Br. J. Pharmacol. (1989), **96**, 29–38

Actions of the $GABA_B$ agonist, (−)-baclofen, on neurones in deep dorsal horn of the rat spinal cord *in vitro*

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1 The electrophysiological actions of the $GABA_B$ agonist, (−)-baclofen, on deep dorsal horn neurones were studied using an *in vitro* preparation of the spinal cord of 9–16 day old rat.

2 On all neurones tested, (−)-baclofen (100 nM–30 μM) had a hyperpolarizing action which was associated with a reduction in apparent membrane input resistance. The increase in membrane conductance was dose-dependent and had a Hill coefficient of 1.0.

3 The (−)-baclofen-activated hyperpolarization persisted in the presence of bicuculline (50 μM) and Mg^{2+} (20 mM).

4 The reversal potential of the hyperpolarizing event was estimated at 102 mV and was made less negative by increasing the external concentration of potassium ions.

5 Over the same concentration range, (−)-baclofen also depressed the polysynaptic composite excitatory postsynaptic potentials (e.p.s.ps) evoked in these neurones by electrical stimulation of the dorsal root entry zone.

6 The potassium channel blockers caesium, applied intracellularly, and barium, applied extracellularly, depressed the postsynaptic response to baclofen but not its effect on e.p.s.ps.

7 We propose that (−)-baclofen has more than one mechanism of action in spinal dorsal horn: a postsynaptic action mediated via an increase in potassium conductance and a presynaptic action that is not associated with potassium channels and may be mediated via calcium channels. Since previous studies have demonstrated little effect of (−)-baclofen on transmitter release in spinal cord, it is possible that the postsynaptic hyperpolarizing action of (−)-baclofen may account for its clinical potency as an anti-spastic agent.

Introduction

γ-Aminobutyric acid (GABA) is widespread as an inhibitory neurotransmitter within the CNS. There is considerable evidence to suggest that its receptors can be divided into two subtypes, $GABA_A$ and $GABA_B$. Muscimol, isoguvacine and 3-aminopropanesulphonic acid are selective agonists at the $GABA_A$ receptor whereas the *p*-chlorophenyl derivative of GABA, (−)-baclofen, is a highly selective agonist for the $GABA_B$ site. Furthermore, bicuculline is a potent, competitive antagonist at the $GABA_A$ receptor but has little activity at the $GABA_B$ receptor.

It is widely documented that $GABA_A$ receptor agonists exert their actions via an increase in membrane chloride conductance. The ionic conductance which results from $GABA_B$ receptor activation is, however, less well established. In isolated dorsal root ganglia, (−)-baclofen does not affect membrane potential or conductance but causes a shortening of the calcium action potential (Desarménian *et al.*, 1984; Schlichter *et al.*, 1984). This may be the result of a reduction in the amplitude of a slowly inactivating calcium current in these neurones (Robertson & Taylor, 1986). In hippocampus (Newberry & Nicoll, 1984) and substantia nigra (Pinnock, 1984), activation of the $GABA_B$ receptor results in postsynaptic membrane hyperpolarization associated with an increase in potassium conductance.

(−)-Baclofen is a highly effective anti-spastic agent at the level of the spinal cord (Fehr & Bein, 1974). *In vivo* studies have shown that applied microelectro-

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phoretically (Curtis *et al.*, 1974; Henry & Ben-Ari, 1976; Curtis *et al.*, 1981; Davies, 1981) or intravenously (Piercey & Hollister, 1979) it has a potent depressant action on the firing of neurones in dorsal horn which may be the result of either a reduction in the release of excitatory transmitter or the depression of the response to this transmitter via a postsynaptic mechanism. Of particular interest is the observation that baclofen will depress the response of dorsal horn neurones to exogenous excitants (Davies, 1981), implying the existence of a postsynaptic mechanism of action. Intracellular recording from motoneurones has failed to show any effect of systemic baclofen on membrane conductance although synaptic activity is markedly depressed (Pierau & Zimmerman, 1973; Fox *et al.*, 1978). Iontophoretic administration of baclofen to these same neurones does, however, reduce both excitability and membrane input resistance (Fox *et al.*, 1978).

Autoradiographic techniques have detected $GABA_B$ binding sites throughout spinal grey matter but demonstrate a clear band of high density binding to the dorsal horn. This is located mainly in laminae 2 and 3, but laminae 1 and 4 also appear to have a considerable number of binding sites (Price *et al.*, 1984). The effect of baclofen on spinal dorsal horn neurones is thus likely to be important in terms of its functional role.

We have therefore examined the action of baclofen on neurones in dorsal horn using an *in vitro* slice preparation of spinal cord from which we could obtain stable intracellular recordings. Some of these results have already been presented in abstract form (Allerton *et al.*, 1988).

Methods

Slice preparation

Methods used were adapted from those of Randić & co-workers (Miletić & Randić, 1980; Murase *et al.*, 1982). Experiments were performed on 9–16 day old Wistar rats. The animals were anaesthetized with ether and cooled by placing on ice for 5–10 min. When the animal was cool to the touch and the breathing was shallow, a laminectomy was performed to expose the lower thoracic and lumbosacral spinal cord together with the dorsal roots. A 1–1.5 cm length of cord was quickly excised with attached dorsal rootlets, the dura removed and each end of the cord cut to leave a 5–8 mm length of lumbar enlargement. Transverse slices (400 μm) were cut with a Vibratome (Lancer) and incubated in oxygenated artificial cerebro-spinal fluid (ACSF) at 36°C for at least 45 min before use.

Intracellular recording techniques

Slices were transferred to a recording chamber where they were continuously perfused with oxygenated artificial cerebrospinal fluid (ACSF). The slice rested upon Sylgard and was completely submerged in ACSF which flowed through the bath at a rate of $3\,ml\,min^{-1}$. It was observed from above under × 80 magnification and the recording electrode was placed in the deep dorsal horn under visual control with a micromanipulator. A bipolar electrode was placed on the dorsal root entry zone in a similar manner.

Drugs at a known concentration were dissolved in oxygenated ACSF and applied directly to the slice via the perfusion system by means of a three way tap. There was a dead time of approximately 1 min between turning the tap and the drug containing solution entering the bath. The flow rate through the bath did not change during this procedure. Since the volume of the bath was only 0.5 ml and the slice was completely submerged, equilibration took place rapidly. Drug removal was achieved simply by returning to drug-free ACSF.

Conventional intracellular recording techniques were used. Glass recording electrodes were filled with 3M KCl and had d.c. tip resistances of 40–100 MΩ when measured in physiological saline. Recording electrodes were placed in the region identified as the dorsal horn of the spinal cord under visual control, deep to the translucent band of the substantia gelatinosa (Rexed's lamina 2). The electrode was connected to a pre-amplifier with the facility for current injection (Digitimer Neurolog NL102). Membrane potential and injected current were amplified and displayed on an oscilloscope (Tektronix 5110) and chart recorder (Gould 2400S). Data were also recorded on digital tape (Sony) for future analysis.

Constant amplitude hyperpolarizing current pulses were injected into the neurone through the recording electrode to allow monitoring of membrane resistance. For initial impalement, pulses of 0.2 nA amplitude and 25 ms duration were injected at a rate of 10 Hz. As the stability of the recording became apparent, the rate of injection was reduced to 0.3 Hz and pulse duration increased (if necessary) to between 25 and 100 ms until saturation of the membrane capacitance occurred as visualized by the display of voltage on an oscilloscope. The time constant of the membrane could be established from analysis of electrotonic responses using this facility on the SCAN VI.2 (Single Channel Analysis) programme.

Current-voltage relationships could be established by variation of the amplitude of the hyperpolarizing pulses and measurement of the resulting voltage displacement (that is, the maximum potential to which

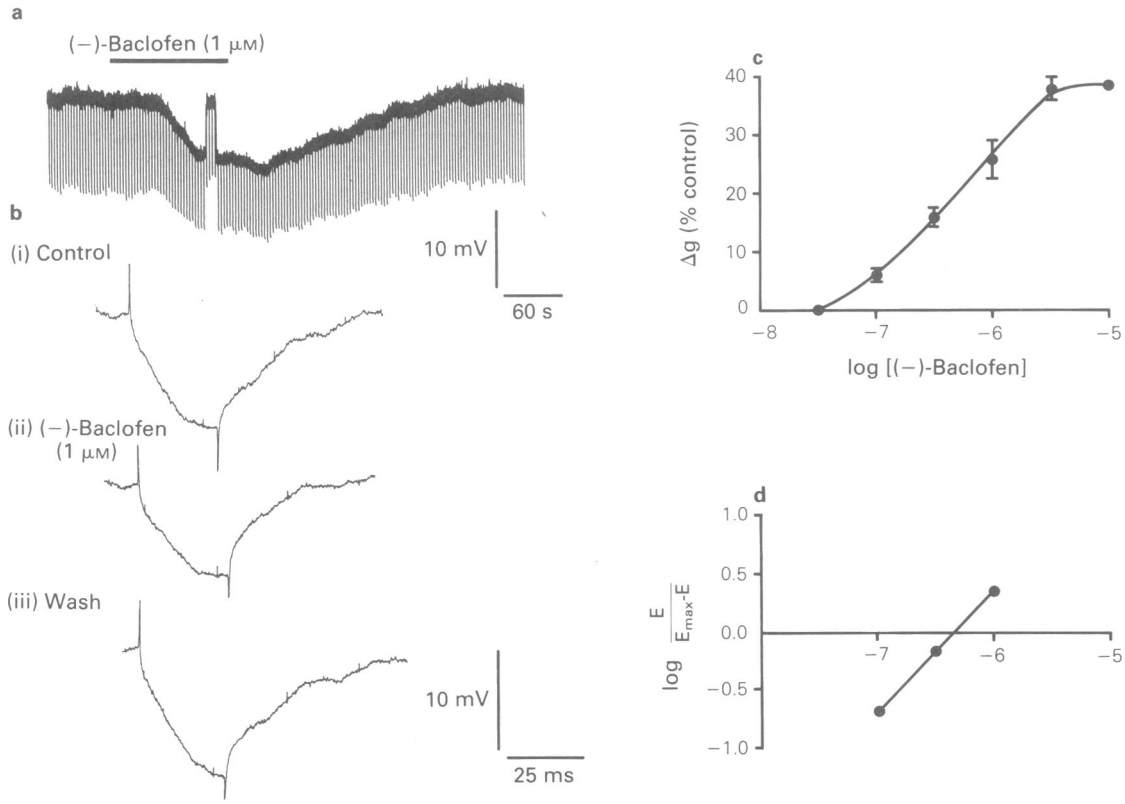

Figure 1 (−)-Baclofen, applied at concentrations of between 30 nM and 10 μM, produced hyperpolarizations in all dorsal horn neurones tested. (a) Chart recorder trace of a deep dorsal horn neurone with a resting membrane potential of −60 mV. Constant amplitude hyperpolarizing current pulses were injected into the neurone through the recording electrode producing the downward voltage deflections observed on the trace which gave an indication of membrane input resistance. (−)-Baclofen was applied for the period indicated by the solid bar, producing a hyperpolarization and a decrease in apparent input resistance. The change in input resistance was still observed when the membrane potential was restored to its original value by injection of depolarizing current showing that it is a drug-induced change and not merely a result of membrane rectification. (b) Membrane voltage response to injection of a 0.2 nA square current pulse (i) under control conditions, (ii) in the presence of (−)-baclofen (1 μM) and (iii) after washout of (−)-baclofen. These responses were elicited on the same neurone illustrated in (a) and therefore represent downward voltage deflections seen on a shorter time scale. Each represents a single voltage response captured on a storage oscilloscope and plotted out on a chart recorder. It can be seen that the membrane capacitance was fully charged and the electrode balanced with the bridge circuit. The change in conductance observed in the presence of the drug from these records is the same as that observed in (a) showing that the frequency response of the pen recorder did not attenuate the voltage response to square current pulses in this case. (c) Dose-dependency of the change in membrane conductance seen in the presence of (−)-baclofen. The mean values plotted are derived from the neurone illustrated in (a) and (b) and from three further neurones. The ordinate scale represents the change in conductance as a percentage of control conductance, while the abscissa scale expresses the logarithm of the concentration of (−)-baclofen. Each point represents the mean of the change observed on the same 4 neurones, except for that at the highest concentration of (−)-baclofen where the value shown is a mean of (−)-baclofen application to 2 of these 4 neurones; vertical bars show s.e.mean. (d) Transformation of the data shown in (b) to a Hill plot. The ordinate scale expresses $\log E/E_{max} - E$, where E is the change in conductance seen at the concentration of (−)-baclofen indicated on the abscissa scale and E_{max} is the maximum change in conductance seen in the presence of the drug (i.e. the response to 3 μM). The gradient of this line, the Hill slope, is 1.0.

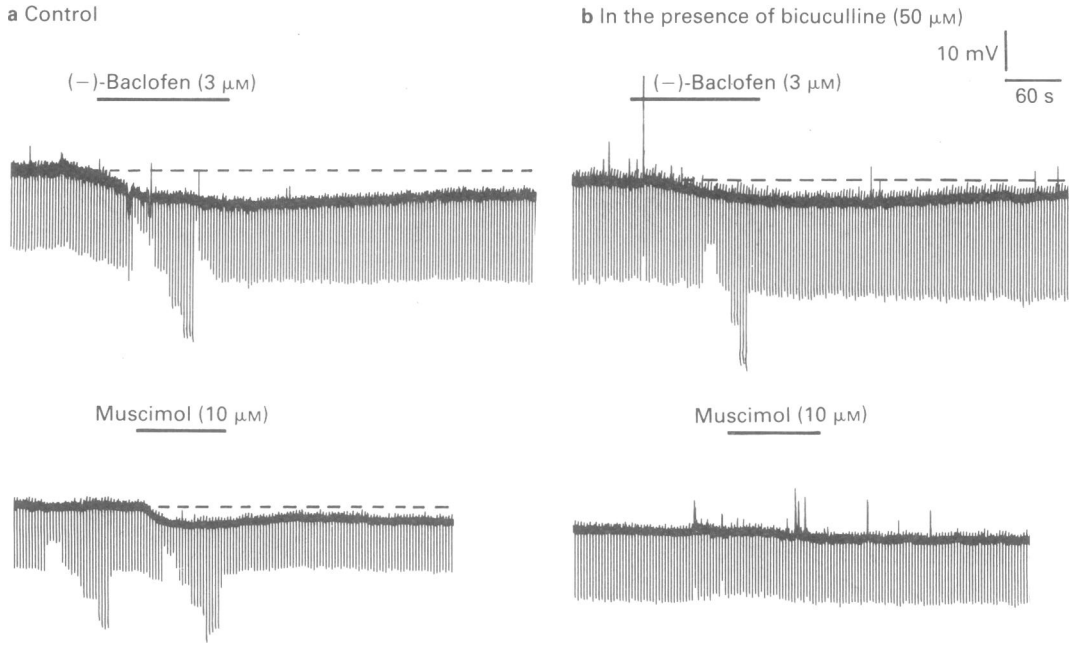

Figure 2 The hyperpolarization seen on application of (−)-baclofen persisted in the presence of a concentration of bicuculline known to block $GABA_A$ receptors. (a) The response of two different neurones to (−)-baclofen at a concentration of 3 μM and muscimol at a concentration of 10 μM respectively applied for the period indicated by the solid bar. (b) In the presence of bicuculline (50 μM) the response to muscimol but not (−)-baclofen was abolished. A hyperpolarization was still seen on application of (−)-baclofen although this response was reduced in the presence of bicuculline. Note also that in both neurones apparent input resistance was increased in the presence of bicuculline, presumably due to antagonism of the effects of endogenous GABA. The irregularity in the size of the electrotonic pulses was due to the variation in the amplitude of the injected current pulses that was necessary during IV plot construction. The maximum conductance change produced by (−)-baclofen on this neurone was a 30% increase during plotting of the IV curve. Note that the amplitude of current injections after the IV curve was not returned exactly to the pre-drug value. Distortion seen at the peak of the response was due to the slow chart speed. Measurements of amplitude were made at a higher speed that did not show this inaccuracy.

the membrane was displaced during passage of current). Voltage displacements were calculated by averaging 3 or more deflections measured on a chart recorder running at a speed of 0.5 mm s^{-1}. These deflections were of the same magnitude as the voltage response observed on the storage oscilloscope ensuring that the frequency response of the pen recorder had not influenced the apparent magnitude of the responses (see Figure 1). At speeds slower than this some distortion could arise but was observable (as in Figure 2).

Stimulation of dorsal rootlets with a bipolar stimulating electrode (pole separation 0.5 mm, insulated to within 0.5 mm of the tip) was used to obtain orthodromic activation of synapses in the dorsal horn region of spinal cord slices. Excitatory postsynaptic potentials (e.p.s.ps) were measured either directly after capture on the digital storage oscilloscope or after capture and plotting out on a pen recorder. Alternatively, e.p.s.ps were analysed off-line using the e.p.s.p analysis facility on SCAN VI.2.

Drugs and solutions

Composition of ACSF used for slice preparation and incubation was (mM): NaCl 124, KCl 5, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.3, $NaHCO_3$ 26 and glucose 10.

Composition (mM) of ACSF used for recording and drug application: NaCl 127, KCl 1.9, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.3, $NaHCO_3$ 26 and glucose 10.

Drugs used were: 4-aminopyridine (Sigma), (−)-baclofen hydrochloride (Ciba-Geigy), (+)-baclofen hydrochloride (Ciba-Geigy), barium chloride (Sigma), (−)-bicuculline methobromide (Sigma), caesium chloride (Sigma), muscimol (Sigma), tetraethylammonium bromide (Sigma).

Results

Results were obtained from a total of 46 neurones located in the deeper laminae (laminae 3–6 of Rexed) of spinal dorsal horn. The mean resting potential of the neurones studied was 67 ± 1 mV (mean ± s.e.mean, n = 38) and the mean apparent input resistance 80 ± 5 MΩ ($n = 39$). The mean membrane time constant was 14.3 ± 1.1 ms ($n = 10$). These membrane properties were determined with a KCl-filled electrode.

Postsynaptic action of (−)-baclofen

In all neurones tested, application of (−)-baclofen (100 nM–30 μM) resulted in membrane hyperpolarization associated with a reduction in apparent input resistance. Full recovery from the drug could generally be obtained after 10 to 20 min wash-out with drug-free saline and desensitization of the response was not apparent on repeat applications of (−)-baclofen to the same neurone. The increased membrane conductance seen in the presence of (−)-baclofen was dose-dependent. Transformation of the data to a Hill plot resulted in a Hill slope of 1.0 (Figure 1). (+)-Baclofen at a concentration of 10 μM had no effect ($n = 3$).

The direct nature of the interaction of (−)-baclofen with receptors on the post-synaptic membrane was confirmed by application of (−)-baclofen in the presence of a high concentration (20 mM) of magnesium ions. Such a concentration of magnesium completely prevents synaptic transmission within the slice but did not affect the response to (−)-baclofen ($n = 3$).

Since (−)-baclofen may have weak $GABA_A$ properties at higher concentrations, it was important to ensure that the observed hyperpolarization was not merely due to activation of the $GABA_A$ receptor. (−)-Baclofen was therefore applied in the presence of the $GABA_A$ antagonist bicuculline (50–100 μM) and the pharmacological effects of this substance were seen to persist. At these concentrations of bicuculline, however, some reduction (although never complete abolition) of the response to baclofen occurred in most neurones. The same concentration of bicuculline abolished completely the effects of the selective $GABA_A$ agonist muscimol (10 μM) (Figure 2).

In order to obtain some indication of the ionic event involved in $GABA_B$ activation, examination of the reversal potential of the event was made. The amplitude of the (−)-baclofen-induced hyperpolarization was reduced in a potential-dependent manner by the injection of hyperpolarizing current into the neurone under study. At a membrane potential of −100 mV, the hyperpolarizing response was abolished indicating that the reversal potential for the event lay around this value (Figure 3).

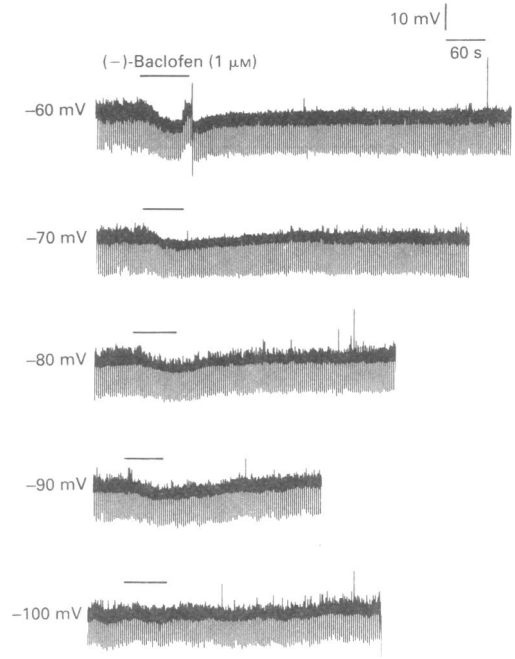

Figure 3 The amplitude of the hyperpolarization seen on application of (−)-baclofen was dependent on membrane potential. A single dose of the drug was applied for the period indicated by the solid bar to a deep dorsal horn neurone whose membrane potential was held at different potentials below rest by injection of hyperpolarizing current. As the membrane was made more hyperpolarized so the amplitude of the drug-induced hyperpolarization was reduced. The membrane potential could not be driven beyond the reversal potential as the relatively high resistance electrodes needed for this study would not pass sufficient current. As more current was passed through the electrode so its ability to monitor hyperpolarizing voltage deflections was reduced as the signal:noise ratio was degraded. The maximum conductance change produced by (−)-baclofen on this neurone was 25%.

A further indication of the reversal potential was obtained by the construction of current-voltage relationships for these neurones in drug-free and (−)-baclofen containing ACSF. The point at which the two lines intersect represents the potential at which no polarizing effect of (−)-baclofen would be observed, that is the reversal potential. Obtained in such a fashion, the reversal potential for (−)-baclofen was found to be -102 ± 2 mV (mean ± s.e.mean, $n = 7$). This was significantly different (Student's t test, $P < 0.01$) from the reversal potential found using a similar procedure on the same neurones for muscimol, -83 ± 3 mV ($n = 5$) (Figure 4).

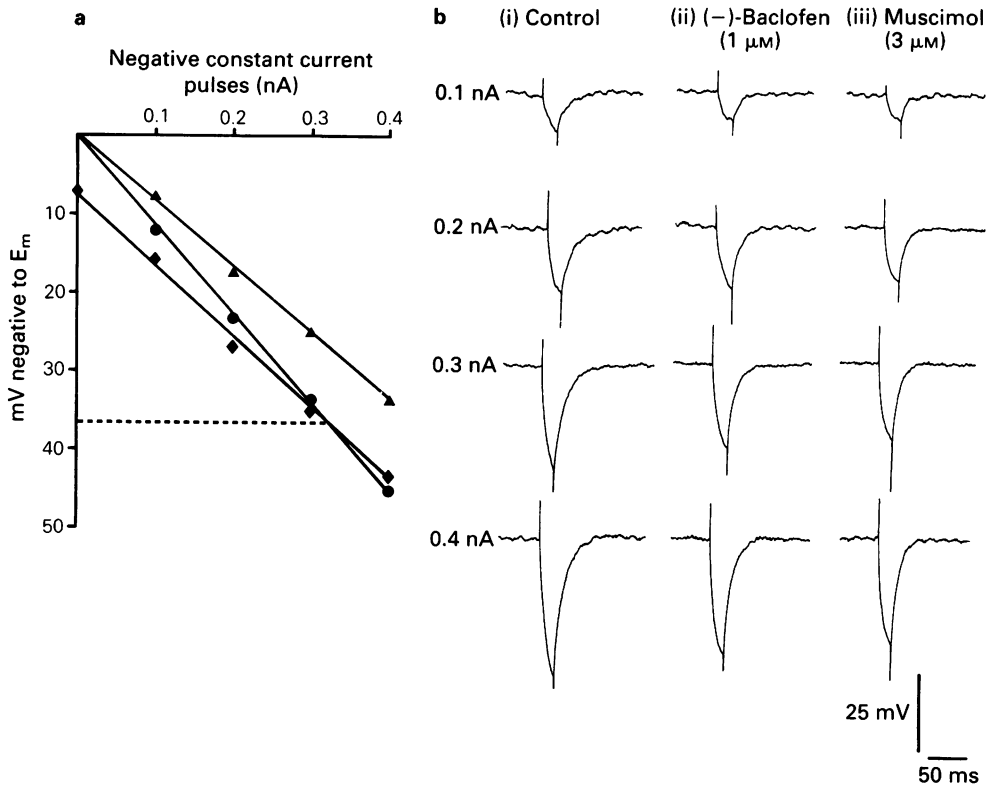

Figure 4 (a) Current-voltage plots were used to estimate the reversal potentials for (−)-baclofen and muscimol on the same neurone. Hyperpolarizing current pulses were injected into the neurone and the resulting voltage displacement measured as described in Methods. On the graph shown, the abscissa scale shows the amplitude of the injected current pulses and the ordinate scale the amplitude of the resulting voltgage displacement relative to the resting potential, E_m. The current-voltage plot obtained in the presence of muscimol (3 μM) (▲) intersects with the plot made under control conditions (●) at the resting membrane potential of −68 mV while that constructed in the presence of (−)-baclofen (1 μM) (◆) intersects with the control 37 mV negative to E_m. Since these points represent the potentials at which no polarizing actions of the drugs would be observed (i.e. the reversal potential), it can be seen that the reversal potentials for muscimol and (−)-baclofen on this neurone are quite distinct. That for muscimol can be estimated at −68 mV, while that for (−)-baclofen is approximately −105 mV. (b) Examples of voltage displacements obtained in response to injection of square current pulses of 0.1 to 0.4 nA in amplitude during construction of the IV plot shown in (a). Voltage responses illustrated were made (i) under control conditions, (ii) in the presence of 1 μM (−)-baclofen and (iii) in the presence of 3 μM muscimol. In (ii) voltage displacements were superimposed upon a 7 mV hyperpolarization. Records shown were captured off-line on a storage oscilloscope and plotted out on a chart recorder.

At the higher injected currents saturation of the membrane capacitance may not have been complete and thus graph (a) may not be accurate at these high currents. It is noteworthy, however, that linearity is preserved and our main conclusion, that reversal potentials for muscimol and baclofen differ, is unaffected by this consideration.

Estimates of the reversal potential suggested that activation of a potassium conductance may be responsible for the hyperpolarizing action of (−)-baclofen. If so, the reversal potential should vary according to the external concentration of potassium ions. It was shown that the reversal potential for (−)-baclofen was made less negative by increasing the external concentration of potassium ions, ($n = 2$) (Figure 5). On extrapolation of the relationship between external potassium concentration and the (−)-baclofen reversal potential, it could be seen that a tenfold change in the external potassium concentration would produce approximately the 60 mV shift in the reversal potential predicted from the Nernst equation for a pure potassium-mediated event.

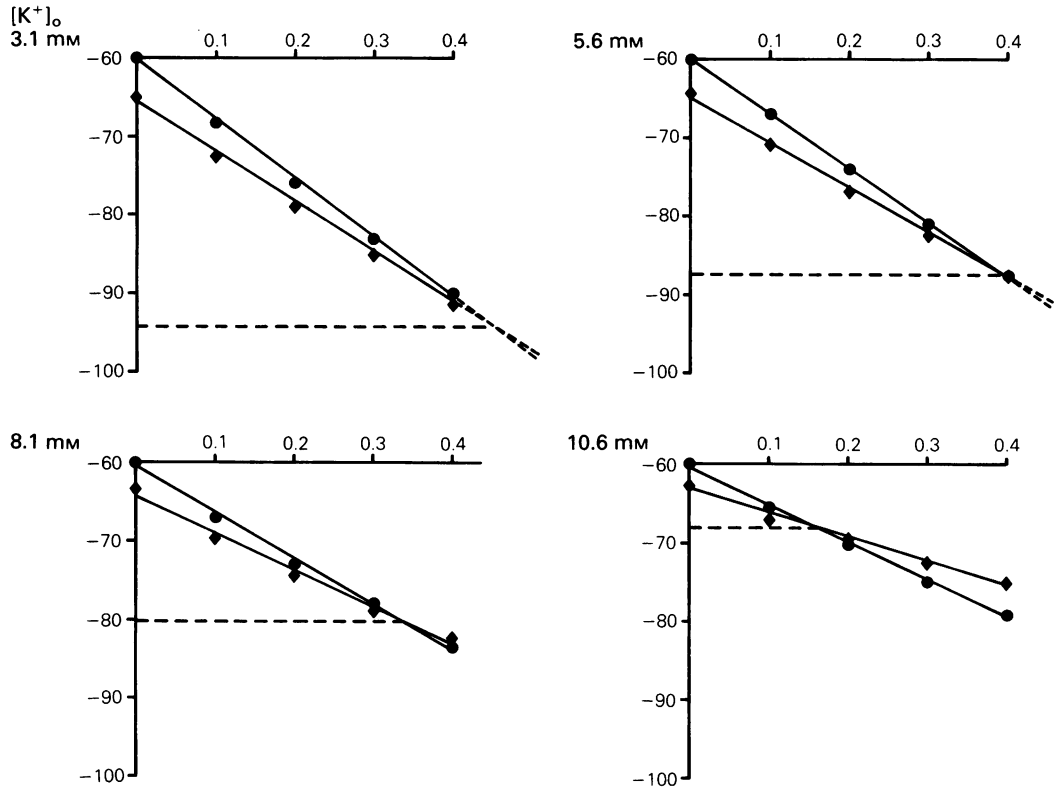

Figure 5 Estimates of the reversal potential for (−)-baclofen made on a single neurone with variation of the external concentration of potassium ions. $[K^+]_o$. External potassium concentration was increased from 3.1 mM to 5.6, 8.1 and 10.6 mM by addition of 1 M KCl to the perfusion medium. Membrane depolarization occurred so the membrane was brought back to its resting potential of −60 mV by current injection. Current-voltage plots were then constructed as described previously in control (●) and (−)-baclofen (1 μM) containing media (◆). The abscissa scale shows the amplitude of the hyperpolarizing current pulses injected into the neurone in nA and the ordinate scale the maximum potential in mV to which the membrane was displaced during these pulses. Again, the intersection of the two lines represents the reversal potential of (−)-baclofen. As the concentration of external potassium ions is raised so the reversal potential for (−)-baclofen becomes less negative.

Effect of (−)-baclofen on synaptic transmission

Polysynaptic composite excitatory postsynaptic e.p.s.ps can be evoked in deep dorsal horn neurones by electrical stimulation of the dorsal root entry zone. They may be up to 30 mV in amplitude and up to 350 ms in duration.

(−)-Baclofen (100 nM–30 nM) depressed the e.p.s.p. in all neurones tested ($n = 15$). This effect occurred along with the postsynaptic response although did not always follow the same time-course. E.p.s.p. depression was dose-dependent and larger than would be predicted if the change was merely due to the increase in postsynaptic membrane conductance (Figure 6(i)).

Effect of potassium channel blockers on the response to (−)-baclofen

Since an increase in membrane conductance can alone produce a reduction in e.p.s.p. amplitude, it was useful to be able to show that e.p.s.p. depression occurred in the absence of any postsynaptic effect. This was achieved by filling electrodes with 3 M CsCl as a substitute for 3 M KCl. As diffusion of ions occurs from the tip of the electrode into the impaled neurone, caesium ions are able to block potassium channels from the intracellular surface of the membrane. Under these conditions the postsynaptic action of baclofen was either substantially reduced or completely abolished, although depression of the

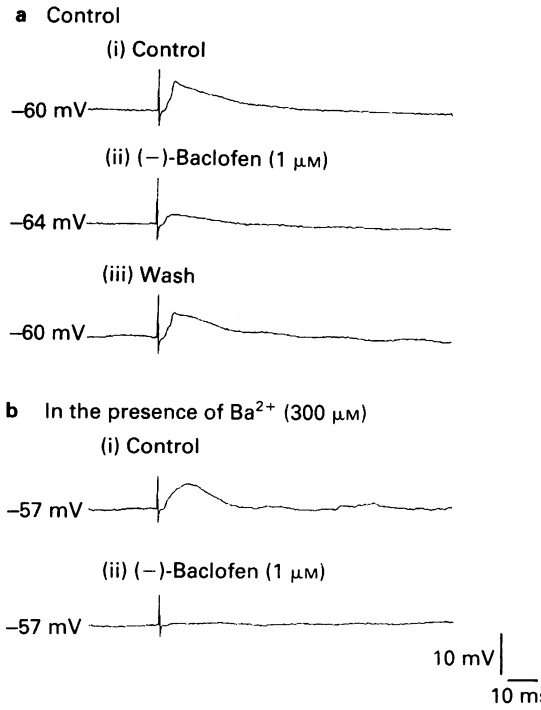

Figure 6 (–)-Baclofen depresses the polysynaptic composite e.p.s.p. evoked in dorsal horn neurones by electrical stimulation of the dorsal root entry zone. This effect persists in the presence of a concentration of barium known to block the hyperpolarizing response to baclofen. E.p.s.ps shown are the average of 10 e.p.s.ps analysed off-line. (a) Shows the control response of the e.p.s.p. to 1 μM (–)-baclofen. The knockdown of the e.p.s.p. seen on application of (–)-baclofen was superimposed upon a hyperpolarization of 4 mV and an increase in membrane conductance. (b) Shows the response of the e.p.s.p. evoked in the same neurone in the presence of 300 μM extracellular barium. Preincubation with barium had taken place for 10 min before (–)-baclofen application and no hyperpolarizing response of the neurone to (–)-baclofen was observed. Depression of the e.p.s.p. was however still observed under these conditions. The stimulus artifact in (b) is smaller as the amplitude of the stimulus was reduced to prevent action potential firing owing to the more excitable state of the neurone. A satisfactory return of the e.p.s.p. to control after (–)-baclofen application could not be obtained in the presence of barium as increasing potassium channel blockade made the neurone highly excitable and electrical stimulation resulted in burst firing of action potentials.

e.p.s.p. was still observed ($n = 6$). Blockade of the postsynaptic action of (–)-baclofen was a time-dependent phenomenon and it generally took up to 60 min of impalement with a caesium-filled electrode before complete abolition of the response occurred. Injection of caesium into the neurone and the resulting potassium channel blockade caused a gradual depolarization of the cell membrane over time.

Extracellular application of barium ions is also known to block potassium channels. Administration of barium chloride at a concentration of 300 μM in the perfusate markedly reduced the postsynaptic action of (–)-baclofen while not affecting its ability to depress e.p.s.ps (Figure 6b(ii)). Barium itself produced a depolarization of the neuronal membrane concomitant with an increase in apparent input resistance. Additionally, barium produced an increase in neuronal activity which could be depressed by both (–)-baclofen and a high concentration (20 mM) of magnesium ions.

In an attempt to learn more about the type of potassium current activated post-synaptically by (–)-baclofen, tetraethylammonium ions (TEA) at a concentration of 10 mM and 4-aminopyridine (4-AP) at a concentration of 50 μM were applied in the perfusate. Although both had effects on cell membrane properties (depolarization and firing of action potentials) neither drug affected the response to (–)-baclofen.

Discussion

The $GABA_B$ agonist (–)-baclofen exerts a direct hyperpolarizing action on the postsynaptic membrane of neurones in deep dorsal horn of spinal cord. The direct interaction of (–)-baclofen with receptors on the postsynaptic membrane is confirmed by the persistence of the effect in the presence of 20 mM Mg^{2+} which blocks synaptic transmission within the slice. The Hill slope associated with such a postsynaptic action is 1.0 implying that it is the result of (–)-baclofen binding to a single site. Estimates of the reversal potential of the postsynaptic event and its modulation by the external potassium concentration indicate that it is mediated by activation of a potassium conductance. This is supported by its sensitivity to intracellularly applied caesium and extracellularly applied barium.

A similar postsynaptic action of (–)-baclofen has been reported in other areas of CNS and is perhaps best characterized in hippocampus. Here, $GABA_B$ receptor activation induces a postsynaptic potassium current which is voltage-dependent and rectification is seen at both depolarized and hyperpolarized potentials (Inoue *et al.*, 1985a; Newberry & Nicoll, 1985). This contrasts with the conductance activated by (–)-baclofen in spinal dorsal horn neurones which, under our experimental conditions, does not rectify over the voltage range studied. However, it should be noted that under control conditions, membrane rectification is observed in hippocampal but

not spinal neurones and so the difference in voltage properties of the (−)-baclofen-evoked potassium currents may merely be a reflection of a difference between the two neurone populations.

In the hippocampal slice, the (−)-baclofen-activated current is blocked by micromolar concentrations of 4-AP (Inoue *et al.*, 1985a) whereas in the spinal cord slice concentrations of 4-AP up to 50 μM had no effect on the response to (−)-baclofen. It should, however, be noted that in hippocampal cultures the potassium current activated by (−)-baclofen was not affected by 4-AP (Brown & Gahwiler, 1987). The insensitivity of the (−)-baclofen response in dorsal horn neurones to TEA is consistent with previous findings in both hippocampal slices (Inoue *et al.*, 1985a) and cultures (Brown & Gahwiler, 1987).

The ionic event associated with postsynaptic $GABA_B$ receptor activation by (−)-baclofen is quite distinct from the chloride-mediated event associated with activation of the $GABA_B$ receptor and distinct reversal potentials for the two events can be obtained on the same dorsal horn neurones. There is however some depression of the response to (−)-baclofen by the $GABA_A$ receptor antagonist bicuculline. This phenomenon has also been observed in hippocampus (Inoue *et al.*, 1985b; Newberry & Nicoll, 1985) and may be the result of lack of absolute pharmacological selectivity of either agent or the ability of bicuculline to block potassium channels at the concentrations used (Heyer *et al.*, 1982).

The second observable effect of (−)-baclofen on deep dorsal horn neurones is the depression of the polysynaptic composite e.p.s.p. evoked by dorsal root stimulation. This still occurs where the postsynaptic response has been blocked by caesium and therefore cannot merely be due to the concomitant increase in postsynaptic membrane conductance.

It is possible that e.p.s.p. depression is the result of baclofen opening a potassium conductance on cell bodies of neurones earlier in the pathway. However, this is unlikely since the baclofen-induced depression of the e.p.s.p. is still observed in the presence of a concentration of bath-applied barium seen to block the post-synaptic response. Barium itself resulted in an increase in the activity of all neurones studied, an observation which may be explained in more than one way. Firstly, since barium is taken up into the neurone through calcium channels, it may be leading to transmitter release. This is unlikely as barium can, but does not consistently, substitute for calcium in transmitter release (Miledi, 1966). Secondly, barium-blockade of potassium channels results in depolarization of the neuronal membrane with depolarization of terminals leading to calcium entry and transmitter release. In either case, the involvement of terminal calcium channels is implicated and supported experimentally by the blockade of barium-induced activity seen on application of a high concentration of magnesium ions which act as a competitive antagonist for calcium at a variety of chemical synapses (Katz, 1969). The observation that baclofen can also depress barium-induced activity implies that it too may act at these sites, but could only be confirmed if intracellular recording from nerve terminals was possible in this preparation.

(−)-Baclofen is an extremely potent anti-spastic agent which, unlike other drugs of this class, depresses monosynaptic as well as polysynaptic extensor reflex transmission in spinal cord (Fehr & Bein, 1974). It has no effect on transmitter release from motoneurone terminals (Glavinovic, 1979) implying a central site of action but has not been found to affect transmitter release from excitatory spinal interneurones, cholinergic motor axon collaterals or from descending fibres in the dorsolateral funiculus (Curtis *et al.*, 1981; Curtis & Malik, 1985). Since (−)-baclofen invariably hyperpolarizes neurones in spinal dorsal horn, this may account for its potent depression of spinal reflex transmission. Although two mechanisms of action of (−)-baclofen on dorsal horn neurones can be distinguished in this spinal cord slice preparation, it is possible that the postsynaptic activation of a potassium current is functionally the more important.

The authors would like to thank Dr R.D. Pinnock for suggesting these experiments and for his helpful discussions thereafter, D. Conner for technical assistance and Ciba-Geigy for their generous gift of the stereoisomers of baclofen. The analysis programme SCAN VI.2 was kindly provided by J. Dempster, University of Strathclyde.

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(Received March 1, 1988
Revised August 10, 1988
Accepted August 22, 1988)