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Activation of type B γ-aminobutyric acid receptors in the intact mammalian spinal cord mimics the effects of reduced presynaptic Ca^{2+} influx

AHARON LEV-TOV*, DONALD E. R. MEYERS†‡, AND ROBERT E. BURKE†

†Laboratory of Neural Control, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892; and *Department of Anatomy, The Hebrew University Medical School, Jerusalem 91010, Israel

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ABSTRACT **Intracellular recordings from mammalian spinal motoneurons *in vivo* show that the type B γ-aminobutyric acid receptor agonist, L-(−)-baclofen, when administered systemically to pentobarbital-anesthetized or decerebrate unanesthetized cats decreases the amplitude of monosynaptic group Ia excitatory postsynaptic potentials (EPSPs), markedly increases tetanic and posttetanic potentiation, and reduces or abolishes synaptic depression during high-frequency synaptic activation and in the posttetanic period. These changes occur without detectable alteration in motoneuron input resistance, EPSP shape, or the invasion of action potentials into the intraspinal group Ia terminal arborizations. The baclofen-induced effects are qualitatively similar to those observed in more accessible synaptic systems when presynaptic Ca^{2+} influx and, concomitantly, transmitter release are reduced. Based on these and other recent findings regarding the mechanism of action of baclofen and the distribution of its receptors in the spinal cord, we suggest that L-(−)-baclofen modifies frequency modulation of Ia synaptic transmission by reducing presynaptic Ca^{2+} influx and the concomitant level of transmitter release from Ia afferent terminals. The drug appears to be a useful tool in studies of the ionic mechanisms that control the release of transmitter and its frequency modulation at inaccessible mammalian synapses.**

The direct excitatory connection made by group Ia (primary muscle spindle) stretch receptor afferents onto α-motoneurons is a key model system for investigation of synaptic transmission in the intact mammalian central nervous system (1–3). However, *in vivo* this chemical synapse (1, 4) is not amenable to manipulations of the external ionic environment that are often used to study the critical role of Ca^{2+} influx in the control of transmitter release and its frequency modulation in more accessible *in vitro* systems (5–8). The type B γ-aminobutyric acid ($GABA_B$) receptor agonist, L-(−)-baclofen, has recently been shown to decrease voltage-dependent Ca^{2+} conductance (9–11) and neurotransmitter release (11–13) in a number of sensory and central neurons. We report here that, when transmitter release from group Ia afferent terminals is reduced by L-(−)-baclofen, the depression induced by high-frequency synaptic activation is reduced or abolished, and tetanic and posttetanic potentiation (PTP) are enhanced. These effects mimic those found in *in vitro* systems under conditions that reduce Ca^{2+} influx and, consequently, the transmitter released (7) from active synapses. Our results provide evidence that the mechanisms of transmitter release at group Ia synapses within the mammalian spinal cord are qualitatively similar to those at better-defined chemical synapses. The results also buttress previous evidence (see ref. 14) that PTP of Ia excitatory postsynaptic potentials (EPSPs) does not result from variations in action potential blockade at branch points within group Ia terminal arborizations (15).

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MATERIALS AND METHODS

Experiments were performed on adult cats of either sex. All surgical procedures were carried out under halothane anesthesia. The left medial gastrocnemius (MG), lateral gastrocnemius–soleus, and posterior biceps and semitendinosus nerves were cut and mounted on bipolar stimulating electrodes. The lumbosacral spinal cord was exposed by laminectomy (L3–L7) and the ventral roots of S1, L7, and L6 were severed. The central ends of the cut S1 and L7 ventral roots were mounted on bipolar hooks for antidromic stimulation. Warm mineral oil pools covered the spinal cord and the exposed left hindlimb tissues. Body temperature was continuously monitored and kept at 36–38°C by a heating pad and infrared lamp.

In three animals, anesthesia was maintained during recording with pentobarbital administered in small i.v. doses (total, <50 mg/kg over ≈12 hr). Two other cats were surgically decerebrated under halothane anesthesia at the precollicular–postmammillary plane and the forebrain was then removed by suction. After decerebration, anesthesia was discontinued and the animals were spinalized at the T13 level and paralyzed with gallamine triethiodide administered i.v. (2.5–5 $mg \cdot kg^{-1} \cdot hr^{-1}$). The paralyzed animals were artificially ventilated and expired CO_2 was maintained between 3.5% and 4%.

Motoneurons were penetrated with beveled glass micropipettes filled with 3 M potassium acetate. Motoneurons belonging to the MG, lateral gastrocnemius–soleus, and posterior biceps and semitendinosus pools were identified by Ia EPSP patterns produced by these muscle nerves at 2–3 times group Ia threshold (16). Motoneuron input resistance, R_N, was measured by the spike height method by intracellular current step injection (17). Monosynaptic group Ia EPSPs were generated by stimulating muscle nerves (MG, lateral gastrocnemius–soleus, or posterior biceps and semitendinosus) at a strength 2–3 times threshold for the most excitable fibers in the nerve. Tetanic depression was studied by stimulating the appropriate nerve at 100 Hz (see Fig. 1). PTP was produced by applying high frequency (500 Hz) stimulus trains lasting 10–20 s. Each such stimulus train was preceded by 60 individual pulses (pretetanic control) and followed by 120 pulses (posttetanic), all applied at 1 Hz. The group Ia EPSPs generated were continuously recorded on an FM tape recorder (bandpass dc to 2.5 kHz) for later analysis.

Abbreviations: Ia, primary muscle spindle afferent; $GABA_B$, type B γ-aminobutyric acid; PTP, posttetanic potentiation; EPSP, excitatory postsynaptic potential; MG, medial gastrocnemius.

‡Present address: Bekesy Laboratory of Neurobiology, University of Hawaii at Manoa, Honolulu, HI.

The recorded data were digitized by using a high-speed analog/digital (A/D) convertor (RC Electronics, Santa Barbara, CA) and an IBM-XT computer. Peak amplitudes of synaptic potentials were measured from these data files with an interactive computer program. Individual EPSP amplitudes (V_i) recorded during (only in the 100-Hz trains) and after the high frequency stimulus trains were divided by the mean amplitude of the 60 pretetanic EPSPs (V_0) to give percentage potentiation (P_i):

$$P_i = 100\,[(V_i/V_0) - 1].$$

The maximal percentage potentiation, P_{max}, during the entire posttetanic period (see Fig. 3) was calculated from an 8-s posttetanic epoch that contained the largest potentiated EPSPs. In each animal, L-(−)-baclofen (CIBA–Geigy; total dose, 0.8–2 mg/kg, dissolved in sterile saline) was administered i.v. while recording intracellularly from a motoneuron.

RESULTS

Systemic administration of L-(−)-baclofen has been shown to produce a marked decline in amplitude of EPSPs in the cat (18, 19) and frog (12, 13) spinal cord. In the present study, the mean amplitude of Ia EPSPs recorded in α-motoneurons after baclofen administration was 0.86 + 0.68 mV (SD), as compared to 4.6 + 2.69 mV (n = 66) in a control population of motoneurons (based on 11 motoneurons from the present study and 55 motoneurons from a previous study of untreated animals; see ref. 14).

Under these conditions, there were substantial changes in the modulation of EPSPs produced by high-frequency activation of group Ia synapses. In the normal cat spinal cord, the amplitudes of composite group Ia EPSPs show rapid initial potentiation during high-frequency (e.g., 100 Hz) tetanization of all of the group Ia afferents in the muscle nerve (14), followed by an asymptotic decline (e.g., Fig. 1*A Left*). In contrast, after i.v. administration of L-(−)-baclofen (1 mg/kg), Ia EPSP amplitudes increased monotonically to reach a steady plateau during similar tetani (Fig. 1*A Right*).

In the control state, prolonged (20 s) very-high-frequency (500 Hz) tetanization (14) of the muscle nerve also produced a mixture of posttetanic depression and potentiation in Ia EPSPs following the tetanus. Posttetanic depression is thought to be caused by partial depletion of transmitter and/or inactivation of release sites by high rates of release (see ref. 7) and is maximal immediately after cessation of tetanic stimulation. At this point, depression coexists with potentiation, keeping PTP at a submaximal level for up to 30 s (14). This sequence of events is shown in Fig. 1*B* (*Left*). The first EPSP elicited in the posttetanic period (at 1 Hz) was smaller than the pretetanic control response but subsequent EPSPs increased to reach maximum PTP at ≈24 s and then decayed slowly to the pretetanic baseline (see also ref. 14). The time course of PTP in a different motoneuron, observed after administration of L-(−)-baclofen, was markedly different (Fig. 1*B Right*); the EPSP was maximally potentiated immediately after cessation of the tetanus and the maximal percentage potentiation (P_{max}; see *Materials and Methods* and ref. 14) was much higher than in control motoneurons. To exclude the possibility that these effects of baclofen might involve interaction with pentobarbital anesthetic (12, 21), the same experiment was repeated in unanesthetized decerebrate–spinal animals. The results were the same (Fig. 2*A*).

Enhanced percentage PTP and minimal or undetectable posttetanic synaptic depression were observed in 32 motoneurons recorded after systemic administration of L-(−)-baclofen (0.8–2 mg/kg) in both anesthetized (n = 17) and unanesthetized (n = 15) preparations. The mean percentage

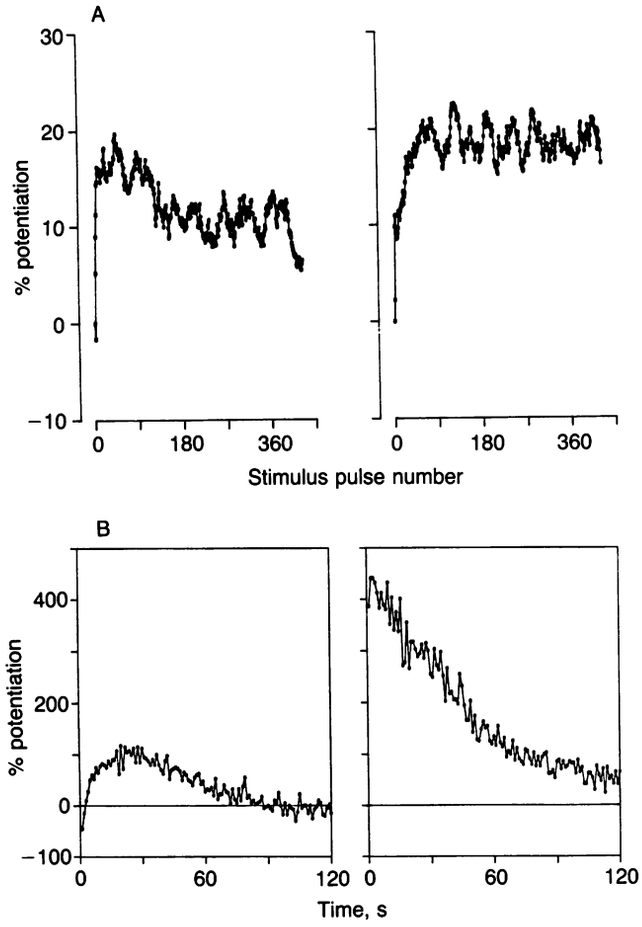

FIG. 1. Effects of L-(−)-baclofen on tetanic and posttetanic potentiation. (*A*) Graphs of percentage potentiation as a function of the pulse number of 425 individual EPSPs recorded in the same MG motoneuron during a 100-Hz tetanus to MG group Ia afferents before (*Left*) and after (*Right*) administration of L-(−)-baclofen (1 mg/kg) to an unanesthetized decerebrate cat. Note the decline in percentage potentiation during the prebaclofen tetanus (*Left*), which disappeared after drug administration (*Right*). Data were smoothed by a modified moving bin method (34) (bin size, 20 responses; bin shift, 1 response). (*B*) Curves of percentage potentiation as a function of time after 500-Hz 20-s tetani to the MG nerve. Ia EPSPs were recorded in two different MG motoneurons, one recorded before (*Left*) and one 67 min after (*Right*) administration of L-(−)-baclofen (1.0 mg/kg) to a pentobarbital-anesthetized cat.

potentiation (P_i) of the first posttetanic EPSP was −2.1% in motoneurons in control animals (i.e., the first posttetanic EPSP was smaller than the mean control amplitude; n = 43; 11 cells from the present study and 32 of 55 motoneurons from a previous study of untreated preparations; ref. 14). In contrast, after baclofen administration the mean P_i of the first posttetanic EPSP was +234%. The maximum percentage potentiation observed in the posttetanic period (P_{max}) also increased from a mean value of +76.5% (n = 66) in the control population to +241.3% after baclofen administration.

The marked baclofen-induced changes in the amplitude and frequency modulation of Ia EPSPs were not accompanied by detectable changes in relevant motoneuron properties. The mean and range of cell input resistance (R_N) values in a population of motoneurons sampled after administration of baclofen (mean ± SD, 1.13 ± 0.68 MΩ; n = 18) were not significantly different (two-tailed t test) from those in cells sampled in the absence of the drug (1.38 ± 1.03 MΩ; n = 14). Moreover, the baclofen-induced effects did not change EPSP shape, as illustrated by the superimposed computer-averaged records of the pretetanic EPSP before and after addition of

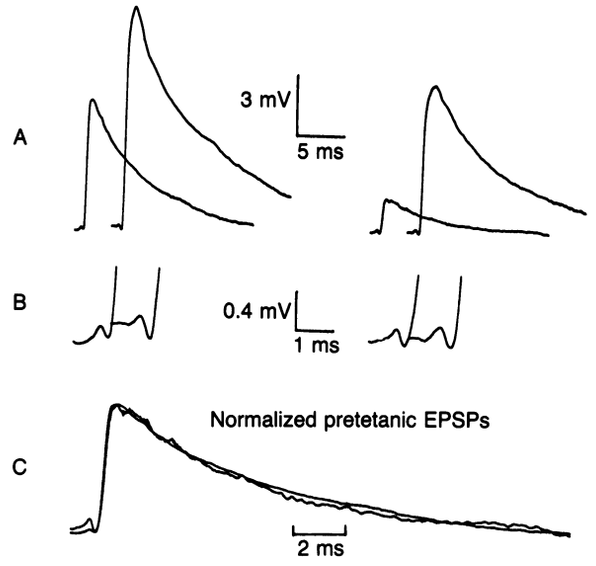

FIG. 2. Baclofen effect on PTP in an unanesthetized decerebrate preparation. (*A*) Computer averaged records (eight sweeps each) of pretetanic (left record in each pair) and posttetanic Ia EPSPs recorded in a MG motoneuron before (*Left*) and 10 min after (*Right*) administration of L-(−)-baclofen (1 mg/kg). Note marked decrease in amplitude of the pretetanic EPSP and relative increase in PTP after the drug. (*B*) Much amplified records of the foot of each EPSP from *A* shown on an expanded time scale. The pretetanic terminal potentials that result from action potential invasion into the afferent arborizations were essentially unchanged after baclofen administration and the posttetanic increase in the terminal potential was identical before and after baclofen. (*C*) Superimposed records of pretetanic EPSPs in *A* after normalization to the same peak amplitude. Shape and duration of the EPSP after baclofen were essentially the same as the predrug response, indicating no significant change in the postsynaptic membrane time constant (the cell input resistance was essentially the same before and after baclofen).

baclofen in Fig. 2*C*. The two EPSPs were virtually identical when normalized to the same peak amplitude.

Fig. 2 also provides evidence that L-(−)-baclofen did not affect the invasion of group Ia afferent terminals by the presynaptic action potentials. Terminal potentials preceding the pretetanic EPSPs (the left trace in each pair of records in Fig. 2*B*) were virtually the same before and after administration of baclofen, as was the increase in terminal potential amplitudes produced by tetanization (right trace in each pair of records; see ref. 14).

In four motoneurons (four different cats), we were able to record pre- and posttetanic EPSP behavior in individual motoneurons before and after i.v. administration of L-(−)-baclofen. In these cells, the drug produced a marked decline in amplitude of pretetanic control EPSPs (for example, Fig. 3 *A–D Upper*). This decrease (from 6.2 to 0.4 mV) was accompanied by a dramatic increase in P_{max} of PTP (from 120% to 507%; *A–D Middle*) and by progressive elimination of the masking effect of posttetanic depression (*B–D*; cf. Fig. 1*B Left*).

DISCUSSION

The main findings in this study are that the $GABA_B$ agonist L-(−)-baclofen markedly increases percentage PTP and reduces, or abolishes, the depressive effects (synaptic depression) of high-frequency synaptic activation. These effects were in all cases preceded by a marked decrease in the amplitude of monosynaptic Ia EPSPs, which was not due to changes in postsynaptic motoneuron input resistance. The lack of significant changes in the shape of EPSPs before and after baclofen (Fig. 3*C*) also argues that postsynaptic factors such as alterations in dendritic electrotonus or "remote" postsynaptic inhibition (1) are unlikely to account for the diminution in pretetanic EPSP amplitudes (see also refs. 18 and 19). Rather, the evidence suggests that baclofen modifies the frequency modulation of the Ia EPSPs by reducing the amount of transmitter released by group Ia synapses. In *in vitro* studies, reduction in quantal transmitter release has been directly demonstrated after L-(−)-baclofen treatment at synapses in the hemisected frog spinal cord (12, 13) and in synapses between cultured spinal neurons (11).

Decreased transmitter release could, in principle, result from failure of complete action potential invasion into the terminal arborizations of group Ia afferents (15). L-(−)-baclofen produces small degrees of hyperpolarization in hippocampal neurons *in vitro* that are associated with an increased K^+ conductance (22–24). If this mechanism were to operate in intraspinal group Ia afferent arborizations, it might reduce the probability for action potential invasion into fine Ia terminal axons. Three lines of evidence make this seem unlikely. First, the pre- and posttetanic "terminal potentials" that reflect action potential invasion into group Ia terminal arborizations (25, 26) were essentially unchanged by baclofen (Fig. 2*B*), even in cases that showed a decrease in EPSP amplitude by a factor of 20. Second, baclofen produces no significant changes in the electrical excitability of mammalian group Ia afferents in the ventral horn (19, 27, 28) or in the intermediate nucleus (29). Finally, the baclofen-induced decrease in quantal content in the dual electrical–chemical Ia synapse in the frog spinal cord is not accompanied by demonstrable changes in the electrical coupling potential (12, 13), which is a sensitive index of the degree of activation of presynaptic terminations (30).

The reduction in pretetanic Ia EPSP amplitudes produced by L-(−)-baclofen and the concomitant changes in the frequency modulation phenomena that we have demonstrated [i.e., lack of EPSP attenuation during tetanic trains (Fig. 1*A*), decrease or abolition of synaptic depression in the posttetanic period (Figs. 1*B* and 3), and marked increase in PTP (Figs. 1*B*, 2, and 3)], closely resemble the changes in frequency modulation of transmitter release at the neuromuscular junction as quantal content is lowered by reduction of the presynaptic Ca^{2+} influx (7, 8, 31–34). GABA and its analog L-(−)-baclofen, acting through the $GABA_B$ receptor, have been shown to decrease the voltage-dependent Ca^{2+} conductance in sensory neurons (9, 10, 14, 20, 35) and spinal cord cells in tissue culture (11). Furthermore, receptor binding experiments have shown that $GABA_B$ receptors are found in many locations in the mammalian spinal cord, including presumed group Ia afferents (36, 37).

It therefore seems that our findings can best be explained as follows: (*i*) the binding of L-(−)-baclofen to $GABA_B$ receptors on Ia afferents reduces the voltage-dependent Ca^{2+} conductance in group Ia synaptic terminals and Ca^{2+} influx evoked by presynaptic action potentials; (*ii*) consequently, quantal transmitter release and Ia EPSP amplitudes are decreased; and (*iii*) when evoked release of the transmitter reaches a sufficiently low level, the synaptic depression usually induced by high release rates during and after high frequency activation is virtually eliminated. The apparent enhancement of percentage PTP is likely due in part to removal of coexistent synaptic depression and presumably also to a shift in the operating point on the sigmoidal relationship between Ca^{2+} influx and transmitter release, as observed in *in vitro* studies of neuromuscular junctions bathed in low Ca^{2+} medium (7, 34). Our data provide additional evidence that the decreased group Ia EPSP amplitudes found by us and others (18, 19) in mammals after baclofen administration is indeed a consequence of decreased presynaptic transmitter release, and that the basic mecha-

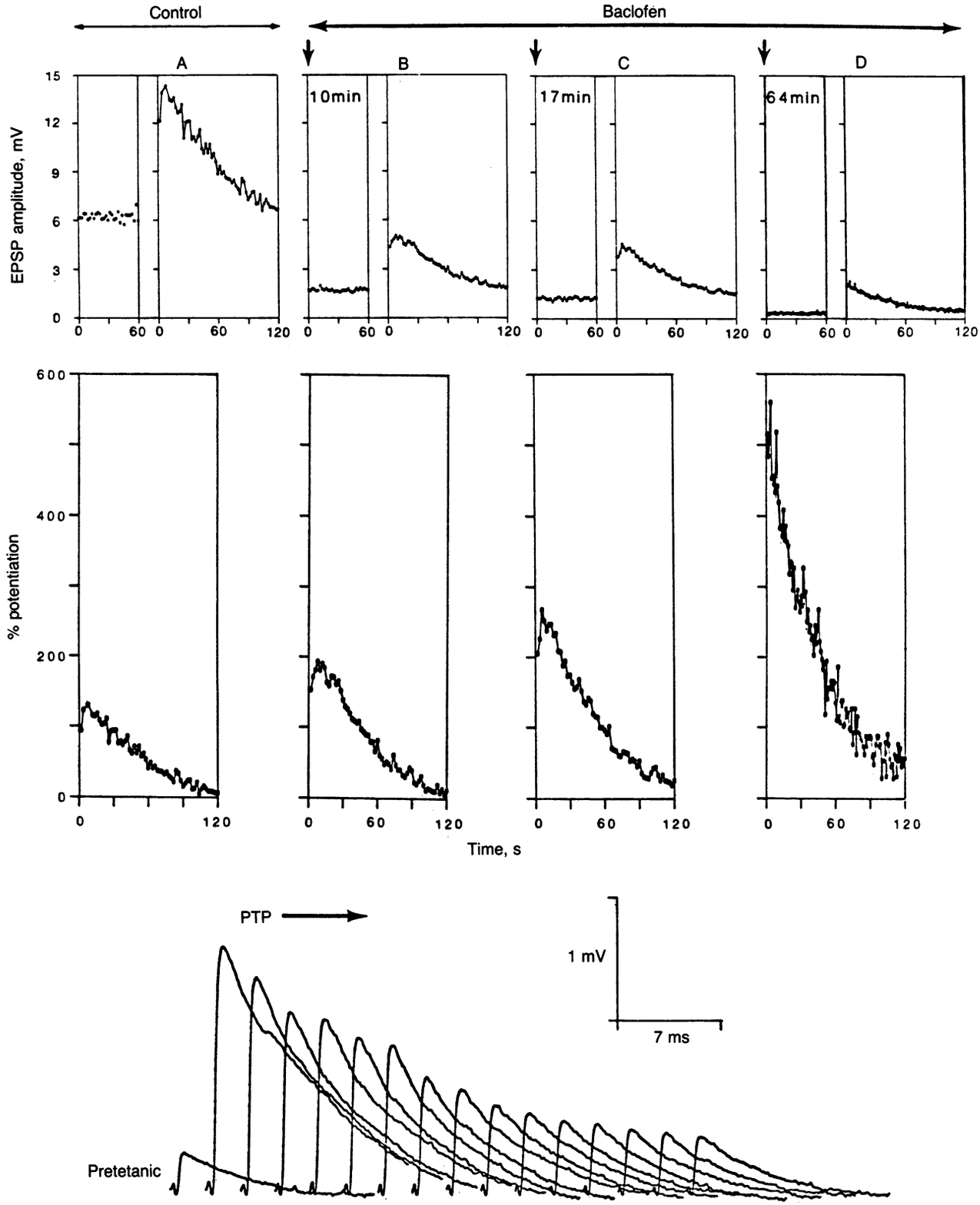

FIG. 3. Time course of changes in Ia EPSP amplitude and PTP after administration of L-(−)-baclofen. Data from four tetanus sequences studied over a 90-min period in the same MG motoneuron before (*A*) and after (*B–D*) administration of L-(−)-baclofen (2 mg/kg) to a pentobarbital-anesthetized cat. (*Upper*) Each panel shows absolute Ia EPSP amplitudes before (*A–D Left*) and after (*A–D Right*) a 500-Hz 20-s tetanic stimulation of the MG nerve. (*Middle*) Graphs of posttetanic changes in percentage potentiation of the data in *Upper*. (*Lower*) Computer-averaged records (eight sweeps each) of pre- (leftmost trace) and posttetanic EPSPs from sequence in *D*. Membrane potential was stable at −76 mV throughout the recording period. Note the steady fall in the amplitudes of both the pre- and posttetanic EPSPs after drug administration, the marked increase in percentage potentiation, and the progressive abolition of synaptic depression with time from sequences *A–D* (synaptic depression was undetectable in sequence *D*).

nisms of transmitter release and its frequency modulation, which have been described in more controlled *in vitro* systems, also apply to central synapses in the mammalian spinal cord. It also seems possible that activation of $GABA_B$ receptors may play some role in the GABAergic mechanism of presynaptic inhibition in this system (see ref. 1).

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