.01 \).
During naturally occurring active sleep and carbachol-induced motor inhibition, large amplitude (>1 mV), repetitively occurring IPSPs bombard somatic motoneurons (Chase et al. 1989; Morales and Chase 1982; Morales et al. 1987a,b; Soja et al. 1991; Xi et al. 1997). These IPSPs are specific to this state, i.e., they are not observed during any other behavioral state. Therefore high-gain membrane potential recordings were examined in the present study to determine whether similar IPSPs were also present during bicuculline-induced motor inhibition.

Prior to the injection of bicuculline, the membrane potential of lumbar motoneurons was characterized by a mixture of small-amplitude, spontaneous depolarizing and hyperpolarizing potentials (Fig. 2A). After the administration of bicuculline, large-amplitude, repetitively occurring hyperpolarizing synaptic potentials (>1 mV) dominated the membrane potential recording (Fig. 2B). The mean frequency of hyperpolarizing synaptic potentials increased from 0.2 ± 0.5 potentials/s (n = 30 cells) to 22.6 ± 1.2 potentials/s (n = 32 cells) after bicuculline administration. The difference between these mean frequencies was statistically significant (P < 0.001).

The discrete, large-amplitude IPSPs were remarkably similar to those that appear during active sleep in the chronic cat and during carbachol-induced motor inhibition (Chase et al. 1989; Morales and Chase 1982; Morales et al. 1987a,b; Soja et al. 1991). In accord with the behavior of other IPSPs, the intracellular application of hyperpolarizing direct current (Fig. 3A) and the injection of chloride ions (Fig. 3B) reversed the polarity, which exhibited equilibrium potentials between −70 and −80 mV. These results confirm that these hyperpolarizing potentials were chloride-dependent IPSPs (Coombs et al. 1955; Morales and Chase 1982).

In two experiments, muscimol, a GABA<sub>A</sub> agonist, was microinjected into the same area of the NPO ~30 min after the first injection of bicuculline into the NPO. Injections of muscimol completely blocked the discrete, large amplitude IPSPs that had been induced following the injection of bicuculline into the NPO (12 cells).

It is well established that the pontine cholinergic system is critically involved in the generation of motor inhibition.
Following the injection of muscimol, the amplitude of the monosynaptic reflex returned to its control value (C2). This represented a 58% reduction in the amplitude. Carbachol administration to this motoneuron (B3) decreased the resting membrane potential by 0.23 mV after carbachol administration (C2). The effect of muscimol injection on carbachol-induced spontaneous IPSPs was abolished and the membrane potential of this motoneuron returned to the precarbachol control value.

The neural mechanisms underlying the phenomenon of reticular response-reversal were examined in the present study before and during bicuculline-induced motor inhibition. Prior to the injection of bicuculline, electrical stimulation of the NRGc elicited a short-latency EPSPs in a majority of motoneurons (17 of 19). An example of this excitatory potential is shown in Fig. 4A. Following the injection of bicuculline, the characteristic large-amplitude IPSP during carbachol-induced motor inhibition (peak amplitude: 2.1 ± 0.9 mV; latency to peak: 50.1 ± 2.0 ms; half-width: 22.7 ± 2.3 ms; duration: 58.2 ± 2.7 ms). The waveform parameters of the NRGc-evoked IPSPs during bicuculline-induced motor inhibition were similar to those observed during naturally-occurring active sleep and carbachol-induced motor inhibition (Fung et al. 1982; López-Rodríguez et al. 1995; Pereda et al. 1990).

Recordings of NRGc-evoked responses were also made from motoneurons following an injection of carbachol and a subsequent injection of muscimol into the NPO. Figure 5C presents records from a motoneuron demonstrating that electrical stimulation of the NRGc was capable of evoking a characteristic large-amplitude IPSP during carbachol-induced motor inhibition, as previously reported (López-Rodríguez et al. 1995).
Following the injection of bicuculline into the NPO, discrete large-amplitude (>1 mV) hyperpolarizing potentials were observed in lumbar motoneurons. These potentials, which were not present during control conditions (prior to the injection of bicuculline), were reversed in polarity both by the intracellular application of hyperpolarizing DC and by the injection of chloride ions. These potentials exhibited equilibrium potentials in the range of −70 to −80 mV. These findings indicate that these hyperpolarizing potentials are chloride-dependent IPSPs. The waveforms of these potentials are remarkably similar to those that occur in lumbar motoneurons during naturally occurring active sleep and carbachol-induced motor inhibition (Chase et al. 1989; Morales and Chase 1981; Morales et al. 1987a,b; Soja et al. 1991). These findings support the hypothesis that the IPSPs that appear during bicuculline-induced motor inhibition originate from the activation of the same group of interneurons that are responsible for the postsynaptic inhibition of lumbar motoneurons during active sleep and during carbachol-induced motor inhibition.

One interesting aspect of the neural control of motoneurons is a phenomenon called “reticular response-reversal” (Chase and Babb 1973). This phenomenon refers to the difference in motor response to activation of the brain stem reticular formation during different behavioral states. Specifically, electrical stimulation of the NPO or NRGCs during wakefulness and quiet sleep elicits EPSPs in motoneurons. The identical stimulus during active sleep elicits IPSPs (Chandler et al. 1980; Chase and Babb 1973; Chirwa et al. 1991; Fung et al. 1982). In the present study, stimulation of the NRGCs evoked a large-amplitude IPSP in lumbar motoneurons during bicuculline-induced motor inhibition, whereas the same stimulus evoked an EPSP during control conditions. An analyses of the waveform parameters of the NRGC-evoked EPSPs and IPSPs during control conditions and during bicuculline-induced motor inhibition indicated that these potentials are similar to those evoked by stimulation of the NRGCs during wakefulness and active sleep, respectively. Based on the present data, we therefore conclude that the same brain stem-spinal cord inhibitory system that is responsible for the postsynaptic inhibition of motoneurons during active sleep is also activated by the injection of bicuculline into the NPO. In other words, this system is under tonic inhibitory GABAAergic control, which can be released by the administration of bicuculline.

A number of studies have presented data indicating that the NPO of the pontine reticular formation is a key region in promoting active sleep and the accompanying pattern of so-
matomotor atonia (for review, see Jones 1991; Siegel 2000; Steriade and McCarley 1990). Anatomical evidence indicates that the NPO receives cholinergic innervation from both the laterodorsal tegmental and the pedunculopontine tegmental nuclei of the dorsolateral pons (Mitani et al. 1988; Shiromani et al. 1988). Microinjections of cholinergic agonists into the NPO reliably induce an active sleep-like state that is indistinguishable from naturally occurring active sleep (Baghdoyan et al. 1984, 1987, 1989, 1993; George et al. 1964; Yamamoto et al. 1990; Yamuy et al. 1993). In addition, a majority of the neurons in the NPO increase their discharge rate during active sleep (McCarley and Hobson 1971; McCarley et al. 1995), although many cells in this nucleus also increase their discharge rate during waking movements (Siegel et al. 1977). For these reasons, cells in the NPO are believed to act as effector neurons that are responsible for active sleep, especially for muscle atonia that is a key characteristic of this state.

On the basis of the well-established fact that bicuculline blocks synaptic transmission at GABA_A receptors, and our experiments that have shown that microinjections of bicuculline into the NPO induce a generalized inhibition of motor activity, we suggest that a GABAergic system is a key component of a “gating” mechanism within the NPO that participates in promoting somatomotor atonia during active sleep. This gating mechanism apparently operates in such a way that during wakefulness and quiet sleep the activity of GABAergic synaptic transmission in the NPO is both tonic and dominant; consequently, there is a sustained inhibition of effector neurons in the NPO. Thus motor inhibition occurs when GABAergic synaptic transmission in the NPO is suppressed, which allows the brain stem-spinal cord inhibitory system to become activated. However, additional anatomical and physiological studies are needed to determine the precise nature of GABAergic synaptic transmission in the NPO during the behavioral states of active sleep and wakefulness and to identify the GABAergic system involved.

It is interesting to note that 20–30 min after the state of motor inhibition was induced by the injection of carbachol into the NPO, the subsequent injection of muscimol into the same area resulted in the disappearance of the large-amplitude spontaneous IPSPs and a great reduction in the amplitude of NRG-evoked IPSPs. Both types of IPSPs were present only during carbachol-induced motor inhibition. At the same time, large-amplitude high-frequency EPSPs dominated the membrane potential record. The amplitude of the IA-monoamine reflex, which was reduced due to the effects of carbachol, recovered to the values that were presented before the injection of carbachol. These results highlight the importance of the interaction of the GABAergic system described in this report with the pontine cholinergic system, which has been shown to be critically involved in the generation of muscle atonia during active sleep (Baghdoyan et al. 1984, 1987, 1989, 1993; George et al. 1964; Yamamoto et al. 1990; Yamuy et al. 1993).

Why are both GABAergic and cholinergic input present in the NPO? We suggest that activation of NPO neurons, which is responsible for the generation of muscle atonia during active sleep, is modulated by both GABAergic and cholinergic systems. Specifically, we hypothesize that the excitatory cholinergic control of the activity of NPO neurons is gated by a pontine GABAergic inhibitory system. This gate mechanism may exert its effects postsynaptically, by direct GABAergic inhibitory effects on NPO neurons, and/or presynaptically, by GABAergic modulation of the release of acetylcholine from synaptic terminals.

The present data demonstrate that the effect of the microinjection of carbachol into the NPO is suppressed by a subsequent injection of muscimol into the same region, suggesting that NPO neurons are postsynaptically inhibited by GABAergic inputs. On the other hand, a recent in vivo microdialysis study has shown that the microperfusion of bicuculline into the NPO significantly increases the release of acetylcholine (Baghdoyan and Vazquez 2000), indicating that a presynaptic GABAergic mechanism may also be involved in the control of cholinergic synapses on NPO neurons.

In summary, after the injection of bicuculline into the NPO, the electrophysiological properties and synaptic activity of lumbar motoneurons exhibit changes that are comparable to those that are present during naturally occurring active sleep and carbachol-induced atonia. These data indicate that bicuculline-induced atonia is due to the postsynaptic inhibition of lumbar motoneurons. Based on the present study, we conclude that the brain stem-spinal cord inhibitory system, which is responsible for the postsynaptic inhibition of α motoneurons during active sleep, is inhibited by pontine GABAergic mechanisms during wakefulness and quiet sleep, and becomes activated during active sleep resulting in atonia of the somatic musculature during this state.

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