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Phaclofen-insensitive presynaptic inhibitory action of (±)-baclofen in neonatal rat motoneurones *in vitro*

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1 Intracellular recordings were made from antidromically identified motoneurones in transverse spinal cord slices from neonatal (12–16 day) rats.

2 Superfusion of (±)-baclofen (0.5–50 μM) reduced the excitatory postsynaptic potentials (e.p.s.ps) and inhibitory postsynaptic potentials (i.p.s.ps) evoked by dorsal root or dorsal root entry zone stimulation in a concentration-dependent manner; the calculated EC_{50} was 2.4 μM. Baclofen in comparable concentrations also reversibly eliminated spontaneously occurring e.p.s.ps and i.p.s.ps.

3 (−)-Baclofen was more effective as compared to baclofen in reducing the synaptic responses, whereas (+)-baclofen at concentrations as high as 50 μM was ineffective.

4 Baclofen (<5 μM) attenuated the synaptic responses without causing a significant change of passive membrane properties and depolarizations induced by exogenously applied glutamate. In addition to synaptic depression, baclofen (>5 μM) caused a hyperpolarization associated with decreased membrane resistance in some of the motoneurones; the glutamate responses were also attenuated.

5 Baclofen reversibly depressed the spike after-hyperpolarization of the motoneurones.

6 GABA (1–10 mM) depressed synaptic transmission and depolarized or hyperpolarized motoneurones. While potentiated by the uptake inhibitor nipecotic acid, the synaptic depressant effect of GABA was not antagonized by bicuculline.

7 The synaptic depressant effect of baclofen was neither blocked by $GABA_A$ antagonists bicuculline and picrotoxin (10–50 μM) nor by the $GABA_B$ antagonist phaclofen (0.1–1 mM).

8 It is suggested that baclofen depresses excitatory and inhibitory transmission in rat motoneurones by primarily a presynaptic mechanism in reducing the liberation of chemical transmitters from nerve endings via a phaclofen-insensitive $GABA_B$ receptor.

Introduction

Baclofen, a lipophilic analogue of γ-aminobutyric acid (GABA) that interacts with a bicuculline-insensitive GABA receptor, known as $GABA_B$ receptor (Bowery *et al.*, 1980; Hill & Bowery, 1981), is an effective antispastic drug (Bein, 1972; Fehr & Bein, 1974). The finding that baclofen inhibits spinal neurotransmission at concentrations (Pierau & Zimmerman, 1973; Curtis *et al.*, 1974; Davidoff & Sears, 1974; Fehr & Bein, 1974; Fukuda *et al.*, 1977; Fox *et al.*, 1978; Curtis *et al.*, 1981; Davies, 1981) much lower than that producing skeletal neuromyal transmission blockade (Glavinovic, 1979) indicates that an important site of its antispastic action resides in the spinal cord.

A number of questions with respect to the spinal action of baclofen remain to be firmly resolved. For example, where is the primary inhibitory site of action in the spinal cord? Results from several studies showed that baclofen inhibits synaptic transmission without causing a significant change of the membrane potential and input resistance of motoneurones, suggesting that baclofen attenuates synaptic transmission by a presynaptic mechanism in reducing transmitter output (Pierau & Zimmerman, 1973; Fox *et al.*, 1978; Curtis *et al.*, 1981; Davies, 1981). Conversely, baclofen has been shown to hyperpolarize motoneurones and to attenuate the membrane excitability; this may contribute to its synaptic depressant action (Fox *et al.*, 1978; Davies, 1981).

The second question relates to the selectivity of baclofen in depressing synaptic transmission. Earlier experiments indicate that baclofen selectively inhibits excitatory transmission to the motoneurones, leaving the inhibitory transmission intact (Pierau & Zimmerman, 1973; Fukuda *et al.*, 1977; Kato *et al.*, 1978; Davies, 1981). On the other hand, baclofen reduces the excitatory and the inhibitory potentials evoked in neurones of the hippocampal and cortical slices (Scholfield, 1983; Blaxter & Carlen, 1985; Inoue *et al.*, 1985; Howe *et al.*, 1987) as well as the inhibitory potential in cultured hippocampal neurones (Harrison *et al.*, 1988). Hence, the suggestion that baclofen affects only excitatory synapses in the spinal cord is intriguing (Pierau & Zimmerman, 1973; Davies, 1981).

Another question concerns the type of $GABA_B$ receptors mediating the inhibitory action of baclofen. Recent studies indicate the presence of two pharmacologically distinct types of $GABA_B$ receptors in the rat hippocampus. The postsynaptic $GABA_B$ receptors mediating membrane hyperpolarization, are coupled to K^+ channels and blocked by the $GABA_B$ antagonist phaclofen, whereas the presynaptic $GABA_B$ receptors responsible for the depression of synaptic potentials, appear to be coupled to different ionic channels and are insensitive to phaclofen (Dutar & Nicoll, 1988). It remains to be established whether the $GABA_B$ receptor mediating the inhibitory action of baclofen in the spinal cord is phaclofen-sensitive or insensitive.

Methods

Neonatal (12–16 day) Sprague-Dawley rats were used in this study. The procedures used in obtaining thin (500 μm) transverse thoracolumbar spinal cord slices have been described in detail before (Dun & Mo, 1989). Slices with ventral and dorsal rootlets removed from the neonatal rats were incubated in Krebs solution at room temperature (21 ± 1°C) until used. The slices were transferred to the recording chamber (<0.5 ml) and superfused with a Krebs solution of the following composition (mM): NaCl 127, KCl 1.9, KH_2PO_4 1.2, $CaCl_2$ 2.4, $MgSO_4$ 1.3, $NaHCO_3$ 26 and glucose 10. The solution was saturated with 95% O_2 and 5% CO_2, and the temperature of the solution reaching the slice was maintained at 34 ± 1°C.

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Intracellular recordings were made from neurones in the ventral horn of the thoracolumbar spinal cord slices by means of glass microelectrodes filled with 3 M potassium acetate; their impedance varied between 60 and 100 MΩ. Electrical stimulation of the ventral and dorsal roots was accomplished via a concentric bipolar electrode positioned close to the respective rootlets. Signals were amplified via an Axoclamp 2A, using the bridge mode and displayed on a Gould Digital Oscilloscope and on a Gould pen recorder. Pharmacological agents, with the exception of glutamate, were dissolved in Krebs solution and applied to the slices in known concentrations. Glutamate (100 mM) dissolved in Krebs solution was applied from a micropipette positioned above the recording motoneurone by a puff of nitrogen (Picospritzer, General Valve Co.) at 350 kPa and a duration of 10 to 20 ms. E.p.s.ps and i.p.s.ps illustrated in the figures usually represent averages of four to eight signals. Unless stated otherwise, (±)-baclofen was used.

The following compounds were used: (±)-baclofen (Research Biochemicals Inc.), phaclofen (Research Biochemicals Inc.), (−)-bicuculline methobromide (Sigma), γ-amino-n-butyric acid (Sigma), picrotoxin (Sigma), sodium L-glutamic acid (Sigma), (±)-nipecotic acid (Research Biochemicals Inc.), (+)-baclofen and (−)-baclofen (gift from Ciba-Geigy).

Results

Data were collected from 108 motoneurones from 78 slices. Neurones in the ventral horn were identified as motoneurones by the appearance of an antidromic spike potential following stimulation of the ventral rootlets. We have previously characterized two types of motoneurones, type I and type II, in the neonatal rat spinal cord slices, according to their axonal conduction velocity and passive membrane properties (Jiang & Dun, 1986, 1987). Type I motoneurones have mean conduction velocity, resting membrane potential, input resistance and time constant of 41 m s^{-1}, −66 mV, 21 MΩ and 3 ms, whereas the corresponding values for type II motoneurones are 12 m s^{-1}, −63 mV; 109 MΩ and 7 ms, respectively. Type I and type II motoneurones may correspond to the α- and γ-motoneurones described in *in vivo* cat spinal cord (Eccles, 1964). Here, as the effects of baclofen on synaptic responses were similar irrespective of the types of rat motoneurones, the results were pooled.

Spontaneous synaptic activities

Spontaneously occurring excitatory (depolarizing) postsynaptic potentials (e.p.s.ps) were recorded in six motoneurones. When recorded at the membrane potential of between −60 and −70 mV, the amplitude of spontaneous e.p.s.ps varied from less than 1 to over 10 mV (Figure 1). Baclofen (1 or 5 μM) reversibly eliminated the spontaneous e.p.s.ps in all 4 cells tested (Figure 1).

GABA at higher concentrations, i.e. 1 to 10 mM, abolished spontaneous activities as well. The inhibitory action of GABA could be potentiated by pretreating the slices with the GABA uptake inhibitor, nipecotic acid (0.1–1 mM; Krogsgaard-Larsen & Johnston, 1975). Figure 1 illustrates a typical experiment in which GABA (0.1 mM) in the presence of nipecotic acid (0.1 mM) reversibly abolished the spontaneous activities in the same motoneurone where the inhibitory effect of baclofen (1 μM) was first determined. Similar results were obtained in three other motoneurones. Nipecotic acid caused a transient reduction of the frequency and amplitude of spontaneous e.p.s.ps in this but not in three other motoneurones. This might be due to a potentiating effect of nipecotic acid on endogenously released GABA from the slice.

Spontaneously occurring inhibitory (hyperpolarizing) postsynaptic potentials (i.p.s.ps) constituted a second type of spontaneous activities that were detected in four motoneurones (Figure 2a). The amplitude of spontaneous i.p.s.ps ranged from less than 1 to 10 mV. Baclofen (1 or 5 μM) eliminated the spontaneous i.p.s.ps in all four motoneurones tested and the effect was fully reversible after washing with drug-free solution (Figure 2a).

Evoked i.p.s.ps

It has been reported previously that stimulation of ventral or dorsal roots elicits a strychnine-sensitive i.p.s.p. in rat

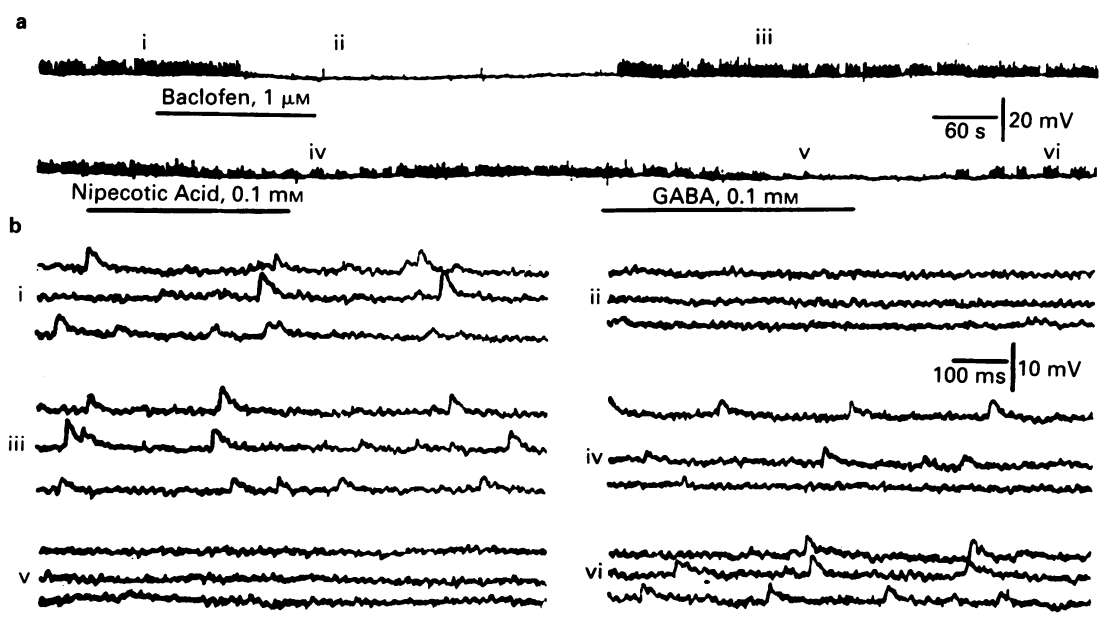

Figure 1 Reduction of spontaneous e.p.s.ps by baclofen and GABA in the presence of nipecotic acid in a rat motoneurone. Spontaneous e.p.s.ps occurred at a high frequency in an otherwise quiescent motoneurone. Superfusion of baclofen (1 μM) as indicated by a solid bar eliminated the spontaneous activities and the effect was fully reversible after 4 minutes of wash. Superfusion of the slice with the GABA uptake inhibitor nipecotic acid (0.1 mM) transiently reduced the frequency as well as the amplitude of spontaneous e.p.s.ps. After superfusing the slice with nipecotic acid, GABA (0.1 mM) reversibly eliminated the spontaneous activities. Representative traces of spontaneous e.p.s.ps taken at times (i)–(vi) marked on the top two traces are shown in the lower panel: (a) are pen recorder traces and (b) are oscilloscope tracings plotted by a Gould Colorwriter. The resting membrane potential was −61 mV.

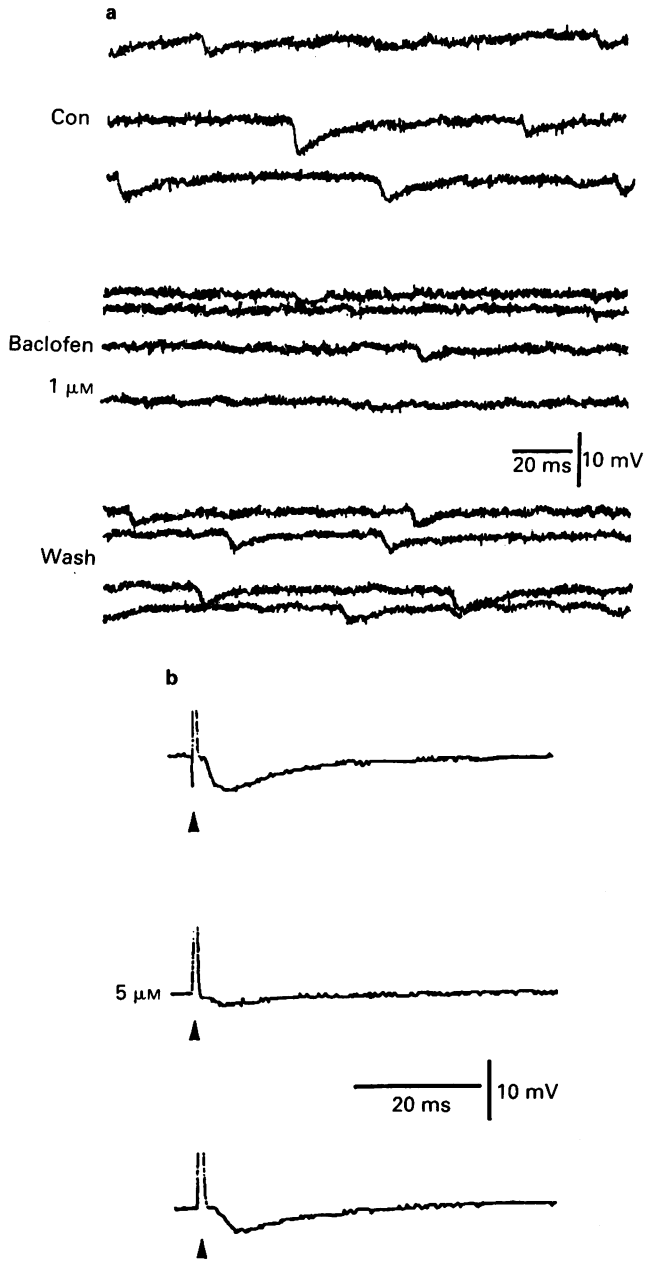

Figure 2 Spontaneous and evoked i.p.s.ps blocked by baclofen in two rat motoneurones. (a) Spontaneously occurring i.p.s.ps before, during and after superfusion of the slice with baclofen (1 μM); (b) i.p.s.ps evoked by dorsal root stimulation as indicated by an arrowhead (upper trace). After superfusing the slice with baclofen (5 μM) for 5 min, the i.p.s.p. was reduced by 65%. The effect was fully reversible. The fast transient preceding the i.p.s.p. was a stimulus artifact. Recordings in (a) and (b) were taken from two different motoneurones which had a resting membrane potential of −74 and −68 mV, respectively.

motoneurones (Dun & Jiang, 1986). In the present study, dorsal root stimulation evoked an i.p.s.p. in five motoneurones (Figure 2b) and a mixed response consisting of an initial e.p.s.p. followed by an i.p.s.p. in another four motoneurones (Figure 3).

In contrast to the earlier report that the inhibitory potential in cat motoneurones was resistant to baclofen (Pierau & Zimmermann, 1973), the i.p.s.ps were sensitive to baclofen in all three motoneurones tested; a representative experiment is illustrated in Figure 2b. Moreover, baclofen preferentially blocked the i.p.s.p. in two motoneurones that displayed a mixed response, resulting in an initial augmentation of the e.p.s.p. amplitude; the latter was eventually eliminated by the continuous superfusion of baclofen (Figure 3). The recovery

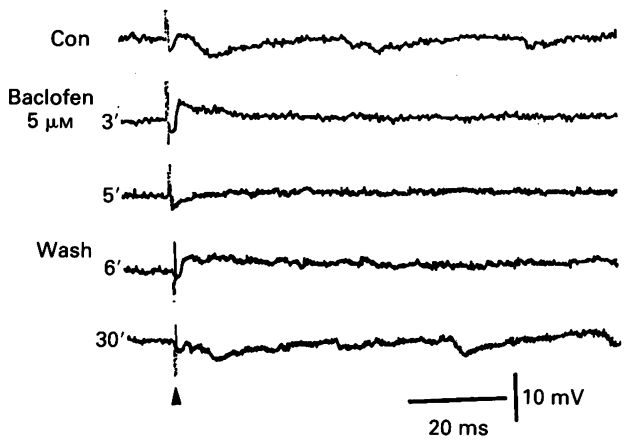

Figure 3 A mixed response of e.p.s.p. and i.p.s.p. blocked by baclofen in a rat motoneurone. Con: dorsal root stimulation as indicated by an arrowhead evoked a small e.p.s.p. followed by an i.p.s.p. with several spontaneous i.p.s.ps superimposed on the tail-end of the trace. Superfusion of baclofen (5 μM) for 3 min eliminated the i.p.s.p., consequently increasing the initial e.p.s.p.; both the e.p.s.p. and i.p.s.p. were eliminated after 5 min. The recovery of the synaptic responses followed a reversed order, that is, e.p.s.p. first returned after 6 min of washing. After 30 min of washing, dorsal root stimulation elicited an i.p.s.p. which was not preceded by a detectable e.p.s.p. This cell had a resting membrane potential of −67 mV.

from the effect of baclofen followed a reversed order, i.e., the e.p.s.p. first returned and then the i.p.s.p. (Figure 3).

Evoked e.p.s.ps

Stimulation of dorsal rootlets evoked a monophasic e.p.s.p. (e.g. Figure 4) or a composite response of two or more e.p.s.ps (Figures 6, 7, 9, 10). The synaptic latency of e.p.s.ps varied in different motoneurones, ranging from 1 to a few milliseconds (Figures 4–10). The short-latency e.p.s.ps probably represent monosynaptic connections from the primary afferents, whereas e.p.s.ps of longer latencies constitute di- or polysynaptic responses. Again, as the effects of baclofen on short and long-latency e.p.s.ps were similar, data were pooled here.

When applied to motoneurones, baclofen consistently reduced the e.p.s.p. amplitude in a concentration-dependent manner; the concentrations used in this study ranged from 0.5 to 50 μM. The dose-effect relationship of baclofen on the amplitude and duration of e.p.s.ps is shown in Table 1; the EC_{50} calculated by the Hill plot was 2.4 μM. Figure 4 shows the effects of three concentrations of baclofen on a motoneurone. The amplitude of e.p.s.ps was depressed in a concentration-dependent manner, whereas only at the highest concentration (50 μM) did baclofen cause a small and transient hyperpolarization.

The depressant effect of lower concentrations ($\leqslant$10 μM) of baclofen was readily reversible several minutes after washing the slices with drug-free Krebs solution (Figures 4, 6, 7). At the higher concentrations (10–50 μM), a wash period of 30 min or more was needed for the response to return to control level (for example, Figure 10).

Table 1 Reduction of e.p.s.p. amplitude and duration by baclofen

	% *reduction* (mean ± s.d.)				
	0.5 μM (n = 6)	1 μM (n = 14)	5 μM (n = 19)	10 μM (n = 9)	50 μM (n = 6)
Amplitude[a]	29 ± 18[b]	33 ± 15[c]	58 ± 21[c]	63 ± 16[c]	90 ± 18[c]
Duration[a]	20 ± 14[b]	27 ± 10[c]	40 ± 20[c]	45 ± 25[c]	87 ± 20[c]

[a] One-way variance analysis: $P < 0.01$.
[b] Paired t test: $P < 0.05$.
[c] Paired t test: $P < 0.01$.

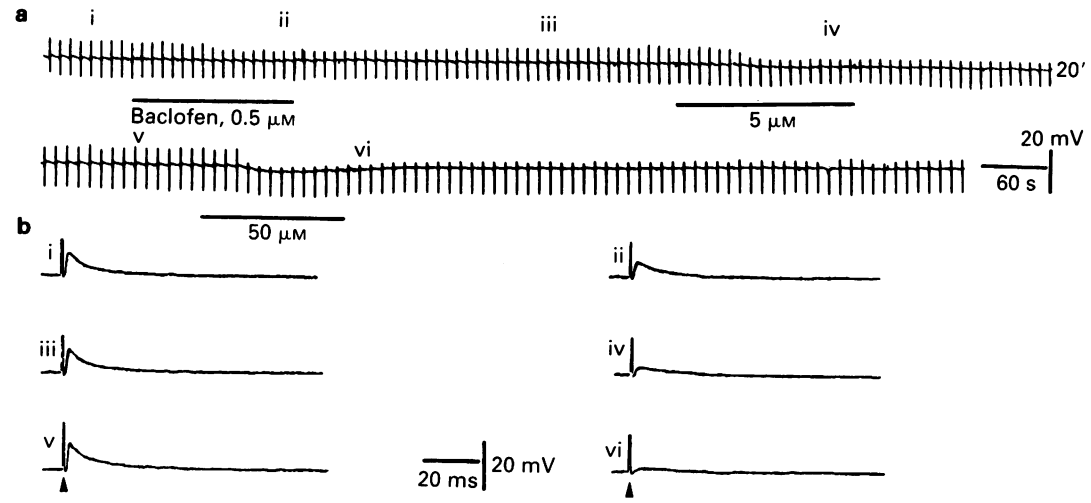

Figure 4 Concentration-dependent depression of evoked e.p.s.ps by baclofen. The upper two traces in (a) represent continuous chart recording interrupted by 20 min of wash at the end of the upper trace. Upward deflections represent e.p.s.ps evoked by dorsal root stimulation and downward deflections are hyperpolarizing electrotonic potentials induced by constant current pulses (not shown). Baclofen was applied in successively higher concentrations as indicated by solid bars and reduced the e.p.s.p. amplitude in a concentration-dependent manner. At concentrations of 0.5 and 5 μM, baclofen decreased the amplitude of e.p.s.ps without causing a detectable change of membrane potential or input resistance as indicated by no apparent change of the amplitude of hyperpolarizing electrotonic potentials. At the concentration of 50 μM, baclofen reduced the e.p.s.ps and caused a small and transient hyperpolarization. Traces in (b) are signal averagings of 4 e.p.s.ps taken at times marked on the upper two traces. The fast transient preceding each e.p.s.p. was a stimulus artifact. The resting membrane potential was -73 mV.

The stereospecificity of the action of baclofen on motoneurones was evaluated by use of (+)- and (−)-baclofen. At concentrations up to 50 μM, (+)-baclofen caused little or no depression of the synaptic responses nor a hyperpolarization in any of the 8 motoneurones tested (Figure 5). On the other hand, (−)-baclofen was consistently effective in reducing the e.p.s.ps in all 10 motoneurones tested (Figure 5); the average reduction caused by (−)-baclofen (2 μM) was 80.8 ± 13.5% (mean ± s.d.). When tested in the same motoneurones, the average reduction of e.p.s.ps caused by baclofen (2 μM) and by the same concentration of (−)-baclofen was 38.2 ± 12.8% and 73.8 ± 8.3%, respectively ($n = 4$).

GABA also depressed the amplitude of e.p.s.ps, but at much higher concentrations (1–10 mM). Even when tested at such high concentrations, the magnitude of reduction was moderate and variable among different motoneurones tested. A more consistent and longer-lasting effect of GABA could be demonstrated by pretreating the slices with the GABA uptake inhibitor nipecotic acid; a typical experiment is illustrated in Figure 6. It should be noted that when compared in the same motoneurones, baclofen at the concentrations that reduced synaptic potentials caused little or no change of membrane potential or input resistance, whereas GABA at the concentrations that produced a reduction of e.p.s.ps invariably caused

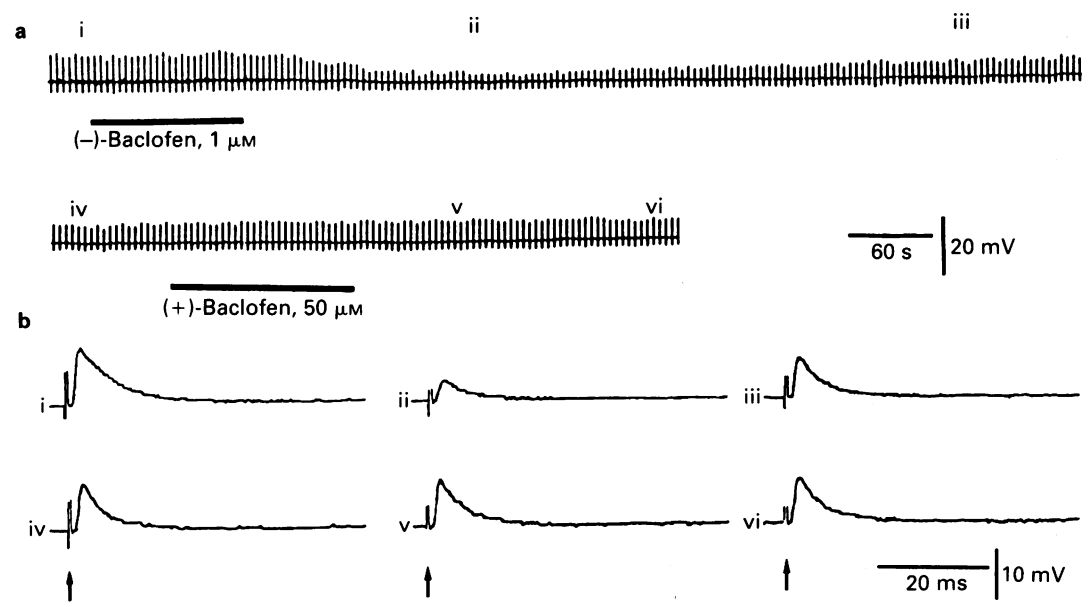

Figure 5 Effects of (−)- and (+)-baclofen on the e.p.s.ps in a rat motoneurone. Traces in (a) are continuous chart recordings. Upward and downward deflections represent e.p.s.ps and hyperpolarizing electrotonic potentials used to monitor membrane resistance change, respectively. Superfusion of (−)-baclofen (1 μM) as indicated by the solid bar reduced the amplitude of e.p.s.ps by 70% without causing a significant change of the resting membrane potential and input resistance; the effect was reversible after washing. Superfusion of (+)-baclofen (50 μM) was without effect. E.p.s.ps taken at times indicated by numbers on upper two traces are shown in panel (b). Recordings were taken from the same motoneurone which had a resting membrane potential of -72 mV.

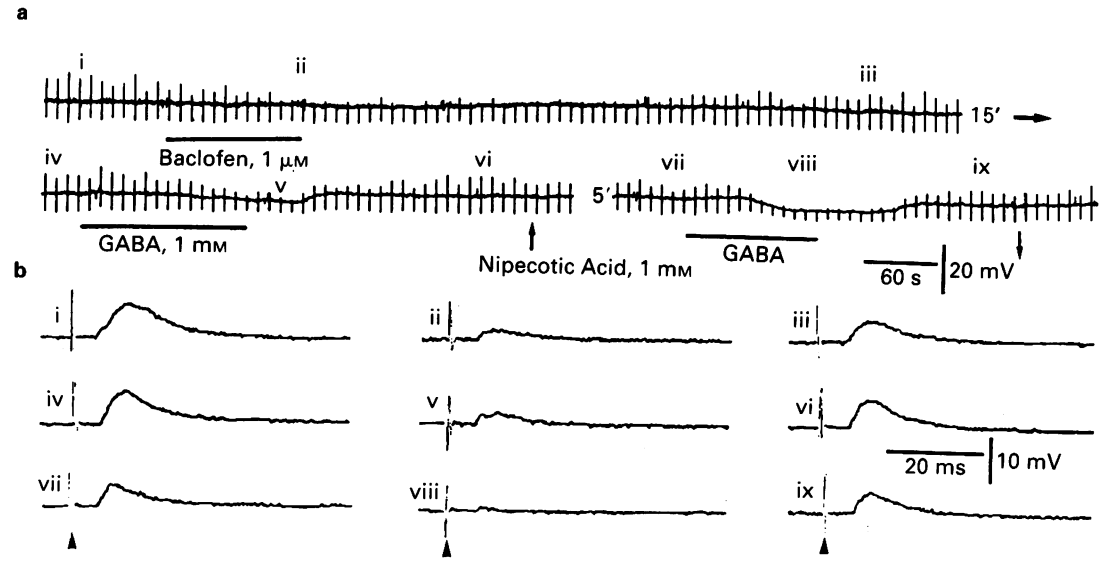

Figure 6 Comparison of the effects of baclofen and GABA in a rat motoneurone. Upper two traces represent continuous chart recordings interrupted by numerals indicating minutes. Upward and downward deflections are evoked e.p.s.ps and hyperpolarizing electrotonic potentials used to monitor membrane input resistance change, respectively. Baclofen (1 μM) reduced the e.p.s.ps by 65%, without causing a significant change of membrane potential and input resistance, and the effect was fully reversible. GABA (1 mM) reduced the e.p.s.ps to about 30% of control level and caused a small hyperpolarization associated with a decrease of membrane resistance, as indicated by a reduction of the hyperpolarizing electrotonic potentials; the effect was rapidly reversed. Nipecotic acid (1 mM) was added to the perfusing solution between two arrows. GABA in the presence of nipecotic acid caused complete abolition of e.p.s.ps and a marked but transient hyperpolarization. E.p.s.ps taken at times indicated by numbers on upper two traces are shown in panel (b). Recordings were taken from the same motoneurone which had a resting membrane potential of −61 mV.

either a depolarization or hyperpolarization; both types of response were associated with a decrease of apparent input resistance (Figures 6 and 10b). The synaptic depressant action of GABA was not antagonized by bicuculline (50 to 100 μM) in any of the 5 motoneurones tested (Figure 7).

Membrane hyperpolarization by baclofen

At the concentration of 5 μM or below, baclofen depressed the e.p.s.ps without causing a detectable change of membrane potentials and input resistances (Figures 4, 6, 7, 10). At higher concentrations, baclofen blocked synaptic transmission and hyperpolarized some but not all the neurones examined. Even at the highest concentration (50 μM), baclofen hyperpolarized 6 of the 10 motoneurones tested. The magnitude of response varied considerably in different cells, ranging from a few (Figure 4) to about 10 mV (Figure 10). The hyperpolarization was associated with a small to moderate (5–30%) decrease in membrane input resistance (Figures 4 and 10). The magnitude of reduction of e.p.s.ps was significantly greater than that of decrease of membrane resistance in a given motoneurone tested.

Further, the time course of depression of e.p.s.ps generally outlasted the hyperpolarization by many minutes. In the experiment illustrated in Figure 10c, baclofen (50 μM) blocked the e.p.s.p. and hyperpolarized the cell in question; the membrane potential returned to the resting level at a time when the e.p.s.p. amplitude showed only a marginal recovery.

Effects on spike after-hyperpolarization

The after-hyperpolarization following the spike elicited by direct intracellular stimulation was reversibly reduced by baclofen (10 μM) in 3 of the 5 motoneurones investigated (Figure 8); the mean reduction was 61%. On the other hand, the peak amplitude and duration of the spike was not affected.

Effects of GABA antagonists

Pretreating the slices with two known $GABA_A$ receptor antagonists bicuculline and picrotoxin (10–50 μM) did not nullify the inhibitory effects of baclofen in any of the 9 motoneurones tested.

Phaclofen, a $GABA_B$ receptor antagonist (Kerr *et al.*, 1987), in concentrations of 0.1 to 1 mM was not effective in antagonizing the inhibitory action of baclofen on e.p.s.ps in any of the 5 cells tested. Phaclofen (0.1 mM) did not cause a significant change of the e.p.s.p. amplitude ($n = 2$), whereas at 1 mM, phaclofen reduced the e.p.s.ps by an average of 36% in three neurones tested; one such experiment is shown in Figure 9. The effect was reversible upon washing. Moreover, the magnitude of reduction caused by baclofen in the presence of phaclofen was greater than that produced by baclofen alone (Figure 9).

Effects on glutamate-induced depolarizations

To learn more about the site of inhibitory action, the effect of baclofen on synaptic responses and on glutamate-induced depolarizations were evaluated in the same motoneurones ($n = 5$). Glutamate or a related excitatory amino acid is thought to be the most likely candidate for a transmitter role in the spinal cord (Fagg & Foster, 1983). In these experiments, glutamate (100 mM) was applied to the motoneurones by pressure, resulting in a rapid membrane depolarization (Figure 10). Baclofen at lower concentrations (≤ 5 μM) depressed the e.p.s.p. without causing a significant change of the glutamate-induced depolarization (Figure 10a). At higher concentrations, e.g. 50 μM, baclofen blocked the e.p.s.p., hyperpolarized the neurone and attenuated the glutamate-induced depolarization (Figure 10c). However, returning the membrane potential to the original level by injection of depolarizing currents did not restore the e.p.s.p. and the glutamate depolarization to the control level (Figure 10c). The recovery of glutamate-induced depolarization preceded the return of e.p.s.ps (Figure 10c). Finally, when tested in the same cell, GABA (10 mM) caused a depolarization and a reduction of the amplitude of e.p.s.ps as well as the glutamate-induced depolarizations (Figure 10b). The effects of GABA were shorter-lasting compared to those caused by baclofen.

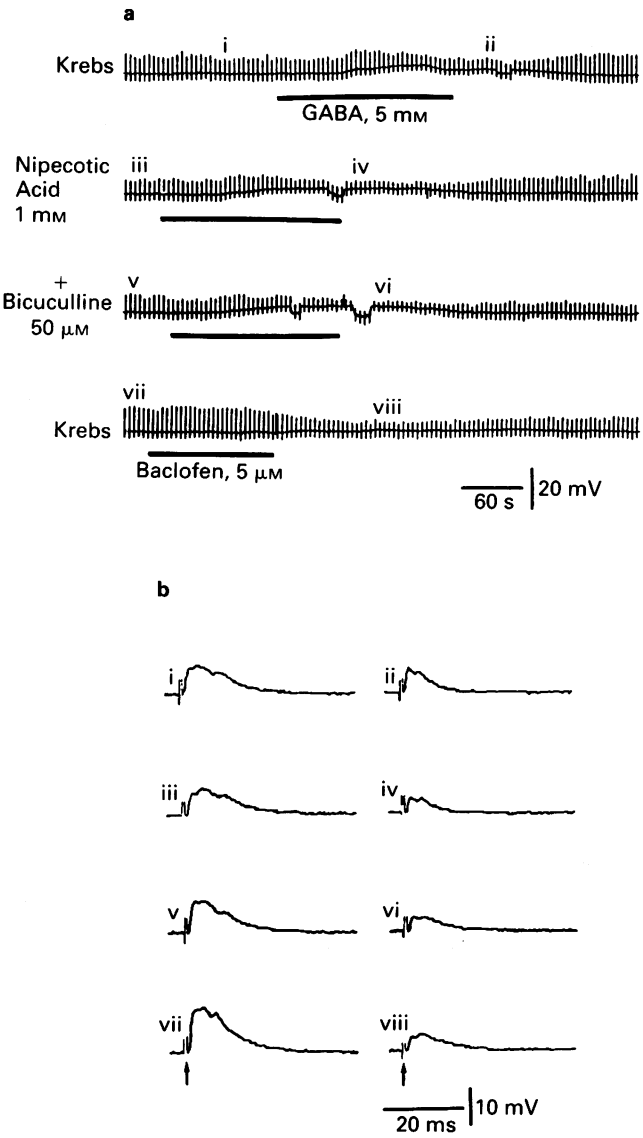

Figure 7 Effects of GABA in the presence of nipecotic and bicuculline in a rat motoneurone. Traces in (a) represent continuous chart recordings interrupted by washing periods (not shown). Upward and downward deflections represent evoked e.p.s.ps and hyperpolarizing electrotonic potentials. In the upper trace, superfusion of GABA (5 mM) as indicated by a solid bar caused a small depolarization, reduction of the amplitude of electrotonic potentials and of e.p.s.ps; the effects were fully reversible. Second trace: in the presence of nipecotic acid (1 mM), GABA caused a larger depolarization and reduction of the e.p.s.ps. At the peak of depolarization, returning the membrane potential to the resting level by current injection did not restore the e.p.s.ps to the control level. Third trace: in the presence of nipecotic acid and bicuculline (50 μM), the effects of GABA were essentially the same as those shown in the second trace. Last trace: superfusion of baclofen (5 μM) reduced the e.p.s.ps but did not cause a noticeable change of membrane potential. E.p.s.ps taken at times indicated by numbers in (a) are shown in (b). Arrows indicate the stimulus artifacts. Recordings were taken from the same motoneurone which had a resting membrane potential of −64 mV.

Discussion and conclusions

The present experiments were designed to address three questions concerning the actions of baclofen in the spinal cord that may underlie its effectiveness as an antispastic agent. In this respect, the findings that (−)-baclofen was more effective than baclofen in reducing the amplitude of e.p.s.ps and that (+)-baclofen was ineffective at concentrations as high as 50 μM indicate that the inhibitory action of baclofen on spinal transmission was stereospecific.

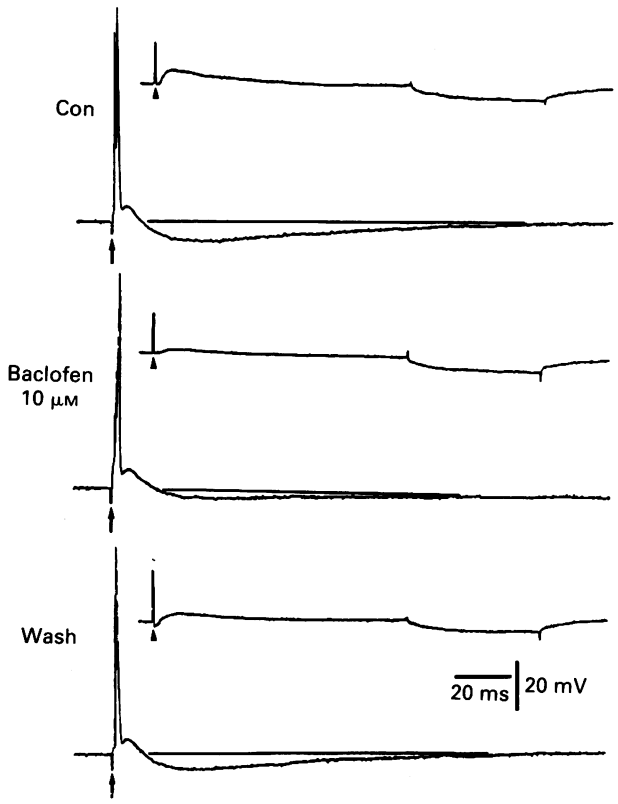

Figure 8 Reduction of spike after-hyperpolarization and e.p.s.p. by baclofen in a rat motoneurone. Action potential elicited by depolarizing current pulse (arrows) was followed by a prominent after-hyperpolarization. Inset: e.p.s.p elicited by dorsal root stimulation was followed by a hyperpolarizing electrotonic potential. Baclofen (10 μM) reduced the amplitude of after-hyperpolarization by over 50% and nearly blocked the e.p.s.p., the action potential and electrotonic potential were not changed. The effect was reversible after wash. The membrane potential of this motoneurone was −68 mV.

First, the selectivity of baclofen with respect to excitatory and inhibitory spinal transmission was examined. That baclofen inhibits central excitatory transmission has been amply demonstrated (Pierau & Zimmerman, 1973; Davidoff & Sears, 1974; Davies & Watkins, 1974; Fehr & Bein, 1974; Fukudo *et al.*, 1977; Fox *et al.*, 1978; Kato *et al.*, 1978; Curtis *et al.*, 1981; Davies, 1981; Lanthorn & Cotman, 1981; Scholfield, 1983; Blaxter & Carlen, 1985; Inoue *et al.*, 1985; Howe *et al.*, 1987; Dutar & Nicoll, 1988; Allerton *et al.*, 1989). On the other hand, the action of baclofen on inhibitory transmission appears to be less consistent; positive and negative results have been reported (Pierau & Zimmerman, 1973; Kato *et al.*, 1978; Davies, 1981; Scholfield, 1983; Blaxter & Carlen, 1985; Inoue *et al.*, 1985; Howe *et al.*, 1987; Harrison *et al.*, 1988).

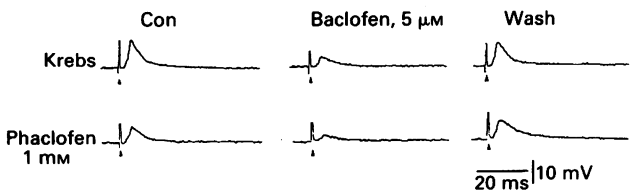

Figure 9 Effect of baclofen not antagonized by phaclofen in a rat motoneurone. Con: e.p.s.p. evoked by dorsal root stimulation. Baclofen (5 μM) reduced the e.p.s.ps to about 35% of control, and the effect was reversible after washing. Phaclofen (1 mM) reduced the e.p.s.ps to 70% of control value. Baclofen in the presence of phaclofen further depressed the e.p.s.ps to 20% of control level. The effect of baclofen and phaclofen was reversible after wash. The fast transient preceding each e.p.s.p. was a stimulus artifact. This motoneurone had a resting membrane potential of −75 mV.

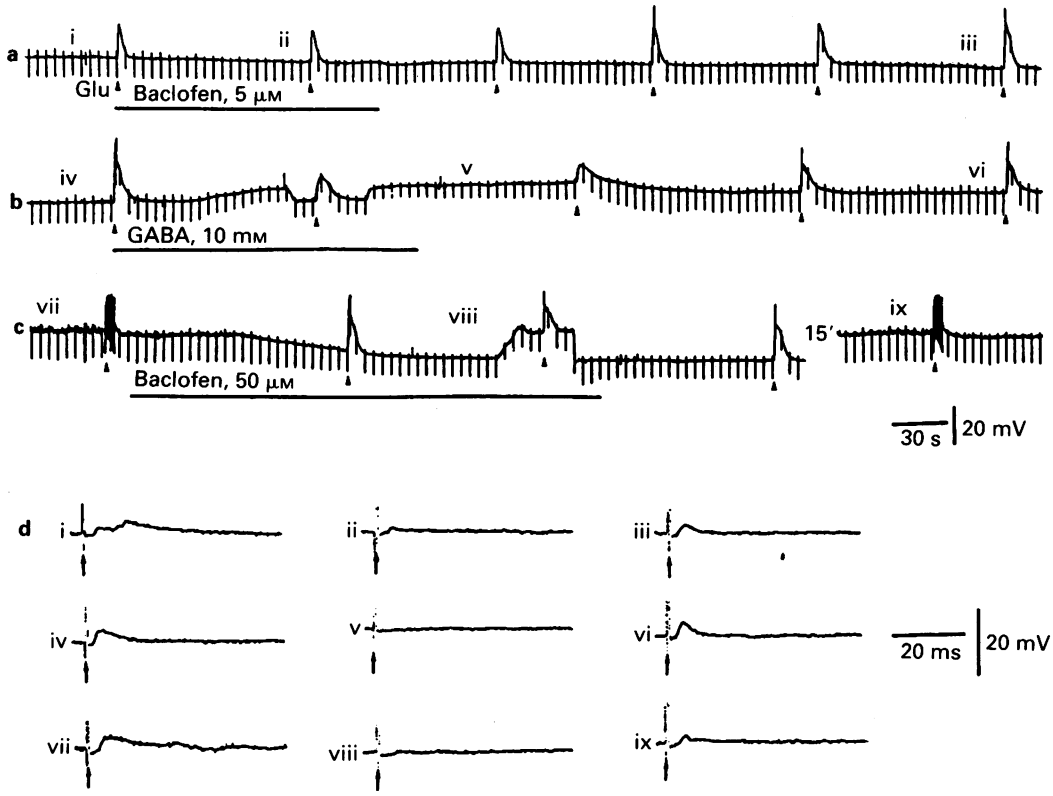

Figure 10 Effects of baclofen and GABA on e.p.s.ps and depolarizations induced by glutamate in a rat motoneurone. A micropipette containing glutamate (Glu, 100 mM) was positioned above the recording motoneurone. Glutamate was applied (indicated by arrowheads) to the vicinity of the motoneurone by a brief pulse of 20 ms and evoked a brisk depolarization and spike discharge, which was truncated by the limited frequency response of the pen recorder. (a) Small upward deflections represent evoked e.p.s.ps the amplitude of which was rather small even during control period. Baclofen (5 μM) eliminated the e.p.s.ps, whereas the glutamate-induced depolarizations were not affected. (b) Superfusion of GABA (10 mM) caused a sustained depolarization and a blockade of e.p.s.ps. Returning the membrane potential to the original level by current injection did not result in a restoration of e.p.s.ps; glutamate was still capable of eliciting a response, though attenuated. A substantial recovery of the e.p.s.p. and glutamate-induced depolarization was seen a few minutes after discontinuation of GABA superfusion. (c) Baclofen at a high concentration (50 μM) blocked the e.p.s.p. and hyperpolarized the neurone, whereas the glutamate-induced depolarization was only moderately reduced. The e.p.s.ps were not restored when the membrane potential was returned to the original level by current injection. After a period of 15 min wash, the glutamate-induced depolarization but not the e.p.s.ps returned to control level. This cell had a resting membrane potential of −72 mV.

Our results show that in concentrations comparable to those suppressing the excitatory responses, baclofen blocked the spontaneous as well as evoked i.p.s.ps in the rat motoneurones. In the two motoneurones where dorsal root stimulation evoked a mixed response of e.p.s.p. and i.p.s.p., baclofen first suppressed the i.p.s.p. and augmented the e.p.s.p. The reason for a preferential blockade of inhibitory transmission by baclofen is not known. One explanation might be that baclofen is more effective in suppressing the polysynaptic than the monosynaptic event; the longer synaptic latency of i.p.s.ps would support this contention.

However, the possibility that abolition of i.p.s.ps by baclofen as observed in the present study is secondary to the blockade of release of excitatory transmitters acting on inhibitory interneurones cannot be entirely excluded. In this respect, paired recordings made from rat hippocampal neurones in culture clearly demonstrated that baclofen inhibits directly the release of GABA from GABAergic neurones (Harrison *et al.*, 1988). This finding in conjunction with earlier results that baclofen reduces the release of a number of putative transmitters including noradrenaline and acetylcholine, in addition to excitatory amino acids (Nistri, 1975; Potashner, 1979; Bowery *et al.*, 1980; Johnston *et al.*, 1980; Collins *et al.*, 1982; Kato *et al.*, 1982) is not in favour of the contention that baclofen selectively inhibits the release of excitatory amino acids, hence excitatory transmission in the central nervous system.

A second question the present study attempted to address is the site of inhibitory action of baclofen in the spinal cord. In aggreement with the majority of earlier reports, our results suggest that baclofen inhibits synaptic transmission by diminishing transmitter release from nerve fibres presynaptic with respect to motoneurones.

Firstly, baclofen at concentrations that depressed synaptic transmission caused little or no change of the electrical properties of motoneurones. Only at higher concentrations did baclofen cause a membrane hyperpolarization and conductance increase as well as synaptic blockade. In the latter cases, the synaptic depression and hyperpolarization exhibited different time courses, and the e.p.s.ps were not restored to the control level upon returning the membrane potential to the resting level by depolarizing currents. These findings indicate that hyperpolarization and synaptic depression are two separate events. Similarly, the magnitude of reduction of synaptic potentials produced by a given concentration of baclofen was much greater than could be accounted for by an increase of membrane conductance. Hence, the site responsible for membrane hyperpolarization on one hand and synaptic depression on the other appears to be distinct.

Secondly, baclofen at the concentrations that depressed synaptic transmission, did not significantly change the postsynaptic chemosensitivity to the putative excitatory transmitter glutamate (Fagg & Foster, 1983). An attenuation of the glutamate-induced responses occurred only in those neurones where higher concentrations of baclofen caused a concomittant membrane hyperpolarization and decreased input resistance. The reduction of glutamate response could

be explained by a short-circuiting effect of baclofen on postsynaptic membrane and is not related to the diminished sensitivity of postsynaptic receptors to glutamate. In this regard, a small reduction of glutamate response as observed here was consistent with a relatively small change of membrane resistance.

Collectively, these findings provide strong evidence that the primary site of inhibitory action of baclofen is presynaptic, although at higher concentrations, the postsynaptic short-circuiting effect may accentuate the presynaptic inhibitory action of baclofen. As baclofen caused a relatively small or no detectable hyperpolarization in the majority of motoneurones investigated, its postsynaptic action was not studied in any detail. Other studies indicate that balcofen hyperpolarizes the membrane by increasing K^+ conductance (Dutar & Nicoll, 1988). On the other hand, GABA in concentrations that diminished e.p.s.ps invariably depolarized or hyperpolarized the neurones. The synaptic depressant effect of GABA was not antagonized by bicuculline, suggesting the involvement of $GABA_B$ receptors in the inhibitory action of GABA.

The last question concerned the type of $GABA_B$ receptors with which baclofen interacts to produce synaptic inhibition in the spinal cord. Recent studies show that baclofen interacts with post- and pre-synaptic $GABA_B$ receptors in the hippocampus, resulting in a hyperpolarization and depression of synaptic transmission, respectively (Dutar & Nicoll, 1988). In addition, the postsynaptic $GABA_B$ receptors are coupled to potassium channels and are blocked by phaclofen, a $GABA_B$ receptor antagonist (Kerr *et al.*, 1987), whereas the presynaptic $GABA_B$ receptors are phaclofen-insensitive (Dutar & Nicoll, 1988). A negative result with respect to phaclofen on the presynaptic inhibitory action of baclofen in cultured hippocampal neurones has also been reported (Harrison, 1988). Our finding that the presynaptic inhibitory action of baclofen persisted in the presence of phaclofen in concentrations that have been shown to be effective in suppressing the $GABA_B$ receptor-mediated hyperpolarization (Dutar & Nicoll, 1988; Hauso & Gallagher, 1988), raises the possibility that the pharmacological characteristics of presynaptic $GABA_B$ receptors in the spinal cord are similar to those described in the hippocampus. It is pertinent to mention that the e.p.s.p. amplitude was consistently and reversibly depressed by phaclofen at high concentrations, suggesting that the latter behaves like a weak agonist in the spinal cord. Phaclofen has been shown to antagonize the inhibitory action of baclofen on cat spinal transmission (Kerr *et al.*, 1987). The reason for the discrepancy between our results and those reported by Kerr *et al.* (1987) is not immediately clear. Species difference notwithstanding, differences in the methods of administration, i.e., bath application vs. microiontophoresis, and the concentrations of phaclofen might explain the discrepancy.

The mechanism by which baclofen reduces the output of transmitters from presynaptic nerve fibres in the spinal cord is of interest and remains to be investigated. Results from the hippocampus indicate that the phaclofen-sensitive $GABA_B$ receptors are coupled to K^+ channels (Dutar & Nicholl, 1988). Studies on somata of sensory neurones show that activation of $GABA_B$ receptors by baclofen reduced the duration of calcium-dependent action potentials and voltage-dependent Ca^{2+} currents (Dunlap, 1981; Robertson & Taylor, 1986; Dolphin & Scott, 1987). If the results from the hippocampus and sensory ganglia can be extrapolated to the spinal neurones, it may be speculated that the phaclofen-insensitive $GABA_B$ receptors are coupled to voltage-dependent Ca^{2+} channels. In this respect, the observation that baclofen reduced the spike after-hyperpolarization, which is thought to be caused by an influx of Ca ions (Barrett & Barrett, 1976) is consistent with the hypothesis that activation of presynaptic $GABA_B$ receptors may reduce Ca^{2+} influx across the terminal membrane, thereby attenuating synaptic transmission (Robertson & Taylor, 1986; Allerton *et al.*, 1989). There is however an apparent disparity between the concentrations of baclofen necessary to cause an attenuation of e.p.s.p. amplitude and inhibition of calcium currents. For example, 100 μM (−)-baclofen produces an average reduction of 28% and 70% calcium currents in cat and rat dorsal root ganglion cells, respectively (Robertson & Taylor, 1986; Dolphin & Scott, 1987), whereas, 50 μM baclofen causes 90% inhibition of e.p.s.p. amplitude in the present study. Thus, a validation of this hypothesis will need a direct testing of the effect of baclofen on nerve terminal membrane calcium currents.

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