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# SELECTIVE DEPRESSION OF SYNAPTIC TRANSMISSION OF SPINAL NEURONES IN THE CAT BY A NEW CENTRALLY ACTING MUSCLE RELAXANT, 5-CHLORO-4-(2-IMIDAZOLIN-2-YL-AMINO)-2, 1, 3-BENZOTHIODAZOLE (DS103-282)

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**1** The effects of a new muscle relaxant, 5-chloro-(2-imidazolin-2-yl-amino)-2, 1, 3-benzothiodazole (DS103-282) have been examined on segmental reflexes and responses of single neurones in the spinal cord of the anaesthetized cat to stimulation of peripheral afferents, ventral roots, acetylcholine and various amino acids. Drugs were administered intravenously and/or iontophoretically.

**2** Polysynaptic reflexes were depressed in a dose-dependent manner by 0.01–0.1 mg/kg DS103-282 whereas monosynaptic reflexes were relatively insensitive to this agent.

**3** In studies on single dorsal horn neurones, iontophoretically and intravenously administered DS103-282 depressed synaptic excitatory responses, polysynaptic responses being much more sensitive to this agent than monosynaptic responses. In contrast (−)-baclofen preferentially reduced monosynaptic excitation.

**4** Doses or ejecting currents of DS103-282 which greatly depressed polysynaptic excitatory responses also reduced spontaneous firing of neurones, but either had no effect or minimal depressant effects on responses to iotophoretically administered excitant amino acids. Acetylcholine-induced excitation of Renshaw cells was depressed by iontophoretically (but not intravenously) administered DS103-282, although ventral root-evoked responses of these cells were insensitive to this agent.

**5** Inhibition of spinal neurones by stimulation of peripheral nerves or by iontophoresis of γ-aminobutyric acid or glycine was unaffected by DS103-282.

**6** These results indicate that DS103-282 preferentially depresses peripherally evoked polysynaptic excitation of spinal neurones probably by an action on the terminals of excitatory interneurones.

## Introduction

A new muscle relaxant drug, 5-chloro-4–(2-imidazolin-2-yl-amino)-2, 1, 3-benzothiodazole (DS103-282), is reported to be more effective than baclofen in reducing muscle tone in spastic patients (Hassan & McLellan, 1980). The mechanism of action of DS103-282 is not known but pharmacological studies in animals suggest action on the central nervous system (Sayers, Bürki & Eichenberger, 1980). It reduces stretch reflexes in spastic patients (Hassan & McLellan, 1980); thus the myotonolytic action of this agent may be mediated at the spinal level. However, the effects of DS103-282 on spinal reflexes in the cat were inconsistent (Sayers *et al.*, 1980) a result not entirely in agreement with this suggestion.

It, therefore, seemed desirable to re-evaluate the effects of this substance on the spinal cord. To this end the effects of DS103-282 have been examined on mono- and polysynaptic reflexes in the cat spinal cord. In addition, in order to assess its possible site and mechanism of action, the influence of this agent on synaptically and chemically induced responses of single spinal neurones has also been determined following systemic and iontophoretic administration. In some experiments comparisons have been made between the effects of DS103-282 and those of baclofen.

## Methods

Experiments were performed on cats (2–3.5 kg) of either sex anaesthetized with α-chloralose (50 mg/kg, i.v.). A cannula was inserted into the brachial vein for intravenous administration of drugs. Blood pressure

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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.

Following a laminectomy extending from L1–L7 vertebrae, the spinal cord was transected at L1. Ventral roots S1, L7 and L6 were cut ipsilaterally and the central ends of S1 and L7 were mounted upon bipolar platinum electrodes for stimulation or recording. The following ipsilateral leg nerves were dissected and their central ends mounted on bipolar silver electrodes for stimulation; posterior biceps and semitendinosus (PBST), gastrocnemius and soleus (GS), flexor digitorum hallucis longus (FDHL), common peroneal (PER), common tibial (TIB) and sural (SUR). The exposed spinal cord and limb nerves were covered with a pool of paraffin oil maintained at 36° to 37°C with the aid of an infra red lamp.

*Reflex experiments*

Mono- and polysynaptic reflexes were recorded mono-phasically from the central ends of transected ventral roots S1 or L7. Monosynaptic reflexes were evoked by single volleys in muscle nerves (PBST, GS or FDHL) at intensities of 4–6 times group I threshold strength (0.8–2 V, 0.1 ms duration at 0.5 Hz). In several experiments two or more monosynaptic reflexes were recorded in response to stimulation of two or more muscle afferents (e.g. Figure 1). Polysynaptic reflexes were similarly recorded but in response to stimulation of cutaneous afferents in SUR, PER or TIB nerves. Stimulus intensities were adjusted to give stable reflexes (usually 3–8 times threshold strength for the fastest afferents in a given nerve). All reflexes were photographed and the area beneath the reflex integrated electronically.

Control reflexes were recorded for at least 15 min before DS103-282 was administered intravenously to ensure stability of the response.

*Single neurone studies*

Experiments were performed using dorsal horn neurones and Renshaw cells. Action potentials of single neurones were recorded by means of the centre barrel (4 M NaCl) of seven barrel micropipettes and were monitored on an oscilloscope and either photographed 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 (PSTH) of synaptic events.

Renshaw cells were identified by their characteristic response to stimulation of ventral roots. Dorsal horn neurones were identified by their position in the cord (mostly in the region of the intermediate nucleus) and their response to afferent volleys as reported previously (Davies, 1981).

The outer barrels of the seven barrel micropipettes contained aqueous solution of the following agents: acetylcholine Cl (0.5 M), Na L-glutamate (0.5 M, pH 7.2), Na L-aspartate (0.5 M, pH 7.2), DL-homocysteate (DLH) (0.5 M, pH 7.0), N-methyl-D-aspartate (NMDA) (0.05 M in 0.15 M NaCl), Na kainate (0.02 M in 0.15 M NaCl), quisqualate (0.02 M in 0.15 M NaCl), γ-aminobutyric acid (GABA, 0.5 M, pH 3.5), glycine (0.5 M, pH 3.5), bicuculline methochloride (BMC) (0.005 M in 0.165 M NaCl), strychnine HCl (0.005 M in 0.165 M NaCl), DS103-282 HCl (0.2 M, pH 5.5) (Sandoz), (−)-baclofen (0.005 M in 0.165 M NaCl).

## Results

*Effects on spinal reflexes*

Table 1 summarizes the effects of DS103-282 on spinal mono- and polysynaptic reflexes. DS103-282

**Table 1** Effects of DS103-282 on spinal monosynaptic and polysynaptic reflexes

| *Reflex* | *Effect** | *Dose* (mg/kg, i.v.)** | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0.01 | 0.025 | 0.05 | 0.1 | 0.15 | 0.2 |
| Polysynaptic | Unchanged | – | – | – | – | – | – |
| | Increased | – | – | – | – | – | – |
| | Decreased | 2(53) | 5(71 ± 12.8) | 5(98 ± 2.0) | 6(100 ± 0) | 2(100) | 2(100) |
| Monosynaptic | Unchanged | 2 | 3 | 3 | 1 | 1 | – |
| | Increased | – | – | – | – | 1(10) | 1(15) |
| | Decreased | 1(30) | 4(21 ± 10.1) | 5(45 ± 6.8) | 4(48 ± 7.7) | – | 3(21 ± 6.6) |

*The numbers refer to the number of reflexes unchanged, increased or decreased with the mean ± s.e. % change in parentheses.

**In the majority of experiments, DS103-282 was administered initially in a dose of 0.01, 0.025 or 0.05 mg/kg thus doses > 0.05 mg/kg are cumulative doses.

was administered intravenously over a period of about 30 s, initially in doses of 0.01–0.05 mg/kg. Subsequently, at intervals of 6–10 min (when no further change in the recorded reflexes was apparent) increasing doses were injected. Arterial blood pressure was significantly increased with all doses of DS103-282. However, in the dose range 0.01–0.05 mg/kg the blood pressure increase was transient, returning to pre-injection levels with 0.5–1.5 min. Doses of 0.1 mg/kg and above produced more prolonged increases in blood pressure (2–5 min depending upon the dose) which were often accompanied by a marked slowing of the heart rate and a subsequent slow, progressive decline in pressure. Polysynaptic reflexes were depressed in a dose-dependent manner in all experiments (Table 1, Figure 1). In contrast, monosynaptic reflexes were generally only significantly depressed over the dose range 0.05–0.1 mg/kg and were in fact enhanced in 3 experiments (1 not included in Table 1) with doses greater than 0.15 mg/kg. This tendency for larger doses to enhance or have less marked depressant effect on monosynaptic reflexes is illustrated for 1 experiment in Figure 1. The depressant action of DS103-282 on monosynaptic reflexes but not on polysynaptic reflexes could be over come by increasing the strength of the afferent stimulus.

*Effects of DS103-282 on mono- and polysynaptic excitation of dorsal horn neurones*

*Iontophoretic studies* The effects of DS103-282 on mono- and polysynaptic excitatory responses of single dorsal horn neurones are summarized in Table 2. Direct comparisons between the effects of DS103-282 and baclofen on a number of cells are also summarized in this table. DS103-282 reversibly reduced synaptic excitation in a dose-dependent manner in many neurones. Polysynaptically evoked excitatory responses were more sensitive to the depres-

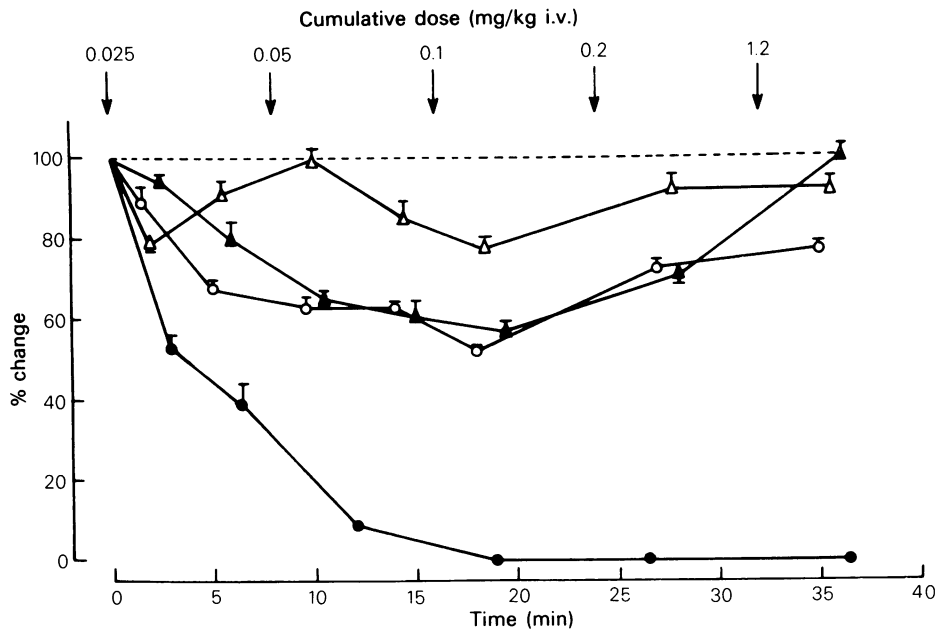


**Figure 1** The graph illustrates the effects of intravenous administration of DS103-282 on 3 monosynaptic reflexes and 1 polysynaptic reflex recorded in L7 ventral root in a single experiment in response to stimulation of ipsilateral leg nerves. Monosynaptic reflexes were evoked from posterior biceps and semitendinosus (PBST) at 4.3 T, flexor digitorum hallucis longus (FDHL) at 4 T and gastrocnemius and soleus (GS) at 5.5 T. A polysynaptic reflex was evoked from sural (SUR) at 4.5 T. All stimulus intensities are expressed in multiples of threshold (T) for the fastest afferent and were of 100 μs duration and 0.5 Hz frequency. Each point on the graph represents the mean area beneath 4 reflexes (bars show s.e.mean) after DS103-282 administration, expressed as a percentage of the mean of 4 control reflexes obtained prior to the first DS103-282 injection. Note the marked effect of DS103-282 on the polysynaptic reflex and reversal of the diminution of monosynaptic reflexes with doses of DS103-282 greater than 0.1 mg/kg. DS103-282 was initially administered in a dose of 0.025 mg/kg. (●) SUR polysynaptic reflex (PSR); (○) PBST monosynaptic reflex (MSR); (△) FDHL MSR; (▲) GS MSR.

**Table 2** Effects of DS103-282 and (−)-baclofen on mono- and polysynaptic responses of dorsal horn neurones

| *Synaptic response* | (Drug nA) | *Duration of ejection* (min) | *No. of cells depressed* / *No. tested* (% depressed)+ | *Recovery times* (min) |
|---|---|---|---|---|
| Monosynaptic | DS103-282 (36.5 ± 6.7) | 2.9 ± 0.49 | $^{5}$/14 (65 ± 21.7) | 5 ± 3.2 |
| | +*(−)baclofen (7 ± 1.2) | 2.1 ± 0.40 | $^{7}$/7 (94 ± 4) | 3.3 ± 1.2 |
| Polysynaptic | DS103-282 (21 ± 2.7)** | 2.4 ± 0.37$^{NS}$ | $^{15}$/16 (67 ± 10.1)$^{NS}$ | 20.6 ± 3.7* |
| | +*(−)baclofen (16 ± 2.4)** | 2.4 ± 0.24$^{NS}$ | $^{6}$/6 (48 ± 11.4)** | 4.7 ± 1.8$^{NS}$ |

+* = (−)-Baclofen was not tested on all cells but was always tested on the same cells as DS103-282.
+ = These values were calculated from peristimulus time histograms computed from at least 32 sweeps analysed in 1 ms intervals.
Significantly different from the corresponding value for monosynaptically activated neurones: $^{*}P<0.05$; $^{**}P<0.01$ (Student's $t$ test). NS not significantly different from the corresponding values for monosynaptically activated neurones.
All values in this table are expressed as means ± s.e.

sant action of DS103-282 than monosynaptic responses in that the former were depressed in a greater number of neurones (93%) by smaller ejection currents of DS103-282 and the duration of the depression was significantly longer than that of monosynaptic responses (Table 2). The differential sensitivity of the two types of synaptic responses to DS103-282 was not due to differences between microelectrodes as care was taken to sample monosynaptically and polysynaptically activated neurones with each microelectrode used and often the effects of this agent were determined on both types of response evoked on the same neurone. An interesting feature of the effect of DS103-282 on polysynaptically evoked responses was that often there was a progressive depression of the response in the first few minutes after terminating the DS103-282 ejection (e.g. Figures 2 and 3). No progressive post-ejection depression of monosynaptic responses was observed. The depression of synaptic excitation was not accompanied by any change in spike configuration although spontaneous firing, when present, was abolished or markedly reduced with a time course paralleling the reduction of polysynaptically evoked responses.

These effects of DS103-282 contrast with those of (−)-baclofen which, although more potent in terms of ejecting currents necessary to depress synaptic responses, was a much more effective depressant of monosynaptic excitation than polysynaptic excitation (Table 2, Figures 3 and 4). The effects of (−)-baclofen were only studied on a small number of neurones. However, the present results are consistent with those obtained on a larger number of neurones in an earlier study (Davies, 1981). The duration of action of (−)-baclofen on monosynaptic excitatory responses was considerably shorter than that of DS103-282 on polysynaptically evoked responses (Table 2). However, the time course of action of baclofen is dependent on the duration and size of ejecting current, longer ejections or higher currents producing more prolonged depression of synaptic responses (Davies, 1981) similar to those seen with DS103-282.

The effects of iontophoretically administered DS103-282 were also determined on responses of 17 dorsal horn neurones (including 12 neurones in Table 2) induced by excitatory amino acids (L-glutamate, L-aspartate and DL-homocysteic acid, NMDA, kainate and quisqualate). Ejections of 10–40 nA (27 ± 3.3 nA, mean ± s.e.) DS103-282 for 2–7 min (2.7 ± 0.29 min) had no significant effect on amino acid-induced responses in 9 neurones whereas synaptic excitatory responses induced in 6 of these neurones were markedly reduced (e.g. Figure 2). Amino acid-induced responses were reduced by 15–80% in the remaining 8 neurones, no consistent differential effect being observed on any particular amino acid. However, the depression of amino acid-induced responses, unlike the depression of polysynaptically evoked responses in 6, or of spontaneous firing in 3, of these neurones, was generally less marked and usually rapidly reversible (within 1–4 min of terminating the DS103-282 ejection).

*Intravenous studies* DS103-282 was administered intravenously in doses of 0.025–0.1 mg/kg while recording chemically and/or synaptically induced ex-

citatory responses of 8 dorsal horn neurones. The results obtained were essentially similar to those observed when DS103-282 was administered iontophoretically. Polysynaptic excitation evoked in 6 neurones was reduced by 35–100% (83 ± 10.7%) (e.g. Figure 2). Background firing when present, was also markedly depressed. These effects of DS103-282 were reversible within 20–40 min of the injection. Monosynaptically evoked excitation was unaffected in 3 neurones but was abolished in 1 neurone by a dose of 0.05 mg/kg DS103-282. However, following recovery of this response 15 min later a subsequent dose of 0.1 mg/kg DS103-282 only produced a 40% reduction in the monosynaptic response. DS103-282 had no significant effect on excitatory responses to DLH, L-glutamate or L-aspartate in any

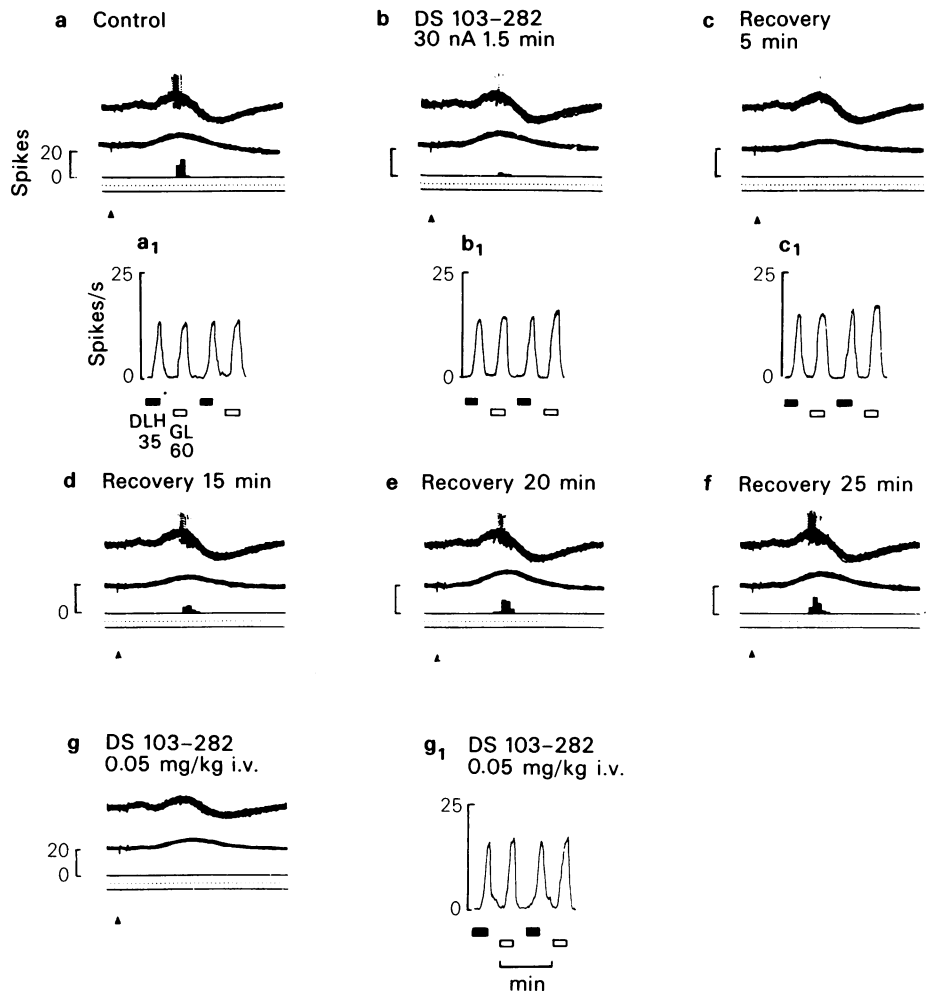


**Figure 2** Effects of DS103-282 on polysynaptically evoked and amino acid-induced excitation of the same dorsal horn neurone. Records (a)–(g) illustrate (from top to bottom) superimposed oscilloscope trace of the polysynaptic excitation evoked by sural nerve stimulation (2 T, 100 μs at 1 Hz), the cord dorsum potential recorded at the dorsal root entry zone of the L7 segment, a peristimulus-time histogram of the synaptic response computed from 32 sweeps analysed in 1 ms intervals and the time scale (ms). The position of the stimulus artefact is shown by a filled triangle below the time scale. Records ($a_1$), ($b_1$), ($c_1$) and ($g_1$) are samples of rate meter records illustrating excitatory responses induced by alternate ejection of DL-homocysteate (DLH, 35 nA) and L-glutamate (GL, 60 nA). (a) and ($a_1$) are control responses, (b) and ($b_1$) were recorded 1.5 min after starting the ejection of DS103-282 30 nA, (c) and ($c_1$) were recorded 5 min after terminating the DS103-282 ejection (note the synaptic response is diminished further at this time); (d)–(f) show progressive recovery of the synaptic responses, (g) and ($g_1$) were recorded 4 min after the intravenous injection of 0.05 mg/kg DS103-282.

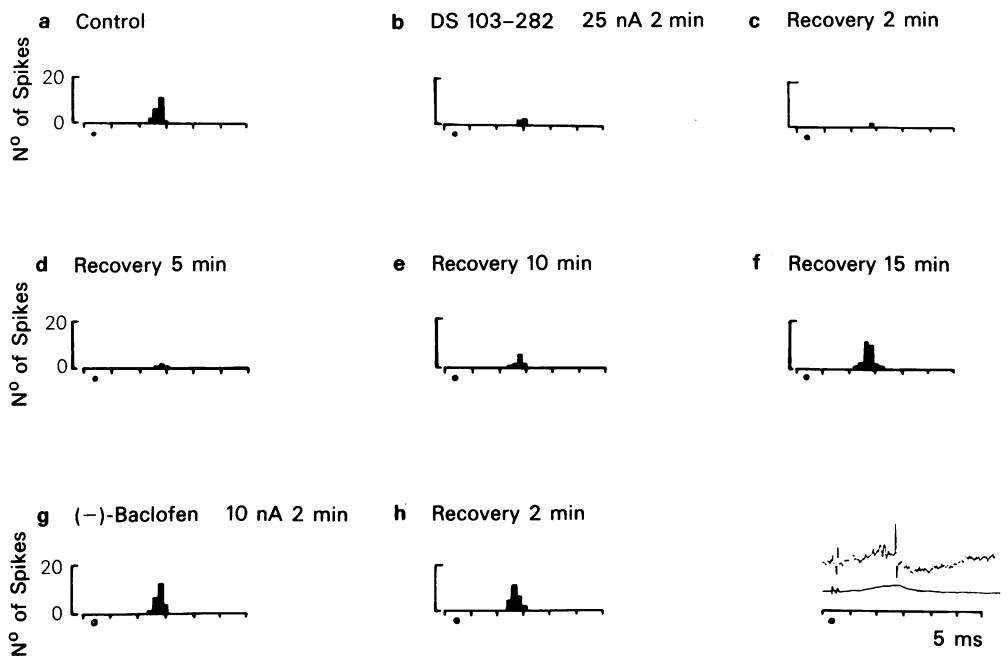


**Figure 3** Records (a)–(g) are peristumulus time histograms of the polysynaptic excitation evoked in a dorsal horn neurone by stimulation of the gastrocnemius and soleus (GS) nerve (4.3 T, 1 Hz, 100 μs pulses) (see inset bottom right) computed from 32 sweeps and analysed in 1 ms intervals. DS103-282 (25 nA for 2 min) produced a marked and prolonged depression of the synaptic response whereas (−)-baclofen (10 nA for 2 min) had no significant effect on this response (cf. Figure 4).

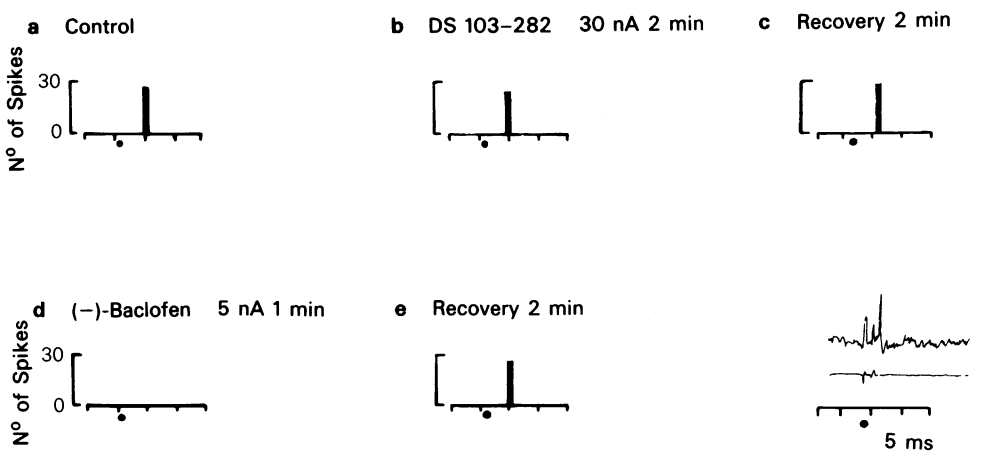


**Figure 4** This illustrates the profound depressant action of (−)-baclofen (5 nA for 1 min) on the monosynaptic excitation of a dorsal horn neurone evoked by stimulation of flexor digitorum hallucis longus (FDHL) nerve (1.3 T, 1 Hz, 100 μs pulses) and the lack of effect of DS103-282 (30 nA for 2 min) on this response. The peristimulus time histograms (a)–(e) were computed from 32 sweeps analysed in 1 ms intervals. Inset (lower right) single oscilloscope sweep of the monosynaptic excitation, cord dorsum potential and time scale.

of the cells tested (4) although all doses produced transient increases in arterial blood pressure.

*Effects on chemical and synaptic excitation of spinal Renshaw cells*

DS103-282 was administered iontophoretically in the vicinity of 9 Renshaw cells which were excited by acetylcholine, excitatory amino acids and S1 or L7 ventral root stimulation (monosynaptic cholinergic excitation; Curtis & Ryall, 1966). Three of these cells were also synaptically excited by stimulation of cutaneous afferents (polysynaptic, non-cholinergic excitation). DS103-282 5–40 nA (26 ± 3.8 nA), for 2–17 min (6.5 ± 1.8 min) had no significant effect on the submaximal ventral root evoked response of any Renshaw cell, but reversibly abolished the primary afferent evoked polysynaptic excitation in 3 cells (e.g. Figure 5). Spontaneous firing, present in 1 cell, was also markedly depressed by DS103-282. Responses to iotophoretic acetylcholine were reduced by 50–100% in 6 neurones and unaffected in 3 cells (Figure 5). Excitation induced by L-glutamate and L-aspartate was unaffected in 6 but was reduced in 3 cells in which responses to acetylcholine were also depressed. However, the depression of responses to acetylcholine was more marked and prolonged than that to amino acids. Indeed, the time course of the depression of responses to acetylcholine was similar to that of the depression of background firing and polysynaptically evoked excitation observed on dorsal horn neurones.

Intravenously administered DS103-282 (0.025–0.1 mg/kg) had no significant effect on ventral root-evoked, acetylcholine or amino acid-induced excitation in tests on 4 Renshaw cells, but polysynaptic excitation evoked in 1 of these was completely abolished (Figure 5).

*Effects of iontophoretically administered DS103-282 on synaptic and amino acid-induced inhibition of spinal neurones*

DS103-282 15–80 nA (33.5 ± 2.6 nA) for 2–9 min (3.8 ± 1.0 min) had no significant effect on the depression of spontaneous or excitant driven firing of dorsal horn neurones (4) or Renshaw cells (3) induced by submaximal iontophoretic currents of GABA or glycine, or by submaximal, strychnine-sensitive, synaptic inhibition of Renshaw cells evoked by stimulation of cutaneous afferents (2 cells). However, spontaneous firing was depressed by DS103-282, but this effect was not antagonized by bicuculline methochloride or strychnine ejected with

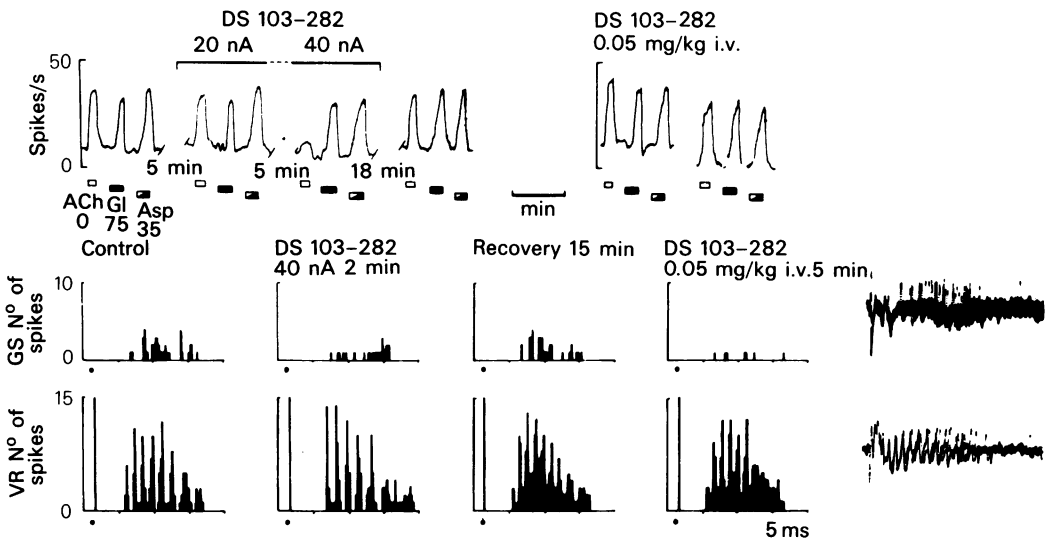


**Figure 5** Effects of iontophoretically (20–40 nA) and intravenously (0.05 mg/kg) applied DS103-282 on chemically and synaptically induced excitation of a Renshaw cell. The upper ratemeter records illustrate excitatory responses to acetylcholine (ACh, 0 nA), L-glutamate (Gl 75 nA) and L-aspartate (Asp 35 nA). The lower records are peristimulus time histograms (PSTHs) of the synaptic responses of the same cell (shown as superimposed oscilloscope records to the right of this figure) evoked by submaximal stimulation of the gastrocnemius and soleus (GS) nerve (5, 6 T, 1 Hz, 100 μs pulses) and L7 ventral root (VR). The PSTHs were computed from 64 sweeps analysed in 100 μs intervals. Note: iontophoretic DS103-282 depressed the GS-evoked and ACh-induced excitation and also spontaneous firing of this neurone. Systemic DS103-282 had no effect on the ACh-induced excitation.

currents which selectively antagonized the inhibitory effects of GABA and glycine respectively on the same neurones.

## Discussion

### *Spinal reflexes*

The present results demonstrate that intravenously administered DS103-282 in doses producing only transient effects on arterial blood pressure markedly reduce segmental polysynaptic reflexes. This effect of DS103-282 appears to be selective since monosynaptic reflexes were relatively insensitive to the depressant action of this agent, and indeed were enhanced in a number of experiments when relatively large doses were administered. These observations are at variance with some of those previously reported in the cat (Sayers *et al.*, 1980). These discrepancies are unlikely to be due to differences in anaesthesia as α-chloralose was used in some of the experiments of Sayer *et al.* (1980). However, these workers evoked segmental reflexes by high intensity dorsal root stimulation. Reflexes evoked in this way may have a much higher safety factor due to summation occurring at the motoneuronal level following stimulation of many different primary afferents in dorsal roots which could account for their higher resistance to DS103-282. In keeping with the present findings, polysynaptic reflexes were reduced by DS103-282 in some intact and Cl spinalized cats (Sayers *et al.*, 1980) but in other experiments in that study reflexes were unaffected. Generally, much higher doses of DS103-282 were employed by Sayers and colleagues (1980) than were used in the present investigation. It is perhaps relevant that monosynaptic reflexes, in particular, were enhanced in the present study on a number of occasions with relatively large doses of DS103-282. One possible explanation for this enhancement may be removal of an underlying tonic inhibition by DS103-282. This could arise if there is an excitatory interneurone in the inhibitory pathway which DS103-282 would be expected to depress (see below).

### *Studies on single neurones*

The results obtained when DS103-282 was administered iontophoretically or intravenously while recording synaptically evoked excitation in spinal neurones are consistent with the study on spinal reflexes in that polysynaptic excitatory events were particularly susceptible to the depressant actions of DS103-282. The greater sensitivity of polysynaptic compared to monsynaptic excitation to iontophoretic DS103-282 is unlikely to be due to differences in the locations of excitatory synapses as monosynaptic responses were more sensitive than polysynaptic responses to (−)-baclofen ejected from the same electrode as DS103-282. Furthermore, systemic administration of DS103-282 would be expected to achieve a more even distribution of drug in the spinal cord than iontophoretic administration and this also selectively reduced polysynaptic excitation. Since iontophorectically administered DS103-282 did not interfere with the depressant actions of the inhibitory transmitter substances GABA or glycine, and did not modify synaptic inhibition, the greater sensitivity of polysynaptic responses compared to monosynaptic responses suggests that the depressant action of this agent is either mainly limited to the postsynaptic membrane of the polysynaptically excited neurone, or to the presynaptic terminals of excitatory interneurones. A general or selective postsynaptic depressant action of DS103-282 seems unlikely in view of the observation that neuronal responses to the putative excitatory transmitter candidates L-glutamate and L-aspartate (Curtis & Johnston, 1974) and other excitatory amino acids were either unaffected or minimally reduced compared with synaptic responses in the same cells by either iontophoretically or systemically administered DS103–282. Indeed, it is possible that the reduction in responses to exogenous excitants observed in some neurones was secondary to the depression of spontaneous background firing produced by DS103-282. The most plausible explanation for the action of DS103-282 reported here is that it in some way interferes with the release of the excitatory transmitter(s) from nerve terminals of excitatory interneurones in the spinal cord. This suggestion is supported by the observations that the $K^+$ evoked release of D[$^3$H]-aspartate from spinal cord slices is depressed by DS103-282 (Davies & Johnson, unpublished observation). However, since the transmitters involved have yet to be identified, the possibility that DS103-282 may act postsynaptically cannot be excluded. It certainly appears that DS103-282 can exert some postsynaptic action since relatively high ejecting currents sometimes depressed responses to excitatory amino acids, and those to acetylcholine were depressed on the majority of Renshaw cells studied. However, since few spinal interneurones, other than Renshaw cells, are excited by acetylcholine the significance of this observation is not clear. Nevertheless, whether the effects of DS103-282 are mediated pre- or postsynaptically, if they are specifically related to one transmitter substance then excitatory interneurones in the spinal cord probably use a similar substance and this transmitter may differ from that utilized by primary afferents. Intracellular studies from spinal neurones coupled with iontophoretic administration of DS103-282 will be necessary to clarify the site of

action of this substance.

The effects of DS103-282 reported here are quite distinct from those of (−)-baclofen which preferentially depressed monosynaptically evoked excitatory responses in spinal neurones. The only similarity between the two substances is that they both appear to act presynaptically but with preferences for different nerve terminals (Curtis, Lodge, Bornstein & Peet, 1981: Davies, 1981).

Thus, in conclusion, DS103-282 selectively depresses polysynaptic reflexes and excitation of spinal neurones evoked polysynaptically by stimulation of ipsilateral leg nerves. These effects may be mediated pre- or postsynaptically. Current evidence tends to favour a presynaptic site of action. It is suggested that the selective action of DS103-282 at synapses of excitatory interneurones in the spinal cord probably contributes to its myotonolytic activity. However, whether its action is limited to the spinal cord remains to be determined. If, as the present results suggest, the action of DS103-282 is limited to certain synapses which may utilize the same transmitter, this compound could be of immense investigational value at other central excitatory synapses in the central nervous system.

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