Br. J. Pharmac. (1983), **79**, 009–011

Spinal interneurone depression by DS103–282

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The depressant effect of 5-chloro-4-(2-imidazolin-2-yl-amino)-2,1,3-benzothiodiazole (DS103-282) on the polysynaptic excitation of interneurones in the cat spinal cord appears to be related to a postsynaptic reduction in the effectiveness of excitatory transmitters rather than to interference with their presynaptic release.

Introduction On the basis of the depression of poly- but not monosynaptic excitation of spinal interneurones without a correlative reduction of excitation by excitant amino acids, or alterations of inhibition by either glycine or γ-aminobutyric acid (GABA), it has been proposed that 5-chloro-4-(2-imidazolin-2-yl-amino)-2,1,3-benzothiodazole (DS 103-282; Sayers Burki & Eichenberg, 1980) acts presynaptically in the feline spinal cord, interfering with the release of transmitters from terminals of excitatory spinal interneurones (Davies, 1982). In these studies microelectrophoretic DS 103-282 did however reduce spontaneous cell firing, and also reduced in a non-specific fashion the effects of several excitatory amino acids on many neurones. Thus a reduction in postsynaptic excitability, to which poly- compared to monosynaptic activation would be most susceptible, could mediate the 'selective' reduction by DS103-282 of polysynaptic excitation of the interneurones (Curtis, Lodge, Bornstein & Peet, 1981). To circumvent this problem we have examined the effect of DS103-282 on amino acid excitation of spinal interneurones, and on synaptic excitation of these cells, where background firing, and thus postsynaptic excitability, was maintained at an approximately constant level by the continuous ejection of an excitant amino acid.

Methods The effects of the amino acid excitants, quisqualate, kainate and N-methyl-D-aspartate, and of the antagonists, DS103-282, (±)-*cis*-2,3-piperidine dicarboxylic acid (2,3-PDA) and (±)-2-amino-5-phosphonovaleric acid (2APV), were studied on interneurones of the L_7 and S_1 spinal segments of cats anaesthetized with sodium pentobarbitone (35 mg kg^{-1}, i.p., supplemented i.v. as required). The centre 3.6 M NaCl-containing barrel of seven barrel micropipettes was used to record extracellular action potentials, the firing rates of which were plotted continuously on a rectilinear ink writing recorder. To examine the depressant effects of DS103-282, the excitants were ejected with currents sufficient to evoke approximately equal but submaximal firing rates (30–80 spikes s^{-1}) for 10–30 s and, after two to three control responses had been obtained for each excitant, the antagonists were ejected with currents of 5, 10, 20, 40 up to 80 nA and the excitation then elicited expressed as a percent of the controls. For each excitant these percentages were averaged across all cells studied.

Interneurones were excited synaptically by electrical stimulation at 1 Hz of dissected ipsilateral hindlimb nerves, and mono- and polysynaptic activity distinguished on the basis of central latencies and responses to repetitive afferent stimulation as detailed previously (Curtis *et al.*, 1981). Antagonist effects were quantified by comparing the areas of peristimulus histograms (obtained by summation of 100 sweeps) of cell firing before and during drug administration.

The outer barrels of the micropipettes contained: N-methyl-D-aspartate (NMDA, 50 mM in 150 mM NaCl, pH 7.5); kainate (5 mM in 150 mM NaCl, pH 7.5); quisqualate (Quis, 5 mM in 150 mM NaCl, pH 7.5); 2,3PDA (200 mM, pH 7); 2APV (50 mM in 150 mM NaCl, pH 7); and DS103-282 (10 mM in 150 mM NaCl or 100 mM, pH 4.7).

Results DS103-282 reduced the excitation of interneurones by quisqualate and NMDA but had no effect on that by kainate. On 8 neurones 80 nA (10 mM in 150 mM NaCl solution) depressed NMDA responses by 36.1 ± 13.9 (s.d.) % and quisqualate responses by 33.4 ± 17.2%. On 3 of these cells 80 nA of 2,3PDA abolished NMDA and kainate responses while only minimally reducing those of quisqualate; and on 7 cells 10 nA of 2APV reduced NMDA responses by 80.4 ± 26.7% without altering Quis responses (see Peet, Leah & Curtis, 1983).

Synaptic excitation was studied with 10 interneurones, which included 4 of those above. When the background firing of interneurones was maintained at an approximately constant rate (which varied between cells, 5–20 Hz) by the continuous ejection of

quisqualate, DS103-282 40-80 nA reduced the poly- but not monosynaptic excitation of 8 of 10 neurones. However, this reduction was only 12.4 ± 12.8% when the antagonist was ejected from solutions of 10 mM in 150 mM NaCl (7 cells) and 18.7 ± 22.3% using the 100 mM solution (3 cells). On all these same interneurones, 2APV also reduced poly- but not monosynaptic excitation, 10–40 nA producing a 42.9 ± 27.8% reduction (Figure 1).

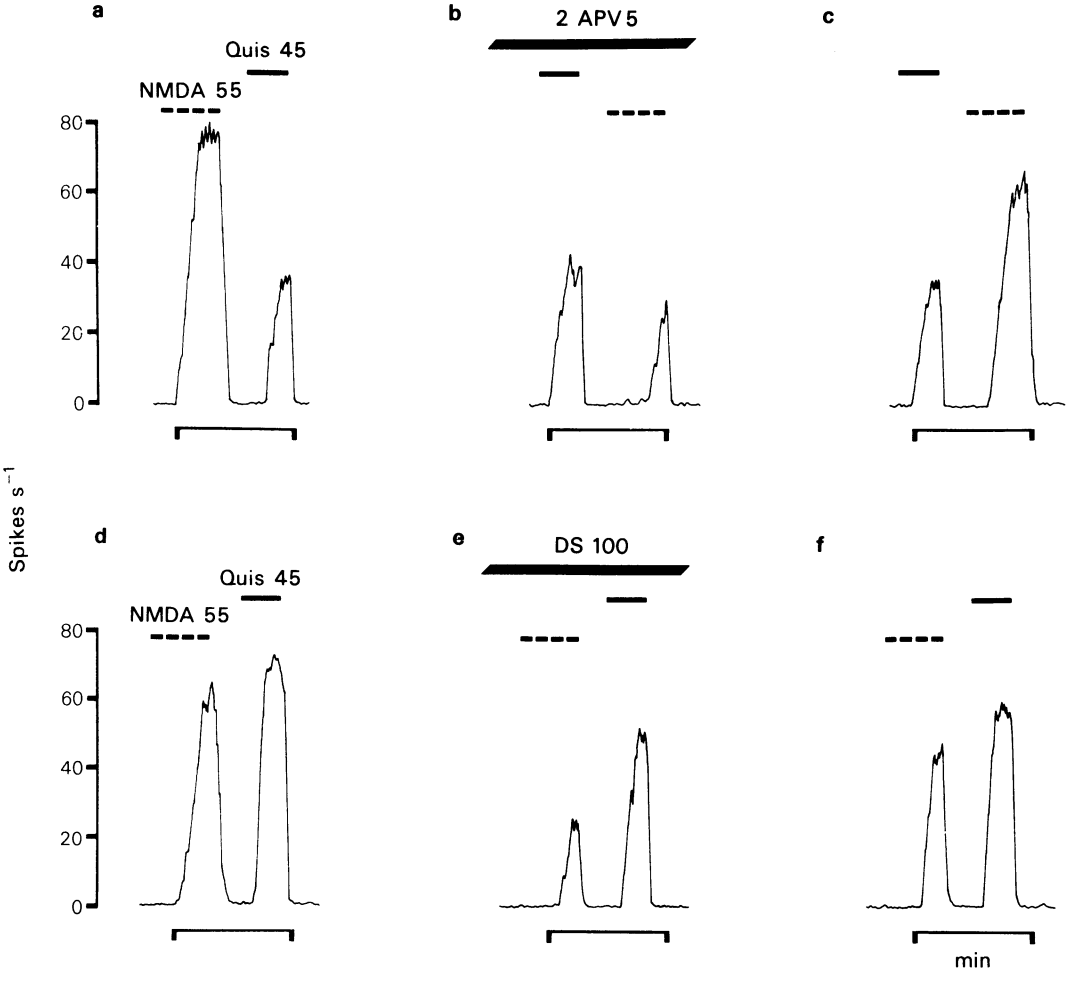

Figure 1 Effects of (±)-2-amino-5-phosphonovaleric acid (2 APV) (a–c) and DS103-282 (d–f) on the excitation of a spinal interneurone by N-methyl-D-aspartate (NMDA) and quisqualate (Quis). The periods of microelectrophoretic ejection are marked by horizontal bars with the figure indicating current (nA). 2APV was ejected from a 50 mM in 150 mM NaCl solution and DS103-282 from a 10 mM in 150 mM NaCl solution. (a) Before; (b) during 2APV (5 nA); (c) 1 min after terminating 2APV. (d) Before; (e) during DS103-282 (100 nA); (f) 1 min after DS103-282. Ordinates: firing rate, spikes per s. Abscissae: time, min. This same interneurone was excited monosynaptically by stimulation of gastrocnemius nerve (2T) and polysynaptically by plantaris nerve (10T). When the background firing was maintained at approximately 10 spikes s^{-1} by continuous ejection of Quis, DS103-282 40 nA (which reduced responses to NMDA by 28% without affecting responses to Quis) for 12 min reduced polysynaptic excitation by 8% without affecting monosynaptic excitation. 2APV 20 nA for 3 min reduced polysynaptic excitation by 40% without altering monosynaptic activation.

Discussion A previous study (Davies, 1982) found no correlation between DS103-282 antagonism of microelectrophoretic excitant amino acids and of polysynaptic activation, and concluded that this agent acts presynaptically. Such a conclusion would seem unwarranted. In the cat spinal cord 2APV antagonizes excitation by NMDA (Davies & Watkins, 1981; Peet *et al.*, 1983), but on some interneurones only the later components of the polysynaptic activation are susceptible to antagonism by 2APV (Peet *et al.*, 1983). Thus for these cells polysynaptic excitation is possibly mediated only in part by NMDA receptors. Moreover, although there is evidence for L-aspartate as the transmitter along spinal polysynaptic pathways, excitation by aspartate is less sensitive to antagonism by 2APV (Peet *et al.*, 1983) or D-α-aminoadipate (Davies & Watkins, 1979) than its analogue NMDA, and the identity of the transmitter(s) acting at NMDA or other receptors for polysynaptic input remains uncertain (Lambert, Flatman & Engberg, 1981; Smith, 1982). Such transmitters may not be identical or related to the excitant amino acids previously examined in relation to the antagonist properties of DS103-282. Thus this agent could act postsynaptically at other than NMDA receptors, or effectively antagonize another unknown excitatory transmitter also involved in polysynaptic excitation, and no relation would be observed between antagonism of NMDA and polysynaptic excitation.

However, the present results show clearly that DS103-282 antagonizes both NMDA and polysynaptically elicited excitation of spinal interneurones. Such a correlation was not unexpected in view of the evidence indicating the involvement of NMDA receptors in polysynaptic excitation of spinal interneurones in the cat (Davies & Watkins, 1979; Peet *et al.*, 1983). Although quisqualate receptors were similarly susceptible to antagonism by DS103-282, no reduction of monosynaptic excitation was detected. This accords with the suggestion that there is as yet no convincing evidence that quisqualate receptors are involved in spinal monosynaptic transmission (Peet *et al.*, 1983).

The reduction by DS103-282 of spontaneous activity observed in this and the previous study (Davies, 1982) is also explicable on the basis of antagonism at NMDA receptors, and when the level of excitation was restored by the ejection of quisqualate, responses to NMDA and polysynaptic inputs were similarly reduced by DS103-282. Thus a postsynaptic mechanism can account for the actions of DS103-282 without the need to propose a presynaptic reduction of transmitter release.

The authors gratefully acknowledge gifts of DS103-282 from Dr E. Eichenberger, Berne, and of 2APV and 2,3PDA from Dr J.C. Watkins, Bristol.

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(Received January 13, 1983.)