

Electrophysiological Properties of Lumbar Motoneurons in the α -Chloralose-Anesthetized Cat During Carbachol-Induced Motor Inhibition

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Xi, Ming-Chu, Rong-Huan Liu, Jack Yamuy, Francisco R. Morales, and Michael H. Chase. Electrophysiological properties of lumbar motoneurons in the α -chloralose-anesthetized cat during carbachol-induced motor inhibition. *J. Neurophysiol.* 78: 129–136, 1997. The present study was undertaken 1) to examine the neuronal mechanisms responsible for the inhibition of spinal cord motoneurons that occurs in α -chloralose-anesthetized cats following the microinjection of carbachol into the nucleus pontis oralis (NPO), and 2) to determine whether the inhibitory mechanisms are the same as those that are responsible for the postsynaptic inhibition of motoneurons that is present during naturally occurring active sleep. Accordingly, the basic electrophysiological properties of lumbar motoneurons were examined, with the use of intracellular recording techniques, in cats anesthetized with α -chloralose and compared with those present during naturally occurring active sleep. The intrapontine administration of carbachol resulted in a sustained reduction in the amplitude of the spinal cord Ia monosynaptic reflex. Discrete large-amplitude inhibitory postsynaptic potentials (IPSPs), which are only present during the state of active sleep in the chronic cat, were also observed in high-gain recordings from lumbar motoneurons after the injection of carbachol. During carbachol-induced motor inhibition, lumbar motoneurons exhibited a statistically significant decrease in input resistance, membrane time constant and a reduction in the amplitude of the action potential's afterhyperpolarization. In addition, there was a statistically significant increase in rheobase and in the delay between the initial-segment (IS) and somadendritic (SD) portions of the action potential (IS-SD delay). There was a significant increase in the mean motoneuron resting membrane potential (i.e., hyperpolarization). The preceding changes in the electrophysiological properties of motoneurons, as well as the development of discrete IPSPs, indicate that lumbar motoneurons are postsynaptically inhibited after the intrapontine administration of carbachol in cats that are anesthetized with α -chloralose. These changes in the electrophysiological properties of lumbar motoneurons were found to be comparable with those that take place during the atonia of active (rapid-eye-movement) sleep in chronic cats. The present results support the conclusion that the neural system that is responsible for motor inhibition during naturally occurring active sleep can also be activated in α -chloralose-anesthetized cats following the injection of carbachol into the NPO.

INTRODUCTION

One of the most striking components of active sleep is atonia of the somatic musculature that occurs as a result of glycinergically mediated postsynaptic inhibition of motoneurons (Chase and Morales 1990; Chase et al. 1989; Morales and Chase 1982; Morales et al. 1987a; Soja et al. 1991). The inhibition of motoneurons, as well as changes in various

physiological processes that arise during active sleep, can also be elicited by the microinjection of cholinergic agonists, such as carbachol, into the rostral pontine reticular formation in the region of the nucleus pontis oralis (NPO) of chronic cats (Baghdoyan et al. 1984, 1987, 1989; George et al. 1964; Mitler and Dement 1974; Yamuy et al. 1993). We have recently reported that muscular activity is suppressed after the injection of carbachol into the NPO in cats that are anesthetized with α -chloralose (López-Rodríguez et al. 1995).

The present study was undertaken to explore the mechanisms that control lumbar motoneuron activity following the microinjection of carbachol into the NPO in α -chloralose-anesthetized cats, and to compare them with the mechanisms that are responsible for the postsynaptic inhibition of lumbar motoneurons that takes place during naturally occurring active sleep. To carry out these studies, the basic electrophysiological properties of spinal cord hindlimb motoneurons in cats anesthetized with α -chloralose were examined with the use of intracellular recording techniques before and during carbachol-induced motor inhibition. Our results indicate that significant changes in the electrophysiological properties of motoneurons occur following the injection of carbachol into the NPO in this preparation and that these changes are due to the postsynaptic inhibition of motoneurons. Moreover, the inhibitory processes that arise during carbachol-induced motor suppression in cats anesthetized with α -chloralose are comparable with those that take place during active sleep in the chronic cat (Morales and Chase 1981; Soja et al. 1991).

Preliminary results of this work have been previously presented (Xi et al. 1995).

METHODS

Surgical procedures

Experiments were performed on four adult cats (3.0–5.0 kg). All surgical procedures were carried out under halothane anesthesia. The trachea was cannulated and the right carotid artery and external jugular vein were catheterized. The following left hindlimb nerves were excised at their distal ends and positioned on silver stimulating electrodes: hamstring (including posterior biceps and semitendinosus and anterior biceps and semimembranosus), gastrocnemius medialis, gastrocnemius lateralis and soleus, tibial distal to the triceps surae branches, and common peroneal. The lumbosacral spinal cord was exposed by laminectomy (L_4 – S_1). The dura was retracted and the right L_7 dorsal and ventral roots were cut distally. The left dorsal roots L_5 , L_6 , L_7 , S_1 , and S_2 were excised

to eliminate the possible 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 gradually substituted for halothane over a period of 5 min to maintain anesthesia. The chloralose solution was filtered before its use to generate a more stable anesthetized preparation (Kohlmeier et al. 1996). Supplementary doses of α -chloralose (20 mg/kg iv) were administered throughout the remainder of the experiment, as needed, to maintain the animal under a deep anesthesia. 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 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 kept at a steady level within the range of normal physiological values (100–120 mmHg for mean blood pressure and 3–5% for end-tidal CO₂). 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).

Intracellular recording and data analysis

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 with glass micropipettes filled with either 2 M potassium citrate or 3 M KCl (tip resistances 10–20 and 5–10 M Ω , respectively). The electrodes were connected to a high-input impedance preamplifier (Axoclamp 2A). High-gain ($\times 100$) DC and low-gain ($\times 10$) 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 (Macintosh IIci) with the use of specially designed software.

The following electrophysiological properties of motoneurons were measured: resting membrane potential, amplitude of action potential, maximum slope of the initial-segment (IS) and somadendritic (SD) spikes, IS-SD delay, 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 (Morales et al. 1987b; Soja et al. 1991). The following is a brief summary of these methods: the resting membrane potential value was determined by measuring the difference between the DC potential recorded intracellularly and that recorded immediately after withdrawing the microelectrode from its intracellular position. Antidromically evoked action potentials were used to measure spike amplitude. The peaks of the first derivative values of the digitized potentials were employed to measure the maximum slopes of the IS and SD spikes. The IS-SD delay was defined as the elapsed time from the first to the second peak of the first derivative of the action potential. Rheobase was determined as the minimum stimulus intensity of a 50-ms intracellular depolarizing current pulse that constantly elicited an action potential. Input resistance was calculated by the "direct" method with the use of computer-averaged voltage responses (100 trials) to the injection of low-intensity (1–3 nA) depolarizing and/or hyperpolarizing current pulses 50 ms in duration (Barret and Crill 1974; Fleshman et al. 1981). 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 correction was made by applying the exponential model proposed by Ito and Oshima in accord with the methodology of Zengel et al. (1985). This procedure involved successively "peeling" exponential terms with the longest time constant from semilog plots of V or dV/dt versus t . The AHP was examined following direct action potentials elicited by passing a short (500- μ s) 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 AHP was measured by determining its duration at half its amplitude. The half-decay width was determined 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 \pm SE of measurements. The statistical level of significance of the difference between sample means was evaluated with the use of the one-tailed Student's t -test ($P < 0.05$). A one-tailed analysis was used because our hypothesis in the present study was that the inhibitory mechanisms responsible for the motor inhibition that occurs in α -chloralose cats following the injection of carbachol would alter the electrical properties of motoneurons in a manner comparable with that observed during naturally occurring active sleep.

Carbachol administration

Carbachol (0.25–0.5 μ l of a 4-mg/ml solution in saline) was injected into the NPO with the use of a 1.0- μ l Hamilton syringe with its tip positioned at P 3.0, L 2.0, H –3.5 (Berman 1968). Motor inhibition following the injection of carbachol was monitored as a decrease in the amplitude of the Ia monosynaptic reflex (Morales et al. 1987b; Pereda et al. 1990), as recorded from the right L₇ ventral root, which was placed on a bipolar silver recording electrode. The reflex was evoked by electrical stimulation of the right L₇ dorsal root at an intensity just suprathreshold for group I afferents.

Histological procedures

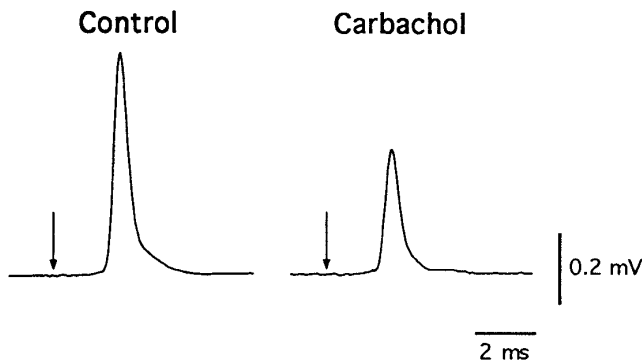
At the end of each experiment, the site of carbachol injection was marked with 0.5 μ l of a 2% solution of Chicago sky blue dye in 0.5 M sodium acetate. The animal was then killed with a lethal dose of Nembutal and perfused with saline followed by a solution of 10% formaldehyde. Coronal serial sections of brain stem tissue were examined to verify the site of carbachol injection.

RESULTS

Monosynaptic reflex

The administration of carbachol into the NPO resulted in a sustained reduction, within 2–6 min, in the amplitude of the Ia monosynaptic reflex (Fig. 1). The suppression of the reflex usually lasted for 2–4 h. The mean reflex amplitude was reduced from 0.79 ± 0.10 (SE) mV during control conditions (before the injection of carbachol) to 0.38 ± 0.04 mV after carbachol administration. This reduction of 51.9% was statistically significant (6 injections, $P < 0.01$). The decrease in the amplitude of the Ia monosynaptic reflex that

A Individual Reflexes



B Mean Amplitude of Reflexes

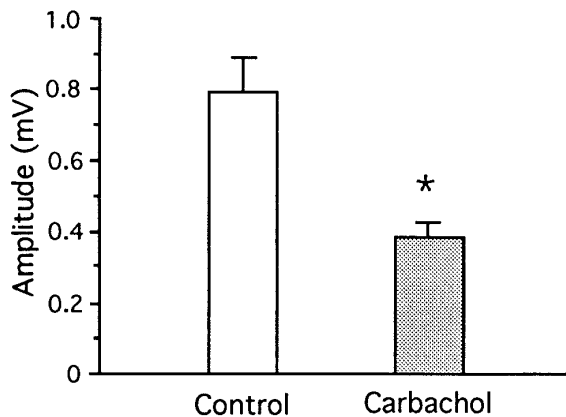


FIG. 1. Suppression of the Ia monosynaptic reflex following injection of carbachol into the nucleus pontis oralis (NPO). *A*: individual reflexes elicited by electrical stimulation of the L_7 dorsal root (\downarrow) before and after carbachol injection. Each trace is an average of 20 trials. *B*: mean amplitude of Ia monosynaptic reflexes recorded before and during carbachol-induced motor inhibition. Brackets: mean \pm SE for each population. Asterisk above bar: statistically significant decrease in mean amplitude of Ia monosynaptic reflex during carbachol-induced motor inhibition.

occurred following the carbachol administration was used as the criterion for the effectiveness of the "carbachol effect"; for the ease of communication in the ensuing presentation, the suppression of reflex activity will be referred to as carbachol-induced motor inhibition or suppression (Morales et al. 1987b; Pereda et al. 1990).

Spontaneous synaptic activity of lumbar motoneurons

Data were obtained from 95 lumbar motoneurons with antidromic action potentials ≥ 65 mV and stable resting potentials. Fifty-five motoneurons were recorded only during control conditions, 37 were recorded during carbachol-induced motor inhibition, and 3 were recorded both before and during carbachol-induced motor inhibition.

During naturally occurring active sleep, large-amplitude (>1 mV) inhibitory postsynaptic potentials (IPSPs) bombard motoneurons (Chase et al. 1989; Morales and Chase 1982; Morales et al. 1987a; Soja et al. 1991). These IPSPs are specific to this state, i.e., they are not observed during any

other behavioral states (Morales and Chase 1982; Morales et al. 1987a). Therefore high-gain membrane potential recordings were examined in the present study to determine whether similar IPSPs could also be recorded during carbachol-induced motor inhibition in α -chloralose-anesthetized cats.

Before the injection of carbachol, the membrane potential of lumbar motoneurons was characterized by a mixture of small-amplitude spontaneous depolarizing and hyperpolarizing potentials (Fig. 2*A*). After the administration of carbachol, large-amplitude hyperpolarizing synaptic potentials (>1 mV) dominated the membrane potential recording (Fig. 2*B*). The mean frequency of hyperpolarizing synaptic potentials increased from 0.2 potentials per second (40 motoneurons) to 24.3 potentials per second (30 motoneurons) after carbachol administration. The difference between these means was statistically significant ($P < 0.001$). The effects of intracellular injection of chloride ions on these hyperpolarizing potentials were examined in four motoneurons. The polarity of the hyperpolarizing potentials was reversed after the injection of chloride ions, which confirms that these hyperpolarizing potentials were chloride-dependent IPSPs (Coombs et al. 1955; Morales and Chase 1982). These discrete, large-amplitude IPSPs were remarkably similar to those that appear during active sleep in the chronic cat (see Fig. 2*A* in Chase et al. 1989; Fig. 2*A* in Morales and Chase 1982; Fig. 1 in Morales et al. 1987a).

Basic electrophysiological properties

ACTION POTENTIAL AND RESTING MEMBRANE POTENTIAL. The action potential amplitude of motoneurons ranged from 67.4 to 91.7 mV before and from 68.9 to 90.8 mV during

A Control



B Carbachol

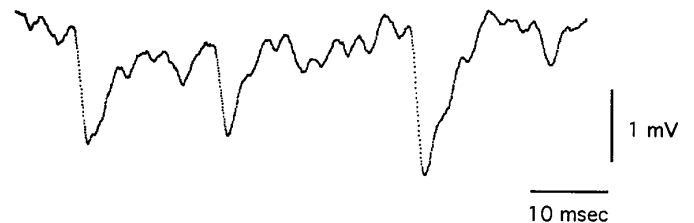


FIG. 2. Membrane potential activity of lumbar motoneurons before and during carbachol-induced motor inhibition. High-gain intracellular records from a single lumbar motoneuron in an α -chloralose-anesthetized cat before (*A*, resting membrane potential -66 mV) and 3 min after injection of carbachol (*B*, resting membrane potential -70 mV). These recordings were obtained with the use of a potassium-citrate-filled microelectrode. Note appearance of discrete, large-amplitude (>1 mV) inhibitory postsynaptic potential (IPSP) following carbachol injection.

carbachol-induced motor inhibition. The mean action potential amplitude of the population of lumbar motoneurons recorded during carbachol-induced motor inhibition was almost identical to that of neurons sampled during control conditions (79.9 ± 0.8 vs. 79.8 ± 0.8 mV, respectively).

The spike potential configuration was studied in motoneurons recorded before and during carbachol-induced motor inhibition. Spikes of motoneurons in both groups revealed two components, i.e., an IS component and a SD component. The maximum slopes of the IS and SD spikes during control conditions and during motor inhibition induced by carbachol were similar (Table 1). The mean IS-SD delay, however, was significantly longer in motoneurons recorded during carbachol-induced motor inhibition ($P < 0.01$, Fig. 3 and Table 1).

Lumbar motoneurons were hyperpolarized during carbachol-induced motor inhibition. The mean resting membrane potential of motoneurons recorded during control conditions was -69.7 ± 0.9 mV; the corresponding value for motoneurons recorded during carbachol-induced motor inhibition was -72.3 ± 0.9 mV. This hyperpolarization of 2.6 mV in mean membrane potential, albeit small, was statistically significant ($P < 0.05$).

INPUT RESISTANCE. The microinjection of carbachol resulted in a sustained reduction in input resistance. Figure 4, B and C, displays sample records from the same motoneuron, showing the voltage response to the injection of 1-nA depolarizing and hyperpolarizing current pulses before and during carbachol-induced motor inhibition. In this particular motoneuron, the input resistance was reduced from 1.9 M Ω before to 1.3 M Ω during carbachol-induced motor inhibition. Overall, the mean input resistance of motoneurons decreased from a control value of 1.8 ± 0.1 M Ω to 1.2 ± 0.1 M Ω (Fig. 4A and Table 1). This reduction of 33.3% was statistically significant ($P < 0.01$).

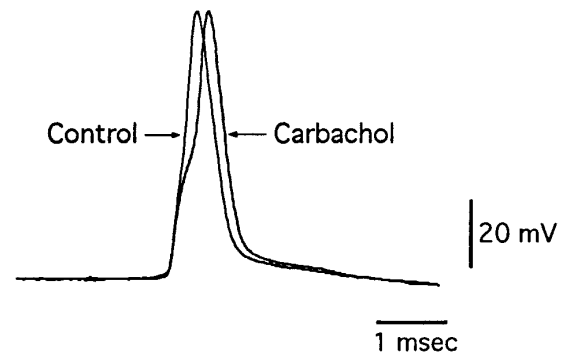
MEMBRANE TIME CONSTANT. There was a significant modification of membrane time constant during motor inhibition induced by carbachol injection. The distributions of mem-

TABLE 1. Electrophysiological properties of lumbar motoneurons

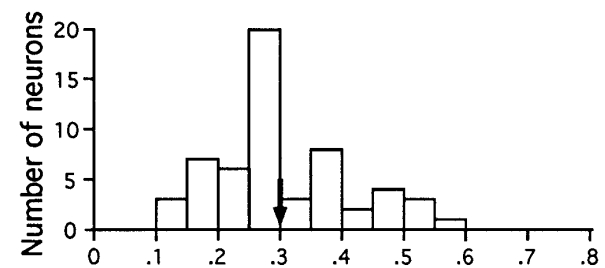
Membrane Property	Control	Carbachol
Spike amplitude, mV	79.8 ± 0.8 (58)	79.9 ± 0.8 (40)
IS spike maximum slope, mV/ms	144.6 ± 3.6 (57)	139.2 ± 4.2 (40)
SD spike maximum slope, mV/ms	291.6 ± 8.6 (57)	284.0 ± 8.0 (40)
IS-SD delay, ms*	0.30 ± 0.01 (57)	0.36 ± 0.02 (40)
Membrane potential, mV†	-69.7 ± 0.9 (46)	-72.3 ± 0.9 (37)
Input resistance, M Ω *	1.8 ± 0.1 (53)	1.2 ± 0.1 (32)
Time constant, ms*	6.0 ± 0.2 (53)	4.2 ± 0.2 (34)
Rheobase, nA*	12.9 ± 1.1 (51)	17.7 ± 1.3 (34)
AHP amplitude, mV†	4.1 ± 0.3 (46)	3.3 ± 0.2 (26)
AHP duration, ms	75.0 ± 3.2 (46)	69.3 ± 4.0 (26)
AHP half-width, ms	29.9 ± 2.2 (46)	25.4 ± 1.8 (26)
AHP half-decay width, ms	20.8 ± 1.7 (46)	17.4 ± 1.4 (26)

Values are means \pm SE, with number of motoneurons sampled in parentheses. IS, initial-segment; SD, somadendritic; AHP, afterhyperpolarization. Carbachol measurements were made during atonia following the injection of 0.25–0.5 μ l solution of carbachol (4 mg/ml in saline) into the nucleus pontis oralis. * $P < 0.01$. † $P < 0.05$.

A Antidromic Spikes



B Control



C Carbachol

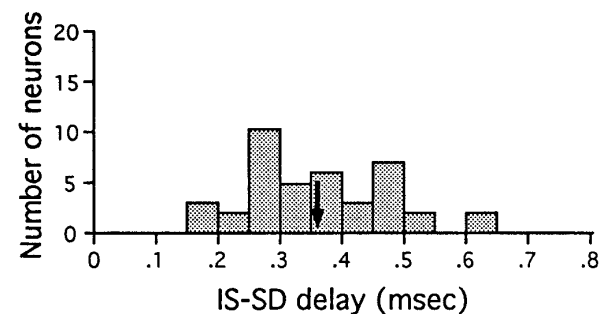


FIG. 3. Increase in initial-segment (IS)-somadendritic (SD) delay during carbachol-induced motor inhibition. A: antidromic action potentials of same motoneuron during control conditions and during carbachol-induced motor inhibition. Note marked increase in IS-SD delay during carbachol-induced motor inhibition. B and C: frequency distributions of IS-SD delay before (open histogram) and during carbachol-induced motor inhibition (shaded histogram). Arrows: mean value of each population.

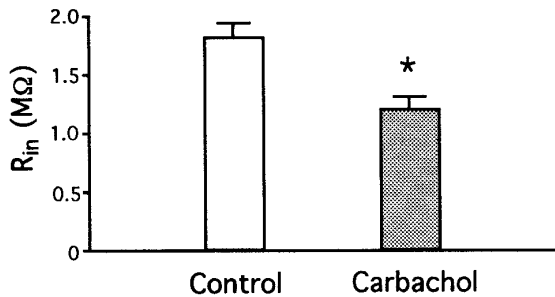
brane time constants obtained from motoneurons during control conditions and during carbachol-induced motor inhibition are presented in Fig. 5. It can be seen that the distribution of the membrane time constants from motoneurons recorded during carbachol-induced motor inhibition was shifted to the left relative to that of motoneurons recorded before the injection of carbachol. There was a significant decrease of 30.0% in the mean membrane time constant from 6.0 ± 0.2 ms before to 4.2 ± 0.2 ms during carbachol-induced motor inhibition ($P < 0.01$).

MOTONEURON EXCITABILITY. The excitability of motoneurons was significantly reduced during carbachol-induced motor inhibition, which is reflected by an increase in rheobase,

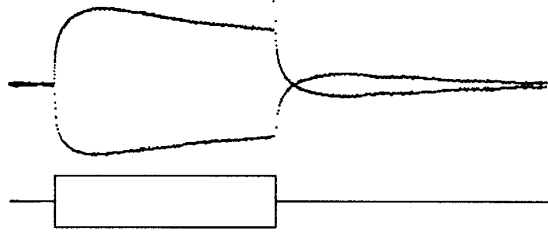
as illustrated in a motoneuron recorded both before and during carbachol-induced motor inhibition (Fig. 6A). There was a statistically significant increase (37.2%) in mean rheobase from 12.9 ± 1.1 nA before to 17.9 ± 1.3 nA during carbachol-induced motor inhibition ($P < 0.01$, Fig. 6B and Table 1).

Threshold depolarization was calculated as the product of rheobase and input resistance for each motoneuron to determine whether the increase in rheobase reflected a change in voltage threshold. The mean threshold depolarization was 19.1 ± 1.4 mV for motoneurons recorded during control conditions ($n = 48$); it was 18.8 ± 1.5 mV for motoneurons during carbachol-induced motor inhibition ($n = 30$). These values were not significantly different, which indicates that the increase in rheobase was not due to changes in voltage threshold, but occurred as a result of the increase in membrane conductance of motoneurons during carbachol-induced motor inhibition. Because motoneuron

A Mean Input Resistance



B Control



C Carbachol

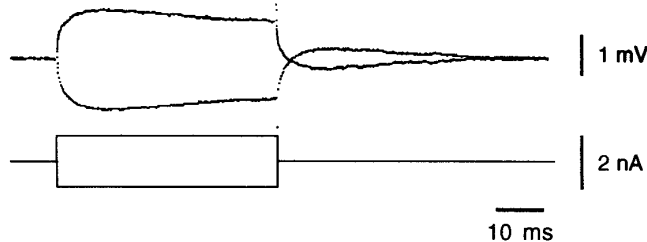
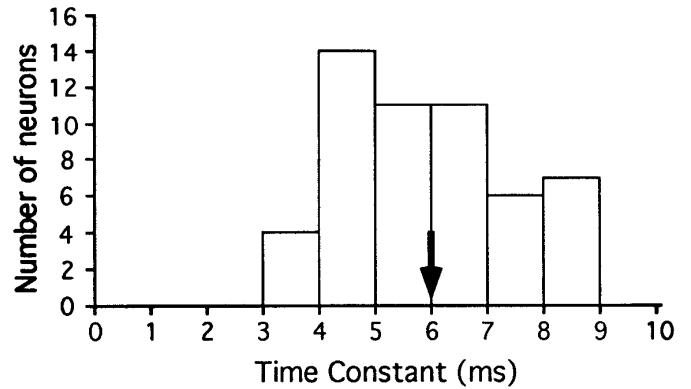


FIG. 4. Decrease in input resistance during carbachol-induced motor inhibition. *A*: mean input resistance of motoneurons. Brackets: mean \pm SE for each population. *B* and *C*: averaged membrane potential responses (*top traces*) to 50-ms current pulses of ± 1 nA (*bottom traces*) in the same lumbar motoneuron before (*B*) and during carbachol-induced inhibition (*C*). Each trace is an average of 100 trials.

A Control



B Carbachol

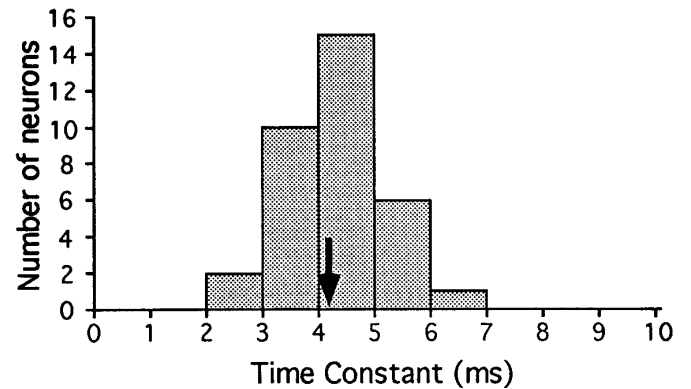


FIG. 5. Frequency distribution of membrane time constant of motoneurons recorded before (*A*) and during carbachol-induced motor inhibition (*B*). Arrows: mean value of 6.0 ms before and 4.2 ms during carbachol-induced motor inhibition.

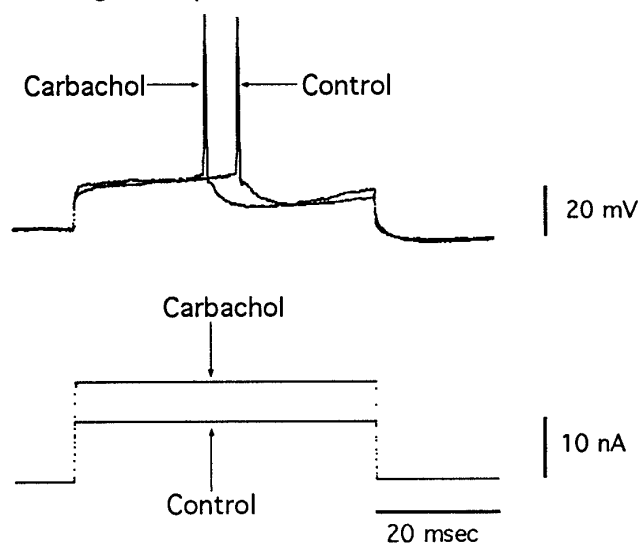
membrane current flow is “shunted” by an increase in membrane conductance, additional depolarizing current would be needed to evoke an action potential, given that the threshold depolarization was constant.

AHP. The amplitude, duration, half-width, and half-decay width of the AHP were measured in motoneurons before and during carbachol-induced motor inhibition. There was a statistically significant decrease of 19.5% in the mean amplitude of the AHP from 4.1 ± 0.3 to 3.3 ± 0.2 mV during carbachol-induced motor inhibition ($P < 0.05$, Table 1). The mean values of duration, half-width, and half-decay width of the AHP were not statistically significantly reduced during carbachol-induced motor inhibition (Table 1).

DISCUSSION

The present results indicate that during motor inhibition produced by the pontine injection of carbachol in cats anesthetized with α -chloralose, large-amplitude IPSPs arise in motoneurons and they are associated with a significant decrease in motoneuron input resistance and membrane time

A Voltage Responses



B Mean Rheobase

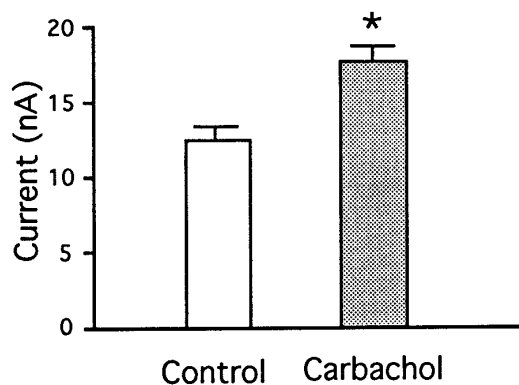


FIG. 6. Decrease in motoneuron excitability during carbachol-induced motor inhibition. *A*: voltage responses and action potentials (*top traces*) elicited by corresponding intracellular current pulses (*bottom traces*) in a single motoneuron recorded before and during carbachol-induced motor inhibition. Magnitude of current injection that consistently elicited an action potential was defined as rheobase value. Rheobase increased in this motoneuron from 10.0 nA before to 16.0 nA during carbachol-induced motor inhibition. *B*: bars represent mean rheobase before and during carbachol-induced motor inhibition. Brackets: mean \pm SE for each population.

constant. These data are consistent with the assumption that these IPSPs increase the membrane conductance to ions with an equilibrium potential more negative than that of the resting membrane potential (Araki et al. 1961; Carlen and Durand 1981; Carlen et al. 1980; Coombs et al. 1955; Smith et al. 1967). The increase in membrane conductance was also associated with an increase in rheobase, because larger-magnitude currents were required to bring the reduced input resistance of the motoneurons to firing threshold. Finally, the increase in IS-SD delay also provides indirect evidence that there is an increase in conductance of the soma membrane on the basis of the IS current being partially shunted before reaching the excitable portion of the SD membrane, which is the basis for postsynaptic inhibition (Brock et al. 1953; Llinas and Terzuolo 1964).

In chronic cats during naturally occurring active sleep, the resting membrane potential of lumbar motoneurons is hyperpolarized compared with quiet sleep or wakefulness (Chase and Morales 1983; Morales and Chase 1978; Soja et al. 1991). In the present study, the mean resting membrane potential of the motoneurons recorded during carbachol-induced motor inhibition was also hyperpolarized relative to that during control condition, but the level of hyperpolarization was not as great as that in the chronic preparation (Morales and Chase 1978). The smaller level of hyperpolarization of the resting membrane potential recorded in the α -chloralose-anesthetized cat in the present study might be due to the fact that the resting membrane potential of motoneurons during the control condition was already hyperpolarized (i.e., from -4.7 to -12.3 mV) compared with that of motoneurons recorded in chronic cats during quiet sleep (see Morales and Chase 1978; Soja et al. 1991). In fact, Nicoll and colleagues (Nicoll and Madison 1982; Nicoll and Wojtowicz 1980) reported that the resting membrane potential of motoneurons is hyperpolarized in α -chloralose-anesthetized frogs. After the injection of carbachol, even though the resting membrane potential of lumbar motoneurons was hyperpolarized the active-sleep-like IPSPs were remarkably evident.

There was a statistically significant decrease in the mean input resistance during carbachol-induced motor inhibition. This change, both in direction and magnitude, was comparable with the 44% decrease in the mean value of this membrane property during naturally occurring active sleep (Morales and Chase 1981; Soja et al. 1991) and with the 41% decrease during the carbachol-induced state of atonia in the decerebrate cat (Morales et al. 1987b).

A significant decrease in the mean membrane time constant was observed in the present study. A similar but larger decrease (52%) in the mean membrane time constant was observed during active sleep in the chronic preparation compared with quiet sleep (Soja et al. 1991). This finding can be understood on the following basis. The membrane time constant is directly proportional to the product of the specific membrane resistance and the membrane capacitance (Rall 1977). Because the membrane capacitance could be assumed to be constant in the present experiment, any change in the membrane time constant may be taken as reflecting a change in the specific membrane resistance. The input resistance of a cell is directly proportional to the specific membrane resistance (Burke 1981). The 30.0% decrease in membrane time constant and 33.3% decrease in input resistance recorded in the motoneurons in the present study are both consistent with a decrease in specific membrane resistance. The decrease in both input resistance and membrane time constant indicates that there was an increase in membrane conductance. Thus there is convincing evidence indicating that lumbar motoneurons are postsynaptically inhibited in the α -chloralose cat following the injection of carbachol in a manner that is similar to the postsynaptic inhibition of motoneurons that is present during spontaneously occurring active sleep.

A reduction in the amplitude of the AHP has been observed in lumbar motoneurons (Glenn and Dement 1981; Soja et al. 1991) and in trigeminal jaw-closer motoneurons (Chandler et al. 1980) of chronic cats during active sleep.

In the present study, there was a significant reduction in the AHP amplitude after the administration of carbachol. The decrease in the AHP amplitude can be accounted for by the decrease in input resistance and the tonic hyperpolarization of the resting membrane potential of motoneurons during carbachol-induced motor inhibition.

It is possible that changes in membrane properties (such as rheobase, input resistance, and membrane time constant) and tonic hyperpolarization reflect two processes: postsynaptic inhibition and disfacilitation. In theory, either of two different mechanisms can produce disfacilitation of spinal cord motoneuron vis-à-vis the present results. First, disfacilitation may occur as the result of a cessation of Ia primary afferent fibers discharge because of the suppression of γ -motoneuron activity during active sleep and carbachol-induced atonia (Kubota et al. 1967; Morales et al. 1987b). However, disfacilitation by this pathway can be excluded in the present study because the ipsilateral dorsal roots of L₅–S₂ were excised and the animal was immobilized with Flaxedil during the recording session. Thus disfacilitation via the cessation of afferent fibers activity is not possible. Second, disfacilitation may occur as the result of the withdrawal of tonic monoaminergic excitatory drive from supraspinal origins, such as the raphe nuclei or the nucleus locus coeruleus. Neurons in these nuclei fire tonically during wakefulness, reduce their discharge rate during quiet sleep, and cease discharging during active sleep (Hobson et al. 1975; see review by Jacobs and Azmitia 1992).

Pharmacological experiments have indicated the possibility that the suppression of hypoglossal motoneurons during carbachol-induced atonia is due to disfacilitation of serotonergic drive, rather than to glycine- or γ -aminobutyric acid-mediated postsynaptic inhibition (Kubin et al. 1992, 1993). Our previous study, however, indicated that during active sleep, tonic hyperpolarization as well as changes in electrophysiological properties of spinal cord lumbar motoneurons are due to glycinergic postsynaptic inhibition, because strychnine suppressed the tonic hyperpolarization and abolished the changes in electrophysiological properties of these motoneurons (Soja et al. 1991). Because the membrane potential activity and electrophysiological properties of lumbar motoneurons after the injection of carbachol in the present study exhibited changes comparable with those that arise during naturally occurring active sleep (Morales and Chase 1981, 1982; Soja et al. 1991), we conclude that the changes in electrophysiological properties observed in the present study are due to postsynaptic inhibition.

On the basis of the following evidence, we propose that the pontine injection of carbachol in α -chloralose-anesthetized cats activates the neural system that is responsible for the postsynaptic inhibition of lumbar motoneurons during active sleep. First, the injection of carbachol into the NPO, which is effective in the induction of an active-sleep-like state in chronic cats, produced general motor inhibition in α -chloralose cats (see López-Rodríguez et al. 1995 for the behavior of electroencephalogram, electrooculogram, and electromyogram in this preparation). Second, discrete, large-amplitude IPSPs, which were only present during active sleep in the chronic cat, were also evident in both lumbar motoneurons (present communication) and trigeminal motoneurons (Kohlmeier et al. 1996) following the injection of

carbachol in α -chloralose cats. Third, the changes in the electrophysiological properties of motoneurons following the injection of carbachol in the α -chloralose cat were comparable with those that occur during active sleep. Fourth, stimulation of the nucleus reticularis gigantocellularis elicited large-amplitude IPSPs after, but not before, the injection of carbachol in α -chloralose cats (López-Rodríguez et al. 1995; M.-C. Xi, R.-H. Liu, and M. H. Chase, unpublished data). These potentials were identical to those that are present in lumbar motoneurons following stimulation of the nucleus reticularis gigantocellularis during active sleep. Fifth, the occurrence of IPSPs in masseter motoneurons that are temporally correlated with pontogeniculooccipital waves during active sleep was also observed in this preparation after the injection of carbachol (Kohlmeier et al. 1996).

We therefore believe that the α -chloralose-anesthetized preparation can be employed to explore the neuronal circuitry involved in the generation of the postsynaptic inhibition of lumbar motoneurons during active sleep. It is a very useful preparation insofar as manipulative procedures can be carried out that would be difficult or impossible in the chronic intact unanesthetized animals, such as long-term intracellular recording, recording simultaneously from pairs of neurons for spike triggered averaging, etc. Additionally, the anesthetized preparation allows one to increase the number of intracellular recordings that can be obtained per animal. Unlike the acute decerebrate cat, this preparation may be used to investigate interactions between structures rostral to the midbrain and those in the brain stem and spinal cord. However, it should be pointed out that there are physiological differences between sleep and the anesthetized state. Therefore results obtained with the use of the α -chloralose-anesthetized preparation should be confirmed in chronic intact, unanesthetized animals.

In summary, the present results indicate that after the injection of carbachol into the NPO in cats anesthetized with α -chloralose, the membrane potential activity and electrophysiological properties of lumbar motoneurons exhibited changes that are comparable with those present during naturally occurring active sleep compared with quiet sleep or wakefulness (Morales and Chase 1981; Soja et al. 1991). On the basis of the present and previous studies (Kohlmeier et al. 1996; López-Rodríguez et al. 1995), we therefore conclude that the brain stem–spinal cord inhibitory system, which is responsible for the postsynaptic inhibition of α -motoneurons during active sleep, is capable of being activated in this anesthetized preparation.

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