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SELECTIVE DEPRESSION OF SYNAPTIC EXCITATION IN CAT SPINAL NEURONES BY BACLOFEN: AN IONTOPHORETIC STUDY

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1 The effects of baclofen have been examined on responses of neurones in the spinal cord of the anaesthetized cat to stimulation of appropriate synaptic pathways, acetylcholine and a range of amino acid excitants. Baclofen and excitant substances were administered by standard microiontophoretic techniques.

2 Small ejecting currents of baclofen (<10 nA) depressed non-cholinergic, excitatory, synaptic responses evoked by stimulation of dorsal roots or muscle or cutaneous afferents. Excitatory monosynaptic responses were particularly sensitive to the depressant action of baclofen.

3 Spontaneous firing in neurones was sometimes reduced in parallel with synaptic excitatory responses, but synaptically evoked inhibition was unaffected and ventral root evoked excitation of Renshaw cells was either unaffected or enhanced by baclofen.

4 Ejecting currents of baclofen which markedly depressed excitatory synaptic responses either had no effect or minimal depressant effects on responses induced by iontophoretically administered acetylcholine and excitant amino acids. However, relatively large currents of baclofen (e.g. 20 to 40 nA) reduced excitatory responses to exogenously administered excitants.

5 It is suggested that baclofen depresses (a) synaptic response by an action on excitatory nerve terminals and (b) responses to exogenous excitants via a postsynaptic action.

6 Comparison of baclofen with a number of other substances indicates that the depression of noncholinergic, excitatory, synaptic responses is unlikely to involve an interaction of this agent with receptors for monoamines, 5-hydroxytryptamine or the inhibitory amino acids, γ-aminobutyric acid and glycine.

Introduction

The γ-aminobutyric acid (GABA) analogue, β-*p*-chlorophenyl-γ-aminobutyric acid (baclofen) is a potent centrally acting muscle relaxant (Birkmayer, 1972). Systemic administration of low doses of baclofen (>2 mg/kg) depresses mono- and polysynaptic reflexes in the spinal cord and transmission through the cuneate nucleus (Bein, 1972; Pierau & Zimmermann, 1973; Davidoff & Sears, 1974; Pierau, Matheson & Wurster, 1975; Polc & Haefely, 1976; Fox, Krnjević, Morris, Puil & Werman, 1978; Kato, Waldmann & Murakami, 1978). These effects of systemically administered baclofen are thought to be mediated presynaptically as excitatory postsynaptic potentials recorded in central neurones are depressed in the absence of any change in membrane potential and conductance (Pierau & Zimmermann, 1973; Fox *et al.*, 1978). However, experiments using the iontophoretic technique of drug administration demonstrate that baclofen depresses the firing of central neurones induced by a number of excitatory substances indicating a postsynaptic depressant action of the drug (Curtis, Game, Johnston & McCulloch, 1974; Davies & Watkins, 1974; Davies & Dray, 1976; Henry & Ben-Ari, 1976; Olpe, Koella, Wolf & Hass, 1977; Fox *et al.*, 1978). It has recently been suggested that the discrepancies between the effects of systemically and iontophoretically administered baclofen may depend on the local tissue concentration attained (Fox *et al.*, 1978), low tissue concentrations (systemic administration) producing only presynaptic effects, high concentrations (iontophoretic administration) resulting in both pre- and postsynaptic effects. In the present experiments an attempt has been made to examine this possibility. To this end, baclofen was administered iontophoretically from micropipettes containing dilute solutions of the agent in NaCl while observing its effects on synaptically evoked and chemically induced excitatory responses on single neurones in the cat spinal cord. The effects of iontophoretically administered baclofen have also been examined on mono- and polysynaptic excitation and synaptic inhibition of spinal neurones since data contained in several reports suggest that systemically administered baclofen influences some synaptic

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events more than others (Pierau & Zimmermann, 1973; Fukuda, Kudo & Ono, 1977; Fox *et al.*, 1978; Kato *et al.*, 1978). However, systemic studies cannot differentiate between effects at the first synaptic relay in the spinal cord and effects at more distal synapses.

In addition, to obtain more information on the pharmacology of baclofen, its effects have been compared with those of a number of other substances which may influence synaptic transmission in the spinal cord.

Methods

Experiments were performed on adult cats of either sex weighing 2.2 to 3 kg. Animals were anaesthetized with pentobarbitone sodium (35 mg/kg i.p.) (6 cats) or, following induction with halothane, α-chloralose (50 mg/kg i.v.) (10 cats). Supplemental doses of an anaesthetic were given as necessary during the course of the experiment. A cannula was inserted into the brachial vein for intravenous administration of drugs. Blood pressure was continually monitored via a cannula inserted into one carotid artery and attached to a pressure transducer. Body temperature was maintained between 37 and 38°C by means of a thermostatically controlled heating blanket.

The spinal cord was exposed by a laminectomy extending from L1 to L7 vertebrae and was transected at L1. Ventral roots S1, L7 and L6 were cut ipsilaterally and the central ends of S1 and L7 were mounted upon silver bipolar electrodes for stimulation. In some experiments the L7 and S1 dorsal roots were also transected and mounted on stimulating electrodes. In others the ipsilateral posterior biceps and semitendinosus (PBST), gastrocnemius and soleus (GS), flexor digitorum hallucis longus (FDHL), sural (SUR) and common tibial (TIB) nerves were also dissected free and mounted for stimulation. The exposed spinal cord and limb nerves were covered with a pool of paraffin oil maintained at 36 to 37°C by an infra red lamp. The dorsal root volleys were recorded by means of a silver ball placed on the L7 dorsal root entry zone into the spinal cord and central latencies of cell firing were measured from the beginning of the negative going-deflection of the dorsal root volley to the shortest latency of firing of action potentials recorded extracellularly via the central barrel (4 M NaCl) of a seven barrelled microelectrode inserted into the L7 segment.

Renshaw cells were identified by their characteristic response to stimulation of L7 or S1 ventral root and other spinal neurones were identified as being mono- or polysynaptically activated by volleys in leg nerves depending upon their central latencies (see results). Leg nerves were stimulated with square wave pulses of 100 μs duration. The strength of stimulation was expressed in multiples of threshold (T) for the lowest threshold fibres.

Neuronal action potentials recorded from spinal neurones were either photographed directly from the oscilloscope screen or electronically counted and displayed on a pen-recorder trace. The counted pulses were also fed into a small computer (Neurolog) which was used to compile peristimulus time histograms of synaptic events.

Drugs were administered iontophoretically from the outer barrels of 7 barrel micropipettes. The drugs used were acetylcholine chloride (0.5 M), Na L-glutamate (0.5 M, pH 7.0), Na L-aspartate (0.5 M, pH 7.0), γ-aminobutyric acid (GABA, 0.5 M pH 3.5), glycine hydrochloride (0.5 M, pH 3.5), noradrenaline bitartrate (0.2 M, pH 4.0), 5-hydroxytryptamine bimaleinate (5-HT, 0.2 M, pH 4.0), Na kainate (0.02 M in 0.18 M NaCl, pH 7.0), Na *N*-methyl-D-aspartate (NMDA; 0.05 M in 0.15 M NaCl, pH 7.0), Na D-α-aminoadipate (DαAA, 0.2 M, pH 7.0), bicuculline methochloride (BMC, 0.005 M in 0.165 M NaCl), strychnine hydrochloride (0.005 M in 0.165 M NaCl), DL-homocysteate (DLH, 0.2 M, pH 7.2), (±)-baclofen hydrochloride (0.01 M in 0.165 M NaCl, pH 3.4) and (−)- and (+)-isomers of baclofen (0.005 M in 0.165 M NaCl, pH 3.5). Noradrenaline and 5-HT were freshly prepared on the day of the experiment.

Results

Results were combined from pentobarbitone and α-chloralose-anaesthetized animals as there were no significant differences between the two groups of animals.

Action of (±)-baclofen on synaptic and chemically induced excitation

Renshaw cells The effects of iontophoretic ejection of (±)-baclofen on the excitatory response evoked in 11 Renshaw cells by submaximal stimulation of dorsal and ventral roots and by iontophoresis of acetylcholine, L-glutamate and/or L-asparate are summarised in Table 1. (±)-Baclofen consistently and markedly depressed excitatory responses evoked by dorsal root stimulation in the majority of neurones (9/11). This reduction was dose-dependent, evident within 1 to 4 min of starting the ejection and was often prolonged, recovery occurring from 3 to more than 30 min after terminating the baclofen ejection (e.g. Figure 1). In tests on the same neurones, currents of (±)-baclofen that depressed dorsal root evoked responses either had no effect (6 cells) or reversibly enhanced (4 cells) ventral root evoked responses and had no effect, or less markedly depressed responses, to iontophoretically administered substances (Table 1 and Figure 1). However, large ejecting currents of baclofen (80 to 100 nA) often reduced responses to excitant amino acids and acetylcholine. Nevertheless,

Table 1 Effects of (±)-baclofen on synaptic and chemically-induced excitation of the same Renshaw cells

Method of activation	*Effect of (±)-baclofen†*		
	Decreased	*Increased*	*Unchanged*
DR stimulation*	9 (58.7 ±11.2)	0	2
VR stimulation*	1 (40.0)	4 (38.7 ± 20.6)	6
L-Glutamate (20–100 nA)	2 (35.0)	0	4
L-Aspartate (10–80 nA)	2 (55.0)	0	8
Acetylcholine (0–40 nA)	2 (45.0)	0	9

* Synaptic responses were evoked by rectangular pulses (0.1 ms, 1 Hz and submaximal intensity) applied to dorsal roots (DR) and ventral roots (VR). † (±)-Baclofen was ejected with currents of 64.8 ± 10.4 nA for 6.5 ± 0.7 min. The figures in each column show the number of neurones in which synaptic and chemically-induced responses were increased, decreased or unchanged. The figures in parentheses refer to the mean ± s.e. percentage change in the responses. These changes were calculated from photographic records of at least 15 oscilloscope sweeps or peristimulus histograms computed from at least 32 sweeps analysed in 250 μs intervals in the case of synaptic responses and from ratemeter records in the case of chemically-induced responses.

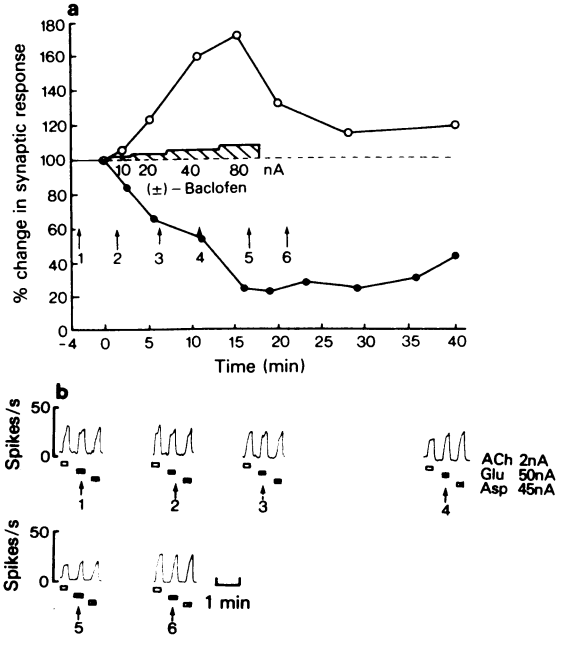

Figure 1 (a) The graph shows the effects of increasing currents of (±)-baclofen (0.01 M solution in NaCl) on the synaptic responses of a Renshaw cell evoked by both ventral (○) and dorsal root (●) stimulation (0.1 ms, 1 Hz submaximal intensity). Each point shows the percentage change in the number of spikes per response. These changes were calculated from peristimulus histograms computed from 32 sweeps analysed in 250 μs intervals. The horizontal line at the 100% point represents the control responses before the ejection of (±)-baclofen and corresponds to 6.8 ± 0.2 and 7.2 ± 0.3 (mean ± s.e.) spikes per sweep for the ventral and dorsal root responses respectively. (N.B. Such large increases in ventral root evoked responses were not generally observed in Renshaw cells, cf. Table 1). In (b) representative ratemeter records of the excitatory responses of the same cell to acetylcholine (ACh 2 nA), L-glutamate (Glu 50 nA) and L-aspartate (Asp 45 nA) obtained at times corresponding to the arrows 1–6 are shown. Compared with the effects on the dorsal root-evoked responses, excitant responses were only weakly depressed by large currents of (±)-baclofen and this effect was rapidly reversible.

for any given cell the reduction of the dorsal root evoked response was greater, more prolonged and preceded any reduction in the response to an exogenously administered excitant. When affected, chemically induced responses also recovered considerably more rapidly from the effects of (±)-baclofen then the synaptically evoked responses. Many of these features of the actions of (±)-baclofen are illustrated for one neurone in Figure 1. None of these effects of baclofen was accompanied by any change in spike configuration. However, spontaneous firing, present in two Renshaw cells, was reversibly depressed by currents of baclofen similar to those affecting synaptic responses.

Dorsal horn neurones The effects of (±)-baclofen on dorsal horn neurones are summarised in Table 2. In essence, (±)-baclofen produced similar effects on these cells to those on Renshaw cells. Synaptically evoked responses were markedly reduced in 7 of 8 cells whereas responses of these cells to excitant amino acids were either unaffected or (in the case of 3 cells with L-glutamate) were reduced to a smaller extent than the synaptic response on the same cell. Closer inspection of the results shown in Tables 1 and 2 suggests that synaptic responses of dorsal horn neurones may be more sensitive to the depressant actions of (±)-baclofen than those of Renshaw cells. This seems to be mainly due to the findings that synaptic responses in 3 cells included in Table 2 were completely abolished by very low currents of (±)-baclofen (5 nA). These cells were monosynaptically activated from dorsal roots whereas the remaining 4 cells in Table 2 were polysynaptically excited and were only weakly depressed by considerably higher ejecting currents of baclofen (40 to 60 nA). In view of this observation a more detailed comparison of the effects of baclofen on mono- and polysynaptically evoked excitation of spinal neurones was made.

Effects of (−)-baclofen on mono- and polysynaptically evoked excitation of spinal neurones.

The (−)-isomer of baclofen was used in this part of the present study in preference to the (±)-racemate because it has been found that the biological activity of the drug resides with the (−)-enantiomer (Olpe *et al.*, 1977; Olpe, Demieville, Baltzer, Bencze, Koella, Wolf & Haas, 1978; Ault & Evans, 1978).

Characteristics of monosynaptic and polysynaptic excitation Neurones activated monysynaptically invariably responded with a single action potential in response to a volley in a low threshold muscle afferent (PBST, GS and FDHL) and followed orthodromic frequencies in excess of 300 Hz. The central latencies of these responses ranged between 0.85 and 1.45 ms (1.16 ± 0.03 mean ± s.e, $n = 22$) and the threshold (T) for excitation was 1.1 − 2.0 T (1.56 ± 0.1 mean ± s.e.) for activation of the fastest fibres (1a) in the dorsal root. The majority of these neurones were located in or around the intermediate nucleus. However, 6 neurones excited monosynaptically by volleys in PBST nerves were found dorsal and medial to motonuclei and these monosynaptic responses were inhibited by a preceding volley in the ventral root of the same spinal segment (L7) when the test-conditioning-interval was greater than 4 ms (e.g. Figure 4). These neurones therefore had characteristics similar to those reported by Hultborn, Jankowska & Lindström, 1971, and were hence classed as 1a inhibitory interneurones.

Polysynaptically activated neurones were located at various depths in the spinal cord. These neurones were excited by (a) volleys in cutaneous afferents with thresholds for excitation of 1.3 to 5 T (3.3 ± 0.4) for the fastest afferents and hence mostly Aα and β fibres were activated (Coombs, Curtis & Landgren, 1956), or (b) stimulation of muscle afferents at intensities

Table 2 Effects of (±)-baclofen on dorsal root (DR) evoked and chemically-evoked responses of dorsal horn neurones

Method of activation	*Effects of (±)-baclofen*† *Decreased*	*Increased*	*Unchanged*
DR stimulation (0.1 ms, 1 Hz, submaximal intensity)	7 (72.8 ±11.4)	0	1
L-Glutamate (30–100 nA)	3 (76 ± 14.0)	0	4
L-Aspartate (40–100 nA)	0	0	7

† Baclofen was ejected with current of 21.8 ± 7.1 nA for 3.1 ± 0.7 min.
The figures in each column show the numbers of neurones in which synaptic and chemically-induced responses were increased, decreased or unchanged. The figures in parentheses refer to the mean ± s.e. percentage change in the responses. For further details see footnote to Table 1.

sufficient to activate groups II or III afferents (i.e. 2 to 10 T for the fastest group 1 afferents). Many of these neurones were activated by more than one input. The central latency of the earliest response in these neurones varied widely but was always greater than 2.3 ms (4.72 ± 0.36 ms, $n = 20$). These latencies are too long to be mediated monosynaptically (Eccles, Fatt & Landgren, 1956; Coombs *et al.*, 1956; Eccles, Kostyuk & Schmidt, 1962; Gregor & Zimmermann, 1972). Unlike responses evoked monosynaptically, those evoked polysynaptically invariably consisted of more than one spike and failed to follow high frequencies of stimulation. Twenty-one neurones, including 4 Renshaw cells, were classed as being polysynaptically activated in this part of the investigation.

Effects of (−)-baclofen The effects of (−)-baclofen on monosynaptically and polysynaptically evoked excitatory response in spinal neurones are summarised in Table 3. Monosynaptically evoked responses were extremely sensitive to (−)-baclofen and on every cell tested were consistently and very markedly reduced by a brief (0.5 to 2 min) ejection of low currents (0 to 10nA). In fact, in 10 of 20 neurones monosynaptic responses were completely abolished by 0.5 to 1 min ejections of 0 to 5 nA of (−)-baclofen (Figure 2) and 100% depression of the responses in the remaining 10 neurones could be achieved with either more prolonged ejections or slightly higher ejecting currents (up to 10 nA). Monosynaptic responses evoked in 1a inhibitory interneurones and in neurones in the intermediate nucleus region of the spinal cord appeared to be equally sensitive to the depressant action of (−)-baclofen (e.g. compare Figure 3 with Figure 4). In contrast, responses evoked polysynaptically were less sensitive to the depressant action of (−)-baclofen, in that significantly smaller reductions in these evoked responses were produced by significantly higher ejecting currents of (−)-baclofen (see Table 3). The differential sensitivity of the two types of synaptic responses to (−)-baclofen was not due to differences between microelectrodes as care was taken to sample monosynaptically activated and polysynaptically activated neurones with each microelectrode used and in many cases the effects of (−)-baclofen were examined on both types of response evoked in the same neurone. Furthermore, the lower sensitivity of polysynaptically evoked responses were not necessarily due to the nature of that response (i.e. usually bursts of spikes) since with several neurones the intensity of the afferent stimulus was carefully adjusted such that only 1 or 2 spikes were evoked per stimulus and these were also less sensitive to (−)-baclofen than monosynaptically evoked responses. Some of these effects of (−)-baclofen are illustrated for one neurone in Figure 3. This neurone was activated monosynaptically and polysynaptically by stimulation of the ipsilateral GS and SUR nerves respectively. The monosynaptic response was abolished within 1 min of starting the ejection of 5 nA (−)-baclofen whereas the polysynaptic response was hardly affected at this time and was only reduced about 50% following an eight fold increase in the baclofen ejection. All the effects of (−)-baclofen described above were reversible; however, the duration of the recovery period depended to some extent on the degree of depression produced and also on the dose of (−)-baclofen (i.e. the size (nA) or duration of the ejection). For example, the more sensitive monosynaptic response in Figure 3 took considerably longer to recover from the effects of (−)-baclofen than the polysynaptic response. However, in another test on the same cell (not illustrated) a brief 1 min ejection of 2 nA (−)-baclofen produced a 100% depression of the monosynaptic response which was completely reversible within 2 min of terminating the ejection.

(−)-Baclofen also depressed spontaneous firing present in 8 of 10 neurones. In 6 of these, spontaneous firing was markedly reduced in parallel with reductions in synaptic firing but in 2 other cells

Table 3 Effect of (−)-baclofen on mono- and polysynaptic excitation of spinal neurones

Synaptic response	*Baclofen* (nA)	*Duration of ejection* (min)	*No. cells depressed/ No. tested* (% depression)⁺	*Time to recovery* (min)
Monosynaptic	6.7 ±0.5*	1.8 ± 0.3^{NS}	20/20 (88 ± 4)**	2.6 ± 0.5^{NS}
Polysynaptic⁺⁺	15.1 ± 2.3	2.4 ± 0.2	18/20 (50 ± 6)	3.2 ± 0.6

Values are mean ± s.e. mean.

⁺ These values were calculated from peristimulus/time histograms computed from at least 32 sweeps analysed in 1 ms intervals. ⁺⁺ Including results from 4 Renshaw cells.

Significantly different from the corresponding value for polysynaptically activated neurones: * $P<0.005$; ** $P>0.001$ (Student's t test). ^{NS} not significantly different from the corresponding values for polysynaptically activated neurones.

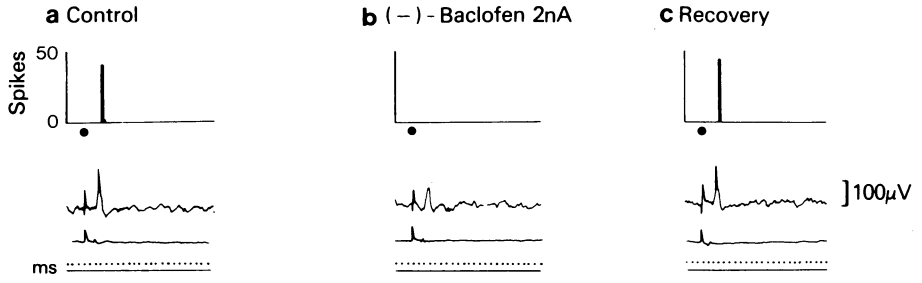

Figure 2 The effects of (−)-baclofen on the monosynaptic excitation of a dorsal horn neurone produced by single volleys in the gastrocnemius-soleus (GS) nerve (stimulus strength 1.4T). The lower trace is the time scale (ms), above this is the cord dorsum potential recorded at L7 segmental level, next is the monosynaptic spike and the top trace is a peristimulus-time histogram of the monosynaptic response computed from 32 sweeps analysed in 1 ms intervals. (a) Control; (b) illustrates complete abolition of the response by a 30 s ejection of 2 nA (−)-baclofen; (c) recovery of the response 10 min after terminating the (−)-baclofen ejection. The dot below the histograms marks the position of the stimulus artifact.

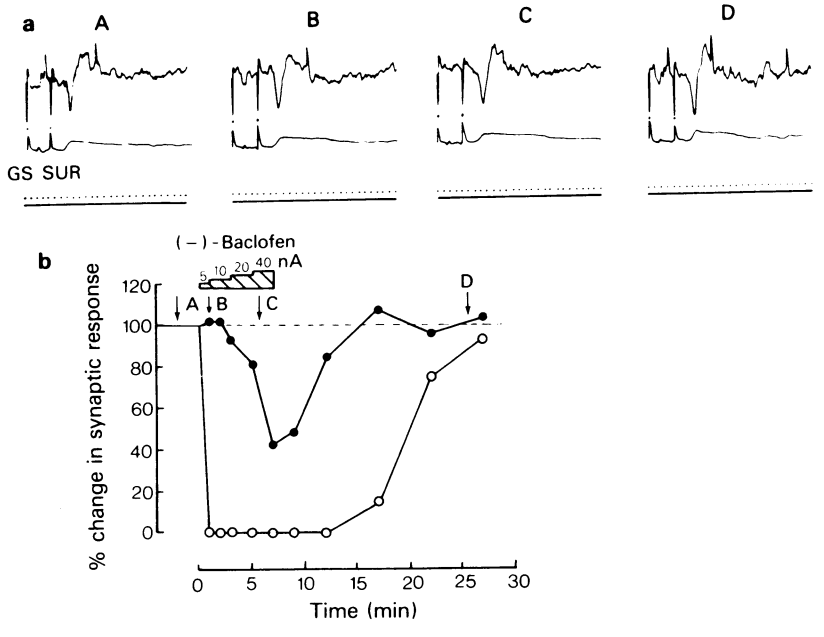

Figure 3 The graph in (b) shows the effects of increasing currents of (−)-baclofen on the mono- (○) and polysynaptic (●) responses of the same dorsal horn neurone evoked respectively by stimulation of the gastrocnemius-soleus (GS, stimulus strength 1.5 T) and sural (SUR) nerve (stimulus strength 1.4 T). Each point shows the percentage change in the number of spikes per response. These changes were calculated from filmed records of 40 oscilloscope sweeps. The horizontal line at the 100% point represents the control responses before the ejection of (−)-baclofen and corresponds to 40 ± 0 and 45 ± 0.1 (mean ± s.e.) spikes per sweep for the GS and SUR responses respectively. In (a) representative oscilloscope sweeps (in descending order) show the synaptic responses, cord dorsum potentials and time scale (ms) obtained at times corresponding to the arrows A, B, C and D marked on the graph. Note, the monosynaptic response was abolished by very low currents of baclofen whereas the polysynaptic excitation was only reduced about 60% by much higher currents.

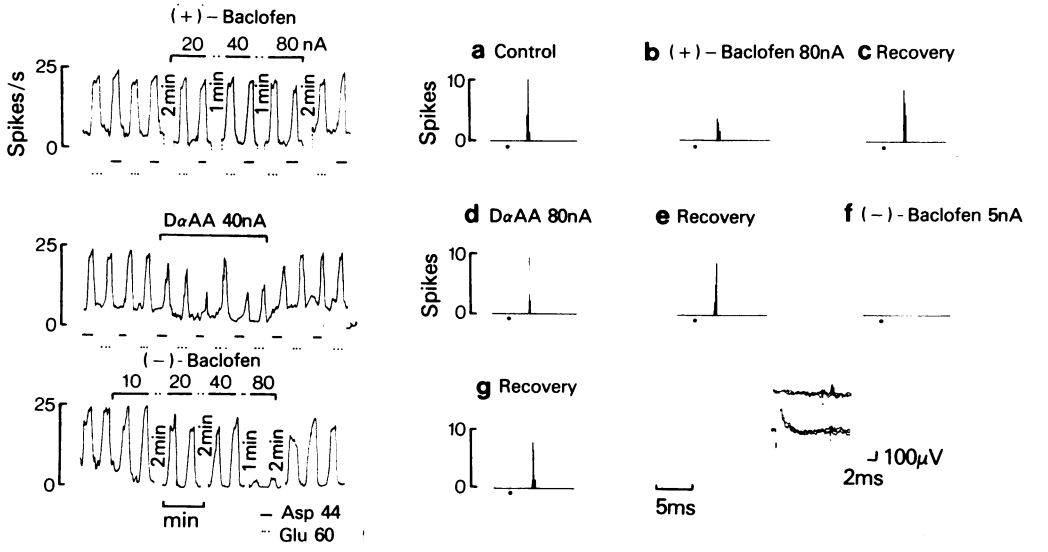

Figure 4 Effects of (+)- and (−)-baclofen and D-α-aminoadipate (DαAA) on L-glutamate- (glu 60 nA) and L-aspartate- (Asp 44 nA) induced excitation and ipsilateral posterior biceps and semitendinosus nerve (PBST)-induced monosynaptic excitation of the same Ia inhibitory interneurone. The ratemeter records on the left illustrate the effects of 20 to 80 nA (+)-baclofen, 40 nA DαAA and 10 to 80 nA (−)-baclofen on amino acid-induced responses. (a–g) Peristimulus-time histograms of the monosynaptic response computed from 32 sweeps analysed in 100 μs intervals. (a) Control response; (b) depression of this response by 80 nA (+)-baclofen for 5 min; (c) subsequent recovery 4 min later; (d) depression produced by 80 nA DαAA for 4 min and recovery 2 min later in (e); (f) 100% depression caused by 5 nA (−)-baclofen for 1 min and (g), recovery 6 min later. Inset: three superimposed oscilloscope traces showing monosynaptic activation of this neurone by stimulation of PBST (stimulus strength 1.2 T) (upper record) and (lower record) inhibition of this response by a preceding antidromic volley in the L7 ventral root indicating that this neurone is a Ia inhibitory interneurone. The dot below these traces and the histograms marks the position of the PBST stimulus artifact.

spontaneous firing was only depressed by large currents of baclofen (30 nA) and was unaffected in 2 cells. This effect of (−)-baclofen was also reversible and recovery preceded that of the synaptic response.

The effects of (−)-baclofen were also determined on responses induced by excitatory amino acids on 21 of the cells included in Table 3 (11 activated monosynaptically, 10 activated polysynaptically). The results obtained were essentially similar to those described above with (±)-baclofen. Thus, doses of (−)-baclofen which greatly reduced synaptically evoked responses only weakly depressed responses to (a) L-glutamate and L-aspartate on 4 of 18 cells and to (b) kainate and NMDA on 1 of 5 cells. Excitant responses induced by DLH were either unaffected or slightly enhanced (10 cells). However, higher ejecting currents of (−)-baclofen (20 to 60 nA) often depressed amino acid-induced excitations but unlike the reduction of synaptically evoked responses, these effects were invariably rapidly reversible on terminating the ejection (e.g. Figure 4).

Effects of (−)-baclofen on synaptic inhibition

(−)-Baclofen ejected with currents (20 to 30 nA for 4 to 6 min) greater than those affecting excitatory synaptic events had no significant effect on the intensity or duration of the submaximal inhibition of spontaneous or acetylcholine maintained firing of 7 neurones (4 Renshaw cells and 3 dorsal horn neurones) evoked by stimulation of the sural or tibial nerves. However, spontaneous firing (3 cells) was markedly reduced and synaptic excitatory responses evoked in 3 of these cells were abolished by considerably smaller ejecting currents. In tests on 4 of these cells, strychnine (20 nA for 2 min) reversibly reduced the evoked inhibition.

Comparison of (−)-baclofen with other substances

The effects of (−)-baclofen on monosynaptic excitatory responses were compared with those of several other substances.

(+)-Baclofen The effects of this isomer were essentially similar to those of (−)-baclofen but in agreement with previous studies (Olpe *et al.*, 1977, 1978; Ault & Evans, 1978) (+)-baclofen was considerably less effective than (−)-baclofen on a current basis. Thus, 39 ± 18.6 nA (+)-baclofen for 4 ± 0.9 min depressed monosynaptic responses in only 2 of 5 neurones by 50% whereas 8 ± 1.2 nA (−)-baclofen for 2 ± 0.5 min depressed responses in all 5 neurones by 68 ± 12.5%. An example of the effects of (+)- and (−)-baclofen on synaptic responses in one neurone is shown in Figure 4.

γ-Aminobutyric acid Comparisons with (−)-baclofen were made on 8 neurones. The effects of GABA were extremely variable, monosynaptic responses being reduced by 10 nA in 2 cells, unaffected by 80 nA in 2 cells and reduced in 4 cells by 60 to 80 nA. However, responses to iontophoretically administered excitants on all 8 cells were markedly reduced by low ejecting currents of GABA (e.g. 5 nA) as was spontaneous firing. By comparison (−)-baclofen (2 to 10 nA) depressed synaptic responses in all 8 cells with no accompanying reduction in response to excitant substances (Figure 5). In tests on 4 neurones, bicuculline methochloride (BMC 10 to 20 nA) completely antagonized the depressant effect of GABA on synaptic responses, whereas similar depressant effects of (−)-baclofen on these cells were uninfluenced by much larger doses of BMC (20 to 40 nA for 5 to 10 min). (The glycine antagonist, strychnine (20 to 40 nA, for 5 to 10 min), also failed to modify the action of (−)-baclofen on monosynaptic responses evoked in 5 neurones, although these currents of strychnine were considerably larger than those necessary to abolish the depressant action of glycine).

Noradrenaline and 5-hydroxytryptamine Presynaptic actions have been linked with the inhibitory effects of noradrenaline and 5-HT (see Langer, 1980) and noradrenaline and baclofen have common structural features (Davies & Watkins, 1974). However, neither noradrenaline (60 to 80 nA) nor 5-HT (50 to 100 nA) had any clear effect on monosynaptically evoked responses evoked in 4 neurones whereas (−)-baclofen (2 to 10 nA) abolished these responses in the same neurones.

D-*α-Aminoadipate* This excitatory amino acid antagonist has previously been shown to interfere with certain synaptic excitatory events in the spinal

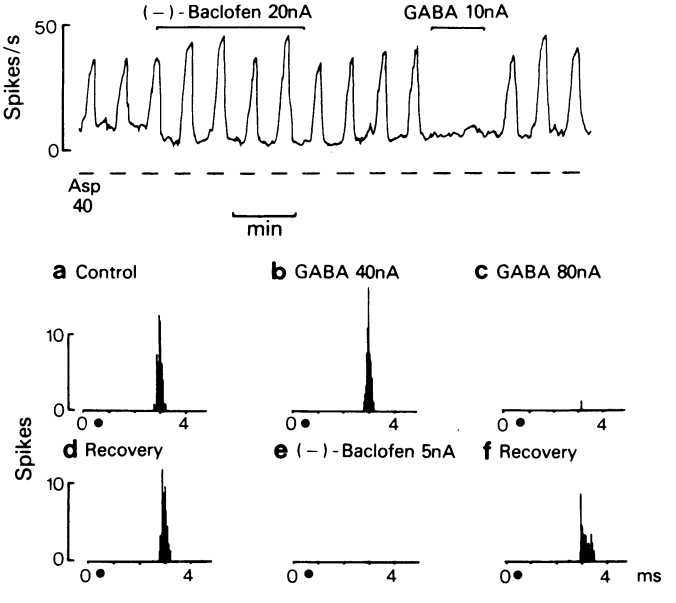

Figure 5 Comparative effects of (−)-baclofen and γ-aminobutyric acid (GABA) on the responses of the same dorsal horn neurone evoked by L-aspartate (Asp 40 nA) and stimulation of the gastrocnemius-soleus (GS) nerve (stimulus strength 1.6 T). The upper ratemeter record shows the marked depression of Asp-induced responses by GABA 10 nA and the lack of effect of (−)-baclofen 20 nA. (a–f) Peristimulus-time histograms of the GS-evoked monosynaptic excitation (dot marks the position of the stimulus artifact). Histograms were computed from 64 sweeps analysed in 100 μs intervals. (a) Control response; (b) 40 nA GABA for 2 min had no effect on the synaptic response while 80 nA for 2 min (c) virtually abolished it. This effect was reversible 1 min after ending the GABA ejection, (d). By contrast, 5 nA (−)-baclofen for 1 min abolished the synaptic response. Recovery was not fully complete 5 min after terminating the (−)-baclofen ejection (f).

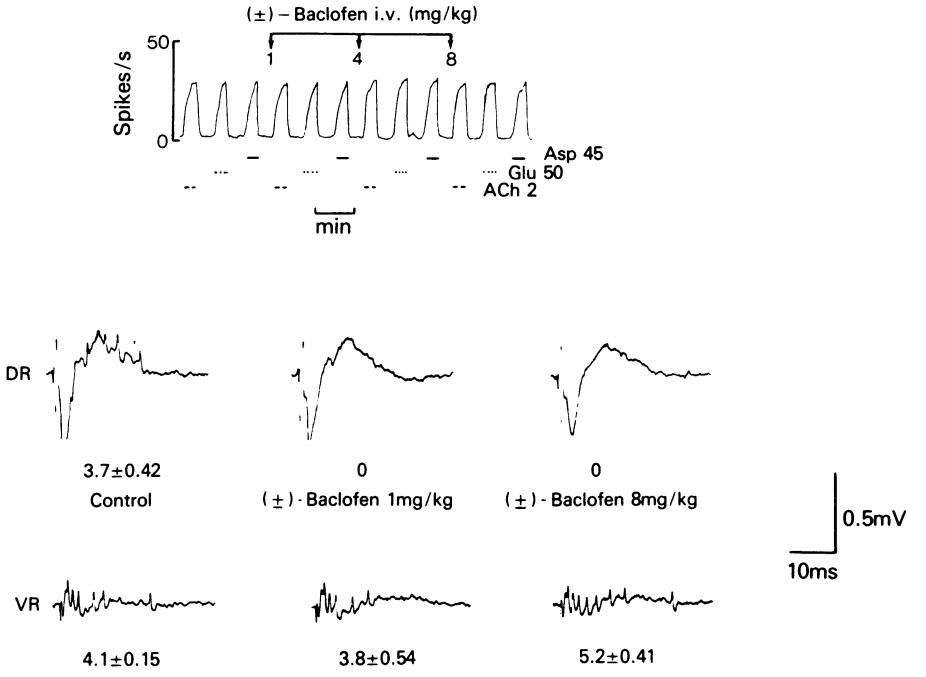

Figure 6 All records are from a single Renshaw cell and show representative oscilloscope sweeps of the responses evoked by submaximal dorsal root (DR) and ventral root (VR) stimulation and ratemeter records of responses of the same neurone to acetylcholine (ACh 2 nA), L-glutamate (Glu 50 nA) and L-aspartate (Asp 45 nA). The numbers below the oscilloscope records indicate the mean ± s.e. number of spikes in 30 consecutive sweeps. Increasing intravenous doses of (±)-baclofen were administered beginning with 1 mg/kg, then 10 min later, 4 mg/kg, and finally 8 mg/kg after a further 10 min. The ratemeter record shows representative responses to the iontophoretic excitants and indicates the complete lack of effect of baclofen on these responses. In contrast, the DR-evoked response was abolished by 1 mg/kg (±)-baclofen and the VR-evoked response was significantly enhanced by 8 mg/kg.

cord (Davies & Watkins, 1979). However, its effects were quite different from those of (−)-baclofen in that responses to exogenous amino acid excitants were considerably more sensitive to the depressant action of DαAA than synaptic excitatory responses (Figure 4). The latter were only reduced in 2 of 7 cells by 80 to 100 nA DαAA whereas the former were reduced in all 7 cells by 10 to 40 nA DαAA (5 to 10 nA (−)-baclofen produced 70 to 100% inhibition of the synaptic response in all 7 cells in the absence of any marked effect on responses to excitants).

Effects of intravenous injection of (±)-baclofen on synaptic and chemically induced excitation of spinal neurones

Prior to the termination of experiments on 6 cats, (±)-baclofen was administered intravenously while recording from Renshaw cells (4) or monosynaptically excited neurones (2). Doses of 1.0 mg/kg completely abolished polysynaptic (Renshaw cells) and monosynaptic responses (dorsal horn neurones) in all cells tested. However, the cholinergic ventral root-evoked responses (Curtis & Ryall, 1966) were unaffected in 2 Renshaw cells and enhanced by 15 to 20% in 2 Renshaw cells. Excitatory responses induced by amino acids and, in the case of Renshaw cells, by acetylcholine, were unaffected by intravenous (±)-baclofen even when doses up to 8 mg/kg were administered (Figure 6). These doses of (±)-baclofen had no significant effect on arterial blood pressure.

Discussion

The present results demonstrate that iontophoretic baclofen ((±)-racemate and (−)-isomer) is an extremely potent depressant of synaptic excitation evoked in spinal neurones by stimulation of dorsal roots, or muscle, or cutaneous afferent nerves. This effect does not appear to be due to either a general or a selective postsynaptic action of the agent, as neuronal responses to a range of chemical excitants, including the putative spinal transmitter candidates, glutamate and aspartate (Curtis & Johnston, 1974),

were either unaffected or minimally reduced compared with synaptic responses on the same cells to iontophoretically or systematically administered baclofen. Hence, these findings are in contrast to those observed with the selective excitatory amino acid antagonist, DαAA (Davies & Watkins, 1979), which more effectively reduced responses to exogenously administered excitants compared with synaptic excitatory responses. Saito, Konishi & Otsuka (1975) suggested that baclofen specifically antagonizes the excitatory effects of one of the putative primary afferent transmitters, substance P, but more recent studies do not support this suggestion (Davies & Dray, 1976; Fotherby, Morrish & Ryall, 1976; Henry & Ben-Ari, 1976; Phillis, 1976). Thus, while the possibility that baclofen antagonizes the postsynaptic action of some, as yet, unidentified excitatory transmitter substance cannot be entirely excluded, the most plausible explanation of the results of the present iontophoretic studies is that baclofen interferes with the presynaptic release of an excitatory transmitter substance. A similar conclusion has been arrived at by other investigators from results obtained following systemic and bath application of baclofen in *in vivo* and *in vitro* experiments respectively (Pierau & Zimmermann, 1973; Davidoff & Sears, 1974; Ault & Evans, 1978; Fox *et al.*, 1978; Kato *et al.*, 1978; Ono, Fukuda & Kudo, 1979). Depression of transmitter release is further supported by recent observations that electrically evoked release of L-aspartate and L-glutamate from slices of guinea-pig cerebral cortex and potassium and protoveratine evoked release of D-aspartate from slices of rat cerebral cortex and cat spinal cord respectively are reduced by baclofen (Potashner, 1979; Johnston, Hailstone & Freeman, 1980).

Previous iontophoretic studies have demonstrated a non-specific depressant action of baclofen on neuronal responses to various excitatory substances in a number of areas of the central nervous system (Curtis *et al.*, 1974; Davies & Watkins, 1974; Davies & Dray, 1976; Henry & Ben-Ari, 1976; Olpe *et al.*, 1977; 1978; Fox *et al.*, 1978). The present results are not at variance with these findings since similar non-specific depressant effects were observed when baclofen was ejected with larger currents than those which markedly reduced excitatory synaptic responses. Hence, these results support the hypothesis that baclofen has pre- and postsynaptic actions on spinal neurones (Fox *et al.*, 1978), the latter only being observed when relatively large doses (currents) of baclofen are administered. However, the relevance of the postsynaptic depressant action of baclofen to its antispastic activity is not clear as large intravenous doses of baclofen did not affect the postsynaptic sensitivity of spinal neurones to iontophoretic excitants in the present study.

While results from previous studies involving systemic administration are consistent with an action on presynaptic nerve terminals they give no indication of which terminals are affected. For example, depression of monosynaptic reflexes may be due to an action on primary afferent terminals, but depression of polysynaptic reflexes (Pierau & Zimmermann, 1973; Fukuda *et al.*, 1977; Kato *et al.*, 1978) and synaptic inhibition in spinal neurones (Kato *et al.*, 1978) could be due to an additional action on the terminals of excitatory and inhibitory interneurones respectively. The present iontophoretic study indicates that the actions of baclofen are limited to non-cholinergic excitatory nerve terminals since neither inhibitory synaptic responses of spinal neurones nor cholinergic synaptic responses of Renshaw cells evoked by ventral root stimulation (Curtis & Ryall, 1966) were depressed. Indeed, the observation that responses evoked monosynaptically were more sensitive to the depressant actions of baclofen than responses evoked polysynaptically indicates that primary afferent terminals are extremely sensitive to baclofen. However, the relatively lower sensitivity of polysynaptic responses to this agent may simply have been because the drug did not diffuse far enough away from the vicinity of the micropipette tip to affect a sufficient number of axon terminals of excitatory interneurones.

It is interesting to note that primary afferent evoked monosynaptic excitation of the 1a interneurones that are involved in evoking direct inhibition of motoneurones (Hultborn *et al.*, 1971) were as sensitive to the depressant actions of iontophoretically administered baclofen as monosynaptic responses evoked in neurones in the region of the intermediate nucleus. Thus, since monosynaptic excitation of motoneurones is produced by volleys in these same muscle afferents it might be expected that direct inhibition and monosynaptic excitation of motoneurones would be equally sensitive to baclofen. However, systemic studies indicate that direct inhibition of motoneurones is more resistant to the depressant actions of baclofen compared with monosynaptic excitation of these neurones (Pierau & Zimmermann, 1973; Fox *et al.*, 1978). The differential sensitivity of these two motoneurone responses to systematically administered baclofen may be due to differences in the degree of convergence of primary afferents onto 1a inhibitory interneurones and onto motoneurones.

Systemically and iontophoretically administered baclofen generally depressed dorsal root evoked excitation of Renshaw cells whereas ventral root evoked responses were enhanced in a proportion of the same cells. This latter effect of baclofen may also be mediated presynaptically, that is, by an increase in the release of acetylcholine from terminals of recurrent α motoneurone axon collaterals, since there was no accompanying increase in the response to ion-

tophoretic acetylcholine (Table 1). The observation that small doses of baclofen (2×10^{-6} M) increase the release of acetylcholine from α motoneurone axon terminals innervating the rat diaphragm (Glavinovic, 1979) is consistent with this suggestion. Incidentally, larger doses of baclofen (2×10^{-4} M) depress acetylcholine release from nerve terminals in this peripheral preparation and decrease the sensitivity of the motor endplate region of acetylcholine (Glavinovic, 1979). Such a dual action of baclofen on cholinergic nerve terminals (depending upon the concentration of the agent) would explain, firstly, the enhancement of ventral root evoked responses of Renshaw cells occasionally observed in the present study and in those of Benecke & Meyer-Lohmann (1974) and, secondly, the absence of such effects on these cells in others studies (Kato *et al.*, 1978).

The mechanism which underlies the presynaptic depressant action of iontophoretic baclofen on non-cholinergic excitation in the present experiments is not clear. An interaction with presynaptic catecholamine or 5-HT receptors appears to be excluded by the observation that neither noradrenaline nor 5-HT reduced synaptic excitation of spinal neurones. Baclofen is structurally related to GABA and recent neurochemical studies suggest that it may suppress transmitter release from central nerve terminals by an action on a novel, bicuculline insensitive, GABA receptor (Bowery, Hill, Hudson, Dodle, Middlemiss, Shaw & Turnbull, 1980). Neither previous nor the present studies support an interaction of baclofen with a classical GABA receptor because of its insensitivity to GABA antagonists (Curtis *et al*, 1974; Davies & Watkins, 1974). The failure of the glycine antagonist, strychnine, to antagonise the effects of baclofen on synaptic excitation also excludes the possibility that this agent acts on receptors for this inhibitory amino acid. Inhibition of excitatory transmitter release could arise through interference with transmitter synthesis. However, baclofen does not reduce the synthesis or turnover of the putative excitatory transmitter substances, L-glutamate or L-aspartate, from cortical slices while strongly inhibiting their evoked release (Potashner, 1979). Direct interference with transmembrane Ca^{2+} fluxes also seems an unlikely explanation for the effects of baclofen in view of its selective action on non-cholinergic excitatory synaptic responses.

Thus, in conclusion, the present experiments indicate that iontophoretically administered baclofen depresses non-cholinergic excitatory synaptic events in the spinal cord by an interaction at some presynaptic site. The close similarity of these findings with those following systemic administration of baclofen suggest that this action of baclofen may be relevant to the beneficial effects of this agent in certain spastic conditions.

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