Synaptic Strength Between Motoneurons and Terminals of the Dorsolateral Funiculus Is Regulated by GABA Receptors in the Turtle Spinal Cord

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Submitted 12 June 2003; accepted in final form 20 September 2003

INTRODUCTION

Gamma-aminobutyric acid (GABA) is the most important inhibitory neurotransmitter in the central and peripheral nervous system (Princivalle et al. 2001; Sivilotti and Nistri 1991). GABA receptors are of three types: GABA_A, GABA_B, and GABA_C. With the exception of GABA_C receptors, which are mainly located in the retina, these receptors are found in the entire nervous system at the postsynaptic level (Princivalle et al. 2001; Sivilotti and Nistri 1991; Towers et al. 2000). In the spinal cord, presynaptic inhibition of afferent fibers occurs via activation of both ionotropic GABA_A and metabotropic GABA_B receptors (Rudomin and Schmidt 1999). Immunocytological studies have shown the existence of GABA_A and GABA_B receptors on transmitters and terminals synaptic on them (Alvarez et al. 1996; Bohlhalter 1996; Magul et al. 1987; Sivilotti and Nistri 1991; Towers et al. 2000; Yang et al. 2001). In neonatal spinal cord, one action potential elicited in one interneuron evoked a unitary compound inhibitory postsynaptic current (IPSC) on motoneurons, mediated by activation of GABA_A and glycine receptors. In the same preparation, a subset of miniature synaptic currents was mediated by activation of GABA_A and glycine receptors (Jonas et al. 1998). Few electrophysiological studies deal with the role of synaptic GABAB receptors (Jonas et al. 1998). In bullfrogs, monosynaptic excitatory postsynaptic potentials (EPSPs) evoked on motoneurons by stimulation of a single muscle afferent in the presence of muscimol were depressed by activation of pre- and postsynaptic GABA_A receptors (Peng and Frank 1989). In the same preparation, activation of presynaptic GABA_B receptors by baclofen inhibited the monosynaptic EPSPs (Peng and Frank 1989). The same action of baclofen has been shown in synapses between Ia afferents and motoneurons (Curtis and Lacey 1998; Edwards et al. 1989; Jiménez et al. 1991). Nevertheless, baclofen depressed monosynaptic motoneuron EPSPs, evoked by stimulation of the ventromedial funiculus, which contains vestibulo- and reticulospinal fibers, by activation of presynaptic GABA_B receptors (Jiménez et al. 1991). No information is available regarding modulation of the dorsolateral funiculus (DLF)-motoneuron synapses by GABA receptors. DLF contains rubro- and propriospinal fibers and evokes mainly EPSPs in flexor motoneurons and IPSPs and/or EPSPs in extensor motoneurons in cats (Kostyuk et al. 1971). In the present study, we have investigated the modulation of synaptic efficacy between terminals of the DLF and motoneurons. We found that this synapse was modulated by activation of postsynaptic muscimol-sensitive receptors and presynaptic baclofen-sensitive receptors.
METHODS

Preparation

Adult turtles 15–20 cm long (Kinosternon) were anesthetized with intraperitoneal injection of pentobarbital sodium (100 mg/kg) and perfused with a cold Ringer solution containing (in mM) 120 NaCl, 5 KCl, 15 NaHCO3, 2 MgCl2, 3 CaCl2, and 20 glucose, saturated with 98% O2, 2% CO2, to obtain a pH of 7.5. A laminectomy was made to isolate the lumbar enlargement. Turtles were subsequently killed by decapitation. Transverse slices of 2–3 mm were cut from the lumbar enlargement. Slices were placed in a recording chamber bathed with Ringer solution at room temperature (20–22°C). All experiments were carried out with the approval of the Cinvestav Experimental Ethics Committee and in accordance with the current Mexican norm for care and use of animal for scientific purposes.

Stimulation of DLF and recording of motoneurons

Intracellular recordings of motoneurons were performed in bridge mode (Axoclamp-2B, Axon Instruments) with a sharp electrodes filled with 1 M potassium acetate (50–60 MΩ). A neuron was classified as a motoneuron according to the criteria described by Hounsgaard et al. (1988): the input resistance, the time constant, the configuration of action potential, and the firing pattern during a depolarizing current pulse were determined for each neuron recorded. Only motoneurons with resting membrane potential more negative than −65 mV and action potential of >80 mV were studied. When voltage excursions were >10 mV, a sag in voltage response during hyperpolarizing current pulses appeared followed by an overshoot at the end of the pulse (Fig. 1D). This is a Cs+-sensitive time-dependent inward rectification (Hounsgaard et al. 1988). This rectification did not affect the results because the control and the drug action were recorded with the same electrode. The input resistance was obtained as the slope of the line fit to the I-V plot built with voltage responses <10 mV (Fig. 1D).

Recordings were digitized by Digidata 1322A (Axon Instruments), visualized in a computer with the Axoscope program (Axon Instruments) and stored in the hard disk for off-line analysis. All the average parameter values are given with the SE. To stimulate the DLF (Fig. 1A), a pair of wire stimulus electrodes was placed on the dorsolateral portion of the spinal cord slice and rectangular pulses (500 μs duration, 0.3–1 Hz) were applied. The threshold (T; 5–10 μA) of the DLF stimulation was determined as the stimulus intensity at which a presynaptic action of muscimol and baclofen.

Histology

At the end of the electrophysiological study, neurons were filled with biocytin (2% in wt) by action of depolarizing current pulses (500 ms, 0.5 nA at 1 Hz for 30 min). After biocytin injection, slices were transferred to a buffer with sucrose (30%). For histochemistry, serial transverse sections (60-μm thick) were cut in a cryostate (Leica, CM 1850) and rinsed in PBS. Slices were first incubated in a hydrogen peroxide solution (1%) in PBS for 30 min to inactivate endogenous peroxides and then incubated in an avidin-peroxidase complex (Vector ABC kit) diluted in PBS and 0.3% Triton X-100 for 2 h. After rinsing in PBS, slices were reacted in diaminobenzidine (10 mg/25 ml 10 mM PBS) and hydrogen peroxide (0.001%, Baker), and nickel sulfate (30%, Sigma).

RESULTS

Motoneuron classification

To determine if the recorded cell was a motoneuron, we searched for distinctive motoneuron properties such as the configuration of the action potential and the subthreshold responses (Hounsgaard et al. 1988). The input resistance and the time constant of the ventral horn neurons recorded were 28.3 ± 3.9 (SE) MΩ (n = 17; 18–55 MΩ) (Hounsgaard et al. 1988) and 36.2 ± 4.1 ms (n = 17; 12–61 ms) (Hounsgaard et al. 1988), respectively. Action potentials had a rise time of 0.52 ± 0.02 ms (n = 20; 0.4–0.5 ms) (Hounsgaard et al. 1988) and a duration of 0.97 ± 0.03 ms (n = 20; 0.9–1.2 ms) (Hounsgaard et al. 1988) as measured 10 mV above threshold. The spike amplitude was 93.5 ± 8.4 mV (n = 20; 95–100.5 mV) (Hounsgaard et al. 1988). All cells included in this study had the typical firing pattern of motoneurons (Hounsgaard et al. 1988). Furthermore, in some of these cells (n = 3) bistability (Hounsgaard and Kiehn 1985) was induced when 5-HT (10 μM) was added to the bath solution (not shown). The morphology of a typical motoneuron filled with biocytin is illustrated in a transverse section of the spinal cord in Fig. 1A. The dimension of the soma and the distribution of the dendritic tree coincided with that reported by others (Hounsgaard et al. 1988; Ruigrok et al. 1985).

Time course of muscimol effect on the motoneuron membrane resistance and on EPSPs

The time course of muscimol effect on the membrane resistance, the amplitude and the half-width of the EPSPs was evaluated recording in the same trace the voltage response and the EPSP elicited by DLF stimulation (2T) every 3 s in Ringer solution and in the presence of muscimol (5 μM). In Fig. 1B, the average of five consecutive traces were shown in each row,
FIG. 1. Histology of a motoneuron and time course of muscimol effect. A: photomicrograph of the transverse section of the spinal cord showing a motoneuron stained with biocytin in the ventral horn, which is delineated with a dashed line. The elliptic area indicates the area electrically stimulated [dorsolateral funiculus (DLF)]. To know the time course of the muscimol effect on the membrane resistance, the amplitude and the half-width of the excitatory postsynaptic potential (EPSP) voltage response to an intracellular hyperpolarizing current (−0.3 nA) and the EPSPs elicited by the DLF stimulation every 3 s were recorded. B: top to bottom, the averages of 5 consecutive sweeps are shown. The column number represents the time elapsed after 5 consecutive sweeps. The 1st trace is the control response (0 s). The next traces were the averages in the presence of muscimol (15–135 s; 5 μM). The input resistance calculated from the average voltage response traces, and values of the amplitude and the half-width of the EPSPs obtained from B, were normalized and plotted against the time (C). Observe that the normalized quantities decreased at the same rate. When the input resistance reached the stable value, both the amplitude and the half-width were also stable. D: the membrane resistance calculated as the slope of the best linear fit to the I-V plot was 14.7 MΩ in control medium (●) and 4.4 MΩ (○) in muscimol. Voltage responses plotted were <10 mV in amplitude. E: the membrane potential was continuously recorded, and the input resistance was monitored by means of the voltage response (bottom) to an intracellular current pulse of −0.3 nA (top). Vertical arrows indicate times at which muscimol was added to the bath solution (left ‡) and at which input resistance started to decrease (right ‡). Insets: 2 amplified voltage responses in control medium (left) and when muscimol effect was stable (right). B and C and D and E were from different neurons.
the first from the top to the bottom correspond to the control responses. Fifteen seconds after muscimol application, the voltage response, the amplitude and the half-width of the EPSP started to decrease (Fig. 1B, 2nd row), and reach the maximal decrement 120 s latter. This was better illustrated when the input resistance, the amplitude, and the half-width of the EPSP, measured from the average traces, were normalized and plotted against the time (Fig. 1C). The three quantities were reduced almost at the same rate, and they reached the maximal reduction at the same time. Similar results were obtained from four cells recorded, suggesting that the input resistance may be the most important factor accounting for the EPSP depression. To know, whether muscimol affected the motoneuron membrane potential, this was continuously recorded monitoring the input resistance by means of the voltage response to a current pulse of $-0.3$ nA (300 ms) every 2 s (Fig. 1E). After some seconds of the control membrane potential recording muscimol was added (Fig. 1E, left ↓), and ~27 s later the input resistance started to decrease (Fig. 1E, right ↓) reaching a stable value after additional 32 s. The membrane potential was not affected.

In this motoneuron, the input resistance was reduced from 14.7 MΩ in control medium to 4.4 MΩ in muscimol (Fig. 1D). Similar results were obtained from 11 cells with an average reduction in input resistance of 54.7 ± 5.4% (mean ± SE).

**Effect of muscimol on monosynaptic postsynaptic potentials (EPSP)**

To evaluate whether the reduction in the amplitude of EPSP and its half-width by muscimol was due to activation of postsynaptic GABA$_A$ receptors, the EPSP time course, the membrane input resistance, and the time constant of the motoneurons were determined in Ringer solution with strychnine added (10 μM) and in the presence of muscimol (5 μM).

Stimulation of the DLF (0.3 Hz, 1.1–1.6T), evoked monosynaptic excitatory EPSPs in motoneurons (Fig. 2A) with a latency of 2.49 ± 0.15 ms ($n = 11$) measured from the stimulus artifact and an amplitude of 2.16 ± 1.16 mV ($n = 11$) (Yamashita 1986).

Muscimol (5–10 μM) consistently depressed the EPSP amplitude by 41.4 ± 13.4% ($n = 11$; Fig. 2A), measured after the muscimol action on membrane resistance was stable, and shortened the half-width by 59.43 ± 8.4% ($n = 11$). The EPSP rise time was not affected, but the time course of the falling phase was faster (14 of 14 cells, Fig. 2A). This was more evident when the EPSPs were normalized (Fig. 2B, left). The time constant calculated from an EPSP semilogarithmic plot was consistently decreased by muscimol (66.8 ± 8.7%, $n = 11$). For the motoneuron shown in Fig. 2B (right), muscimol decreased the membrane time constant from 43.4 ms in control medium to 15.25 ms. After muscimol washout, the membrane time constant returned to 42.7 ms. In cat spinal cord motoneurons, most of the synaptic current is capacitive; this makes the EPSP amplitude independent of the postsynaptic membrane resistance unless the latter is reduced in such a way that the membrane time constant is comparable to the EPSP time to peak (Finkel and Redman 1983). On average, the membrane time constant was reduced by muscimol from seven times EPSP time to peak in control medium to 2.5 times. According to the theory (Finkel and Redman 1983), this strongly suggests that the decrement of the membrane resistance could account for the EPSP depression. In motoneurons where mono- and polysynaptic EPSP were elicited by DLF stimulation, EPSP shortening could be due to a muscimol-induced block of polysynaptic EPSPs. However, in two neurons, polysynaptic EPSPs were blocked by stimulation of the DLF at a higher frequency (10 Hz), resulting in a reduction of the half-width by 12.5% on average and the membrane time constant by 25%.

**FIG. 2.** Muscimol depressed EPSP. A: the motoneuron EPSP elicited by DLF stimulation at 1.5T. Top: in control medium (strychnine, 10 μM); middle: in the presence of muscimol (5 μM) recorded after muscimol action on input resistant was stationary; bottom: after muscimol washout. To show muscimol effect on time course, EPSPs from A were normalized (B, left). The effect of muscimol on membrane time constant is evaluated by plotting the natural logarithm of the EPSP amplitude (B, right). Washout of muscimol recovers the EPSP time course (B, left) and the membrane time constant (B, right). Picrotoxin blocked muscimol effect, time course (C, left) and the membrane time constant (C, right) were recovered. All traces in this and in next figures were averages of 50 responses elicited at 0.3 Hz. A and B and C are from different neurons.
These reductions were smaller than those produced by muscimol. In addition, baclofen clearly blocked polysynaptic EPSPs in two neurons with a reduction of the half-width by ~20% but without any significant change in the membrane time constant. This means that blocking polysynaptic EPSPs could contribute to EPSP shortening but does not explain the large reduction in both the half-width and the membrane time constant in the presence of muscimol. The fact that the EPSP shortening and the reduction of the membrane time constant induced by muscimol were reverted by bicuculline (n = 3) and picrotoxin (n = 3), GABA<sub>A</sub> antagonists, suggests that receptors could mediate such depression (Fig. 2C). In the neonatal spinal cord, strychnine applied at concentration >1.5 µM partially blocked GABA<sub>A</sub> receptors on motoneurons (Jonas et al. 1998). To clarify this point, EPSPs in Ringer solution and the presence of muscimol (5 µM) were recorded. When the muscimol action was stable, strychnine (10 µM) added to the bath solution did not change the EPSPs (n = 3, data not showed), suggesting that the glycine receptor antagonist (10 µM) did not block GABA<sub>A</sub> receptors activated by muscimol. One possibility may be that GABA<sub>A</sub> receptors have higher affinity for strychnine on neonatal rat than on turtle motoneurons.

To assess if EPSP depression was also due to activation of muscimol-sensitive presynaptic receptors, paired-pulse facilitation (PPF) protocol was applied. Facilitation of the second EPSP (A<sub>2</sub>) relative to the first EPSP (A<sub>1</sub>) is a consequence of a nonlinear relationship between [Ca<sup>2+</sup>]<sub>o</sub> and neurotransmitter release. It is believed that the probability of release after a presynaptic action potential is higher due to some residual calcium that remains at the sites of transmitter release (Katz and Miledi 1968, 1970; Zucker 1989). PPF could be induced either by reduction of [Ca<sup>2+</sup>]<sub>i</sub> or by presynaptic inhibition affecting Ca<sup>2+</sup> entry at the terminals (Chen and Regehr 2003; Katz and Miledi 1968, 1970). In cat Ia-motoneuron synapses, presynaptic inhibition, mediated mainly by activation of GABA<sub>A</sub> receptors, induced PPF (Stuart and Redman 1992) and depressed the EPSP by decreasing the number of quanta release without changing the quanta size (Clements et al. 1987). In Fig. 3A, a pair of EPSPs elicited with a stimulus strength of 1.5T and a time interval of 100 ms in control medium (top), in muscimol (middle), and after muscimol washout (bottom) are shown. To compare the time course and the relative amplitude between the first and the second EPSP, they were normalized by taking the amplitude of the first EPSP as 100% for each condition. As illustrated in Fig. 3B, the amplitude ratio in control medium was A<sub>2</sub>/A<sub>1</sub> = 1.08. In the presence of muscimol, both EPSPs were depressed in approximately the same proportions with an amplitude ratio of 1.13. In this cell, muscimol reduced both the input resistance and the membrane time constant by 76 and 72.5%, respectively, which corroborates the strong action on motoneuron excitability exerted by activation of postsynaptic muscimol-sensitive receptors. The average amplitude ratio (A<sub>2</sub>/A<sub>1</sub>) in control condition was 1.13 ± 0.05 (n = 11) and with muscimol it was 1.13 ± 0.04 (n = 11). When a paired two-population t-test was applied to both samples of data, they were not significantly different with a P < 0.05, suggesting that muscimol did not activate muscimol-sensitive presynaptic receptors and supporting the possibility that postsynaptic muscimol-sensitive receptors could account for the EPSP depression.

**Tonic release of GABA**

To know whether GABA<sub>A</sub> receptors were tonically activated, a GABA<sub>A</sub> receptor antagonist was added to the bath solution. Bicuculline (40 µM) increased the EPSPs amplitude by 22.3 ± 2.3% (n = 4) and the membrane input resistance by 24.6 ± 0.66% (n = 4, Fig. 3C). If the DLF terminals were not regulated by presynaptic GABA<sub>A</sub> receptors, then our result suggests that GABA<sub>A</sub> receptors on motoneurons may be tonically activated. On rat spinal cord motoneurons, a subset of spontaneous miniature IPSCs has been reported to be mediated by activation of GABA<sub>A</sub> and glycine receptors (Jonas et al. 1988). It remains to know whether unitary and spontaneous compound IPSCs are evoked on turtle motoneurons.
Effect of baclofen on the DLF-motoneuron synapse

The effect of baclofen (10 μM) on DLF terminal-motoneuron synapses was evaluated. The major action of baclofen was a systematic depression of the EPSPs in all cells (64.8 ± 5.5%; n = 7; Fig. 4A). In contrast, the half-width of the EPSP decreased by 0 to 27% (n = 6; Fig. 4B). In all cells, baclofen produced a small reduction in the membrane resistance (7.7 ± 2%; n = 6; Fig. 4C). The small decrement in the membrane resistance could account for a small decrement in the postsynaptic membrane resistance (7.7 ± 2%) could not explain the large EPSP depression (64.8 ± 5.5%). One possibility is that baclofen affected the neurotransmitter release from the DLF terminals.

Paired-pulse protocol to evaluate baclofen action

To evaluate whether baclofen inhibited neurotransmitter release from DLF terminals, a paired-pulse protocol was applied. Figure 4D shows that baclofen increased the EPSP amplitude ratio (A2/A1) from 0.9 in control medium to 1.08. In all cells (n = 6), the change in amplitude ratio from 0.95 ± 0.17 in control medium to 1.075 ± 0.19 in baclofen was statistically different when a paired two-population t-test (P < 0.05) was applied. This result suggests that baclofen may inhibit neurotransmitter release from DLF terminals.

DISCUSSION

Presynaptic control of vestibulo-, reticulo-, and rubrospinal terminals synapsing on motoneurons is not mediated by activation of GABA_A receptors (Curtis and Malik 1984; Curtis et al. 1984; Rudomin et al. 1991). It is thought that neurotransmitter release from vestibulospinal terminals could be inhibited by GABA_B receptors as is the case for Ia afferents or interneuron terminals synapsing motoneurons (Curtis and Lacey 1998; Edwards et al. 1989; Jiménez et al. 1991; Jonas et al. 1998; Lev-Tov et al. 1988; Peshori et al. 1998; Stuart and Redman 1992). The present findings provide evidence that the strength of the DLF terminal-motoneuron synapse may be

FIG. 4. Baclofen effect on the DLF-motoneuron synaptic efficacy. EPSP elicited by DLF stimulation at 1.5T (A, top) was depressed by baclofen (10 μM) added to the bath solution (A, bottom). Normalized EPSPs in control medium and in baclofen had the same time course (B). Input resistance was reduced from R_i = 56 MΩ (C, ○) in control medium to R_i = 54.4 MΩ (C, ◦) in baclofen. To assess whether EPSP depression by baclofen was due to inhibition of neurotransmitter release from DLF terminals, a paired-pulse protocol was applied. The amplitude ratio A2/A1 changed from 0.9 in control medium to 1.08 in baclofen, which is better appreciated when EPSPs were normalized (D) taking the maximal amplitude of the first EPSP as 100% for each condition. A–D were from the same neuron.
controlled by activation of presynaptic GABA\textsubscript{B} receptors and postsynaptic GABA\textsubscript{A} receptors.

\textit{GABA\textsubscript{A}-induced postsynaptic inhibition}

In all recorded motoneurons, muscimol produced a large depression in amplitude and a shortening of the EPSP falling phase, which were always accompanied by a decrement in the membrane resistance and a large reduction of the membrane time constant, making it comparable to the EPSP time to peak. Theoretically, this finding (Finkel and Redman 1983) agrees with the hypothesis that muscimol depresses the EPSPs by a postsynaptic action. This conclusion is supported by the result that muscimol decreased the input membrane resistance, the amplitude, and the half-width of the EPSPs at the same rate. Additionally, muscimol did not change the EPSP amplitude ratio obtained with the paired-pulse protocol. This suggests that muscimol-sensitive receptors were not activated at the presynaptic level. Our results are consistent with two findings: first, vestibulo- and reticulospinal terminals synapsing on motoneurons are not subjected to presynaptic inhibition of the type acting on Ia terminals (Rudomin et al. 1991), which is characterized by a terminal depolarization (PAD) and mainly mediated by activation of GABA\textsubscript{A} receptors (Rudomin and Schmidt 1999), and second rubrospinal terminals do not have bicuculline-sensitive receptors as determined by measurement of their terminal threshold in the presence of GABA (Curts and Malik 1984). The fact that muscimol-induced shortening and depression of EPSPs and the decrease in the membrane resistance were blocked by bicuculline or picrotoxin indicates the involvement of GABA\textsubscript{A} receptors. Therefore although we cannot completely rule out a presynaptic effect of muscimol, it seems more likely that the synaptic efficacy of DLF terminals synapsing on motoneurons is controlled by activation of postsynaptic GABA\textsubscript{A} receptors.

\textit{Presynaptic inhibition of neurotransmitter release by baclofen}

Our results show that baclofen—a GABA\textsubscript{B} receptor agonist—produced a large EPSP depression of \(\sim68\%\) in all motoneurons recorded without any significant postsynaptic change, suggesting that such depression could be presynaptic in origin. This possibility is supported by the significant increment in EPSP amplitude ratio obtained with the paired-pulse protocol. Similar result was obtained in Ia-motoneuron synapse (Stuart and Redman 1992) and in the retinogeniculate synapse where activation of presynaptic GABA\textsubscript{B} receptors by baclofen depressed EPSC amplitude, turned paired-pulse depression into PPF, and decreased calcium influx in the presynaptic terminals (Chen and Regehr 2003). Presynaptic inhibition induced by baclofen has been predicted by PPF and has been confirmed by intracellular, whole cell, and double recordings (Mouginot et al. 1998; Shen and Johnson 1997; Takahashi et al. 1998). Therefore it is reasonable to assume that GABA\textsubscript{B} receptors should have been activated on DLF terminals and thereby blocking neurotransmitter release. This interpretation is in line with other findings showing that baclofen presynaptically depresses synaptic transmission in the spinal cord (Curts and Lacey 1998; Jimenez et al. 1991; Jonas et al. 1998; Lev-Tov 1988; Peng and Frank 1989a; Rudomin et al. 1991; Russo et al. 1998; Stuart and Redman 1992). As in descending fibers in the cat (Jimenez et al. 1991), baclofen never completely eliminated the postsynaptic response to low-threshold stimulation of DLF terminals, but it produced a strong depression and sometimes abolished the postsynaptic response to Ia afferent, dorsal root, and spinal axon stimulation in cats (Jimenez et al. 1991), in turtles (Russo et al. 1998) and in rats (Jonas et al. 1998), respectively. The differential effect of baclofen could be due to not all DLF terminals having GABA\textsubscript{B} receptors, the density of GABA\textsubscript{B} receptors being less in DLF terminals than in intraspinal terminal afferents, and the density of GABA\textsubscript{B} receptors is similar in both kind of fibers but with different affinity to baclofen (Jimenez et al. 1991). The presence of GABA\textsubscript{B} receptors in motoneurons, in dorsal horn neurons, and in primary afferents has been revealed by immunocytochemical studies (Towers et al. 2000; Yang et al. 2001). The small baclofen-induced decrement in motoneuron membrane resistance may be explained by activation of a Ba\textsuperscript{2+}-sensitive current. This current is activated by baclofen in motoneurons and interneurons in the spinal cord and leads to a decrease in membrane resistance and a downregulation of plateau potentials in both types of neurons (Russo et al. 1998; Svirskis and Houngaard 1998). A postsynaptic effect of baclofen could not be excluded. AMPA receptors, which mediate motoneuron EPSPs, need phosphorylation to be activated (Song and Huganir 2002). Baclofen acting through G-protein-coupled pathways could affect phosphorylation of AMPA receptors and thereby reducing the EPSPs. In the calyx of Held giant synapse, that possibility was discarded because baclofen depressed the postsynaptic response only by reducing calcium influx at the presynaptic terminal (Takahashi et al. 1998). In addition, AMPA receptors dephosphorylated during LTD did not change PPF (Lee et al. 1998) as occurs with DLF-motoneuron synapse in the presence of muscimol.

\textit{Functional implications}

Presynaptic inhibition via axo-axonic GABAergic synapses controls neurotransmitter release from thick myelinated primary afferents by activation of GABA\textsubscript{A} receptors (PAD) (Rudomin and Schmidt 1999). The prominent postsynaptic effect that activation of GABA\textsubscript{A} receptors by muscimol or synaptic inputs showed strongly suggests that GABA\textsubscript{A} receptors also may play an important role in controlling Ia-motoneuron synaptic efficacy by shunting the postsynaptic element. Physiological activation of motoneuron GABA\textsubscript{A} receptors has been shown. In rat spinal cord motoneurons, unitary and miniature spontaneous IPSCs mediated by GABA\textsubscript{A} and glycine receptors were evoked (Jonas et al. 1998). Activation of cat interneurons by Group I afferents produced a slow strychnine-resistant inhibitory potential on motoneurons (Rudomin et al. 1990) that was blocked by picrotoxin. Likewise, Kellerth and Szumski (1966) found strychnine-resistant and picrotoxin-sensitive postsynaptic inhibition elicited on motoneurons. Therefore it could be speculated that motoneuron GABA\textsubscript{A} receptors, like GABA\textsubscript{B} receptor, may down regulate or prevent activation of plateau potentials when they are tonically activated (Svirskis and Houngaard 1998). In conclusion, according to our results, GABA may regulate the DLF terminals-motoneuron synaptic strength by activation of presynaptic GABA\textsubscript{B} and postsynaptic GABA\textsubscript{A} receptors. The presynaptic GABA\textsubscript{B} receptors inhibit...
neurotransmitter release and the postsynaptic GABA<sub>A</sub> receptors decrease motoneuron excitability.

**ACKNOWLEDGMENTS**

We thank Dr. Jorn Hounsgaard, Dr. Ratil Russo and Dr. Pablo Rudomin for helpful comments to improve this paper and Dr. Rafael Gutiérrez and QFB Jasmin Maqueda for help with the motoneuron histology. The authors thank N. MackAulay for reviewing the grammar and to R. M. Martínez-Ferreira for typing and reviewing the manuscript.

**GRANTS**

This work was partially supported by Grant 37152 from the National Council of Science and Technology (Conacyt-México).

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