Interneurones mediating presynaptic inhibition of group II muscle afferents in the cat spinal cord

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1. To investigate whether dorsal horn interneurones with input from group II muscle afferents induce depolarization of sensory fibres, simultaneous recordings were made from single interneurones in the sacral segments and from sacral dorsal root filaments using the spike-triggered averaging technique.

2. The spike potentials of eighteen out of thirty-eight interneurones tested were followed by dorsal root potentials (DRPs). The DRPs occurred at latencies of 2 and 6–8 ms. Interneurones evoking DRPs at latencies of up to 2 ms are considered likely to be last-order interneurones in pathways of presynaptic inhibition, while those inducing DRPs at longer latencies are considered likely to be first-order interneurones. The former were activated by peripheral afferents with somewhat longer latencies than the latter. However, all interneurones were co-activated by group II muscle and cutaneous afferents, indicating that the depolarization of group II muscle afferents, which these afferents induce, may be mediated by the same interneurones.

3. DRPs evoked by electrical stimulation of peripheral nerves were recorded from both sacral and midlumbar dorsal root filaments. The amplitudes of these DRPs were closely related to the potency with which group II afferents of various nerves activate dorsal horn interneurones in the sacral and midlumbar segments and group II afferents contributed to them more effectively than group I afferents. The second stimulus in a train was more effective than the first, while a third stimulus had little additional effect, indicating that the interneurones involved are relatively easily activated.

4. Intraspinal stimuli applied within the dorsal horn, at the sites where the largest field potentials of group II origin were recorded, evoked distinct DRPs. However, the location of the first- and last-order interneurones in pathways of primary afferent depolarization (PAD) could not be differentiated by this approach because the same stimuli induced positive potentials, which masked the onset of DRPs and precluded localization of the sites from which DRPs might be evoked monosynaptically.

Primary afferent depolarization (PAD) of group II muscle afferents terminating in the midlumbar and in the sacral segments of the cat spinal cord is evoked predominantly from different nerves (Riddell, Jankowska & Huber, 1993, 1995). The patterns of origin of PAD in these segments are, however, strikingly similar to the patterns of input to dorsal horn interneurones located in the same segments (Edgley & Jankowska, 1987; Jankowska & Riddell, 1993, 1994). These observations suggest, therefore, that dorsal horn interneurones are responsible for the presynaptic control of transmission from group II afferents and that they operate primarily within the segments in which they are located. If this is so, then the activity of some sacral dorsal horn interneurones ought to be followed by negative dorsal root potentials (Schmidt, 1973; Rudomin, 1990). The present study was undertaken to explore this possibility.

METHODS

Preparation

Experiments were performed on seven cats under deep anaesthesia. Anaesthesia was induced with one dose of sodium pentobarbitone (40 mg kg^{-1} I.P.) and maintained with several doses of chloralose (up to 50 mg kg^{-1} I.V.). The adequacy of anaesthesia was verified using withdrawal reflexes before the cats were paralysed with gallamine triethiodide and by monitoring blood pressure and pupillary reflexes after

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paralysis. The same cats were also used for the studies reported in the accompanying paper (Riddell *et al.* 1995), where a more detailed description of the preparation and of the animal care is given.

The following left hindlimb peripheral nerves were dissected: the quadriceps (Q), sartorius (Sart), gracilis (Grac), posterior biceps and semitendinosus (PBST), anterior biceps and semimembranosus (ABSM), gastrocnemius–soleus (GS), plantaris (Pl), deep peroneal (DP, i.e. tibialis anterior and extensor digitorum longus, from which the nerve branch to the extensor digitorum brevis and the skin was removed), the caudal branch of sural (Sur), superficial peroneal (SP), cutaneous femoris (CF) and pudendal (Pud) nerves. The spinal cord was exposed by laminectomy from the fourth lumbar to the sacral segments and at the level of the Th12–13 segments. The dura mater was opened over all of these segments. Ventral roots were left intact.

Recording and stimulation procedures

Dorsal root potentials (DRPs). DRPs were recorded from the proximal parts of transected natural filaments of the S1, S2 and L5 dorsal roots (C in Fig. 1). The origin of fibres entering the spinal cord through the sacral filaments was determined by identifying those peripheral nerves which evoked afferent volleys in the distal parts of the sectioned filaments (B in Fig. 1); this could not have been done for the much shorter L5 filaments. DRPs were evoked in three different ways: (i) by electrical stimulation of peripheral nerves; (ii) by activating single interneurones expected to mediate the DRPs; and (iii) by electrical stimuli applied within the region of dorsal horn where these interneurones are located. Peripheral nerves were stimulated (via electrodes labelled A in Fig. 1) by single stimuli or by two or three stimuli 3·3 ms apart, which were near threshold (T) for the most excitable group II afferents ($1{\cdot}8T$–$2{\cdot}0T$) or near maximal for group II afferents ($5T$). In order to induce a tonic discharge of interneurones the electrodes used to record from them (E in Fig. 1) were filled with a 0·5 M solution of DL-homocysteic acid (DLH) in a 0·5 or 0·75 M solution of sodium chloride with a pH of 9 set by Tris buffer (1·0–1·5 μm tip diameter, 5–10 MΩ resistance). The interneurones were activated by DLH, which was either allowed to diffuse from the electrode tip, or was ejected by ionophoresis using negative current of up to 15 nA; the intensity of the current was adjusted to produce a regular discharge of the interneurones at a rate of 5–10 s^{-1}. When activation of the interneurones by leakage of DLH was to be prevented, a positive retaining current of up to 15 nA was passed through the electrodes. Intraspinal stimuli used to evoke DRPs (0·2 ms in duration up to 5 μA) were applied through microelectrodes filled with a 2 M solution of sodium chloride.

The presence of DRPs following the spike activity of single interneurones was tested by simultaneously recording electrotonically conducted changes in the membrane potential of afferent fibres within a DR filament (C in Fig. 1). Only filaments in which sizable short latency DRPs (of at least 50 μV and with latencies < 4 ms from incoming group I volleys) were evoked by single stimuli of $5T$ applied to the PBST nerve were used to investigate the actions of single interneurones. Anticipating that only very small DRPs would be evoked by single interneurones, records from the dorsal roots were averaged using the spike-triggered averaging technique, as in previous studies of the synaptic actions of single neurones (see e.g. Jankowska & Roberts, 1972; Rudomin, Solodkin & Jiménez, 1987). An averager (Nicolet type 1170) was triggered by interneuronal spike potentials (with a pretrigger delay of more than 30 or 40 ms) and averages were obtained of 64–3000 individual responses recorded from a dorsal root filament (with a time resolution of between 10 and 60, usually 20 or 40 μs per address).

Interneurones. Interneurones with input from group II afferents were searched for in the dorsal horn of sacral segments at the levels at which maximal cord dorsum and field potentials were evoked by group II afferents of the PBST nerve (Jankowska & Riddell, 1993, 1994). The neurones selected for testing responded to stimulation of the PBST and/or GS nerves with an intensity near maximal for group II afferents ($5T$, single or double stimuli 3 ms apart at latencies of 1·5–2·9 ms; the latencies were measured from the onset of incoming group I volleys recorded with silver ball electrodes in contact with the cord dorsum a few millimetres from the site of entry of the microelectrode (D in Fig. 1). None of the neurones selected were antidromically activated by stimuli applied to the ipsi- or contralateral lateral funiculi at the Th12–13 level (with 0·2 ms current pulses of up to 1 mA). The interneurones were recorded from extracellularly.

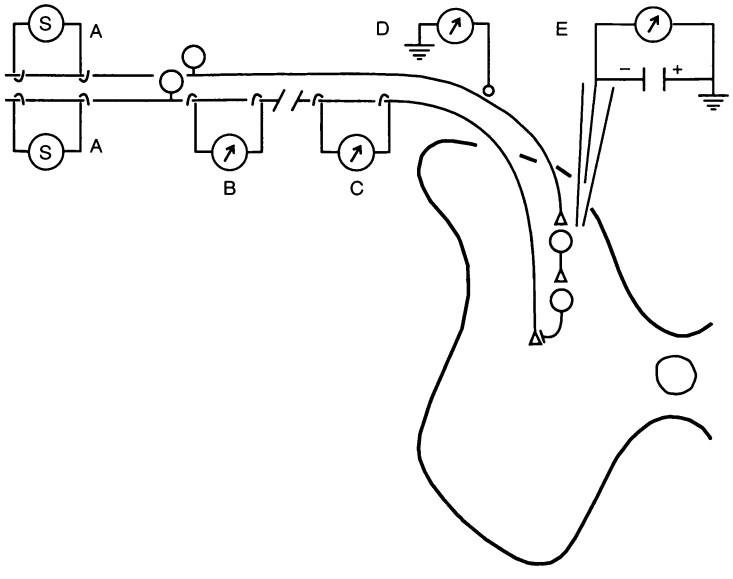

Figure 1. Diagram of the experimental arrangement

A, electrodes used for stimulation of peripheral nerves. B, electrodes used to record responses of sensory fibres in different peripheral nerves from the distal part of a sectioned dorsal root filament. C, electrodes used for recording of dorsal root potentials from proximal parts of the same dorsal root filament. D, electrodes used for recording of afferent volleys reaching the spinal cord via intact dorsal root fibres. E, electrodes used for recording from interneurones and ejecting DLH to activate them.

RESULTS

DRPs evoked by stimulation of group II afferents in peripheral nerves

Except for brief mentions in two early reports on presynaptic inhibition (Eccles, Kostyuk & Schmidt, 1962*a*; Eccles, Magni & Willis, 1962*b*), DRPs evoked by group II muscle afferents have not previously been described. To enable a comparison of the latencies and amplitudes of DRPs evoked by single interneurones with those evoked by a population of group II afferents, we therefore began by investigating the properties of DRPs evoked by electrical stimulation of peripheral nerves.

On the basis of investigations of the origin of primary afferent depolarization of group II afferents (Riddell *et al.* 1995), the PBST nerve would be expected to evoke the largest DRPs of group II origin in dorsal root filaments containing afferents terminating in the sacral segments and the Q nerve the largest DRPs in dorsal root filaments containing afferents terminating in the midlumbar segments. The contribution of group II afferents to DRPs was therefore investigated in the first instance by analysing the effects of stimulation of the PBST nerve on dorsal root filaments entering the sacral segments at the level of Onuf's nucleus and the effects of stimulation of the Q nerve on midlumbar dorsal root filaments. The sacral filaments selected for analysis ($n = 14$) were the most caudal of those through which group II afferents of the PBST and GS nerves were found to enter the spinal cord, and the midlumbar filaments ($n = 3$) were the most rostral of those entering the L5 segment. DRPs were evoked by stimuli supramaximal or near maximal for group I afferents (usually 2*T*) to estimate the effects of group I afferents alone, and by stimuli near maximal for group II afferents (usually 5*T*) to define the additional effects of group II afferents. To estimate the segmental latencies of DRPs, they were evoked by the minimal number of stimuli that were effective, usually one or two. As illustrated in Fig. 2*D*, the second stimulus of a train usually induced a larger response than the first (due to temporal facilitation), while the third stimulus had a smaller additional effect.

Figures 2*A* and *B*, 4*A–C*, and 6*C* and *D* show that group II afferents contributed to the sacral DRPs evoked by stimulation of PBST more effectively than group I

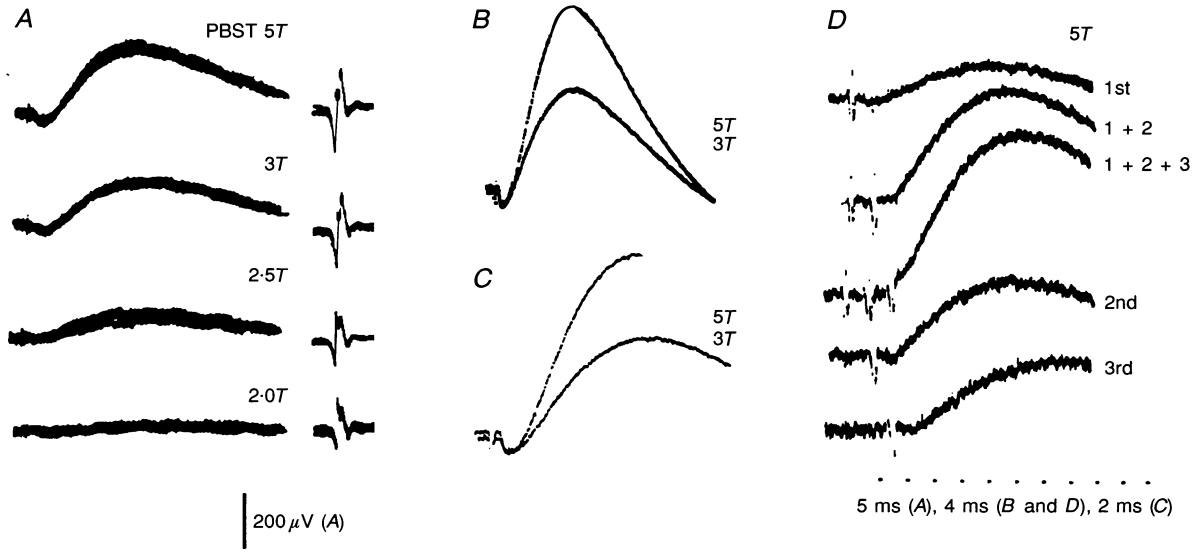

Figure 2. DRPs evoked by group II afferents in sacral dorsal root filaments

A, comparison of DRPs evoked in the most caudal filament of the S1 dorsal root by single stimuli of different intensity applied to the posterior biceps and semitendinosus (PBST) nerves. The corresponding afferent volleys evoked by these stimuli are shown to the right of the DRP records. Note that stimuli of 3*T* produced a virtually maximal group I volley but evoked DRPs that were smaller than those evoked by stimuli of 5*T* (near-maximal for group II muscle afferents). The difference between the responses to stimuli of 3*T* and 5*T* is therefore attributable to group II afferents. *B* and *C*, superimposed averaged records of the same DRPs as shown in *A*. *D*, comparison of DRPs evoked by different numbers of stimuli in the most caudal filament of the S2 dorsal root in another experiment. The records show, from top to bottom, DRPs evoked by a single stimulus, by two stimuli, by three stimuli, by the second of two stimuli (the response to a single stimulus having been subtracted from the response to two stimuli) and the response to the third of three stimuli (the response to two stimuli having been subtracted from the response to three stimuli). In this and the following figures, stimulus intensities are indicated above the records in multiples of threshold stimuli for a given nerve. The voltage calibration applies only to the DRPs in *A*.

afferents, and Fig. 3 shows that the same was true of the contribution of group II afferents of Q to midlumbar DRPs. As can be seen from these records, distinct DRPs could be evoked by stimulation of a peripheral nerve at $5T$ when stimuli of $2T$ (maximal for group I afferents) were without effect (Fig. 2), and even when stimuli of $2T$ were effective much larger DRPs were evoked by stimuli of $5T$ (Figs 3, 4*B* and 6*C* and *D*). In both sacral and midlumbar dorsal root filaments, the mean amplitudes of DRPs evoked by stimuli of $5T$ were more than twice those of DRPs evoked by stimuli of $2T$ ($n = 30$). Furthermore, since stimuli of $2T$ may activate not only group I but also the most excitable fraction of group II afferents (Jack, 1978) the contribution of group II afferents to these DRPs is likely to be under- rather than overestimated by this comparison. An increase in the stimulus intensity from $5T$ to $20T$ did not produce any further increase in amplitude of DRPs.

Group II afferents of other muscle nerves also evoked DRPs in sacral and midlumbar dorsal roots, but of smaller amplitude than those evoked from PBST and Q. When the actions of single stimuli of $5T$ applied to various muscle nerves were compared, DRPs were evoked in the sacral filaments by (in order of effectiveness, mean amplitudes and numbers of observations given in parentheses): PBST (89 μV, $n = 12$), GS (24 μV, $n = 8$), Q (21 μV, $n = 9$), DP (19 μV, $n = 9$), Pl (12 μV, $n = 6$), Sart (8 μV, $n = 8$), Grac (3 μV, $n = 6$) and in the three midlumbar filaments by: Q (310 μV), DP (252 μV), PBST (176 μV), Sart (143 μV) and ABSM (16 μV). It will be noted that in the sacral segments DRPs evoked from the PBST nerve were 4 times larger than those evoked from the Q and DP nerves, while in the midlumbar segments they were not much more than half the size of the DRPs evoked from Q. In general, the relations between the sizes of DRPs evoked from different nerves closely correspond to the effectiveness of group II afferents of these nerves in evoking PAD of group II afferents (see Figs 4 and 7 in Riddell *et al.* 1995). The only discrepancy is in the relatively larger DRPs evoked by DP than by Pl in sacral filaments, and by PBST than by Sart in midlumbar filaments. The amplitudes of DRPs evoked from different nerves in sacral dorsal root filaments also closely correspond to the proportions of sacral interneurones responding to stimulation of these different nerves (see Fig. 2*C* in Jankowska & Riddell, 1994).

The relative amplitudes of DRPs evoked from cutaneous nerves were likewise in keeping with the relative potency of these nerves in inducing PAD of group II afferents (Riddell *et al.* 1995) and in activating dorsal horn interneurones (Jankowska & Riddell, 1994). The cutaneous nerves from which DRPs were evoked (by single stimuli of $2T$) were (in order of effectiveness, mean amplitudes given in parentheses) in sacral filaments: CF (200 μV, $n = 6$), Sur (125 μV), Pud (121 μV, $n = 7$) and SP (99 μV, $n = 8$) and in the three midlumbar filaments: SP (310 μV), CF (212 μV), Sur (120 μV) and Pud (17 μV). DRPs of cutaneous origin are illustrated in Fig. 4*D–G*.

The latencies of DRPs evoked by group II muscle afferents differed considerably. For instance, the latencies of DRPs evoked from the PBST nerve in the sacral dorsal roots (by single stimuli) ranged between 2·5 and 5 ms (from the group I incoming volleys) and those of DRPs evoked from the Q nerve in midlumbar dorsal roots ranged between 3·0 and 5 ms. However, the latencies of DRPs evoked by a pair of stimuli (measured in relation to the second stimulus) were usually shorter than those of DRPs evoked by single stimuli. Therefore, in order to determine minimal latencies, DRPs evoked by single stimuli of $5T$ were subtracted from those evoked by two stimuli of $5T$ (as in Fig. 2*D*), DRPs evoked by stimuli of $2T$ having previously been subtracted from both. The latency of the potential evoked by the

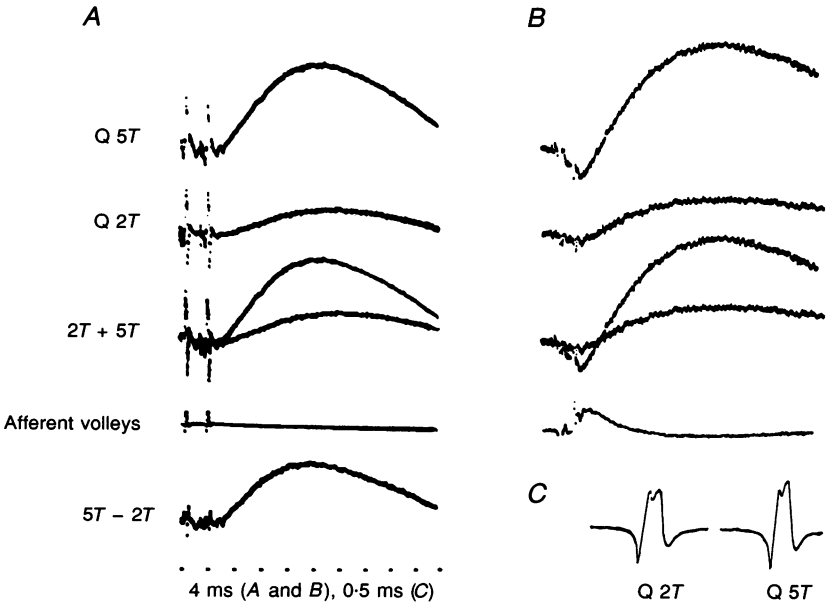

Figure 3. DRPs evoked by group II afferents in a midlumbar dorsal root filament

A and *B*, comparison of DRPs evoked by stimuli of $2T$ and $5T$ applied to the quadriceps (Q) nerve in two L5 dorsal root filaments (averages of 32 (*A*) and 16 records (*B*)). The records are, from top to bottom: DRPs evoked by stimuli of $5T$; DRPs evoked by stimuli of $2T$; superimposed traces of the above; cord dorsum potentials with afferent volleys; and (in *A* only) the difference between DRPs evoked by stimuli of $5T$ and $2T$. Expanded records of the afferent volleys evoked by stimulation of Q at $2T$ and $5T$ are shown in *C*.

second of the two stimuli was then measured with respect to the second afferent volley. One of the problems with such measurements was that the onset of the DRPs was not usually very sharp and could generally only be determined to within the nearest millisecond. Paradoxically, this problem was greatest for the largest DRPs because these were preceded by positive potentials which masked their onset. Stronger stimuli therefore appeared to evoke DRPs of longer latencies than weak stimuli (see Fig. 4*B*). The positive potentials (illustrated in Figs 3*B*, and 4*B* and *C*) were evoked by stimuli of 5*T* but not 2*T*, and resembled the monosynaptic field potentials generated by group II afferents, which can be recorded from the surface of the lateral funiculus (Jankowska & Riddell, 1993).

Minimal latencies of DRPs evoked by group II afferents in the PBST nerve were 2·5–3·0 ms from group I volleys and were estimated to be about 1·8–2·3 ms from group II volleys in both the midlumbar and sacral segments. The corresponding data for other nerves were: 3·0–4·0 ms and 2·2–3·2 ms from group I and group II afferent volleys of Q and Sart in the midlumbar segments, and 4·0 and 2·1 ms from group I and group II afferent volleys of DP in the midlumbar segments. Latencies with respect to group II volleys were estimated as described by Jankowska & Riddell (1993).

Figure 4*D–F* shows that the positive potentials preceding DRPs evoked from the Sur, Pud and CF nerves were particularly large and that measurements of the minimal latencies of DRPs evoked by cutaneous afferents were therefore subject to errors similar to those for DRPs of group II origin. The minimal latencies of DRPs evoked from cutaneous nerves were estimated to be about 2 ms. No differences were seen between the latencies of DRPs evoked from the different cutaneous nerves within the midlumbar or sacral segments.

DRPs evoked by single interneurones

Characteristics of DRPs following the activity of sacral interneurones

Negative dorsal root potentials were observed following discharges of eighteen of thirty-eight interneurones on which tests were performed using spike-triggered averaging. Figure 5*A–G* shows examples of these DRPs. The DRPs are ranged from those evoked at shortest latency (Fig. 5*A*) with respect to the interneuronal

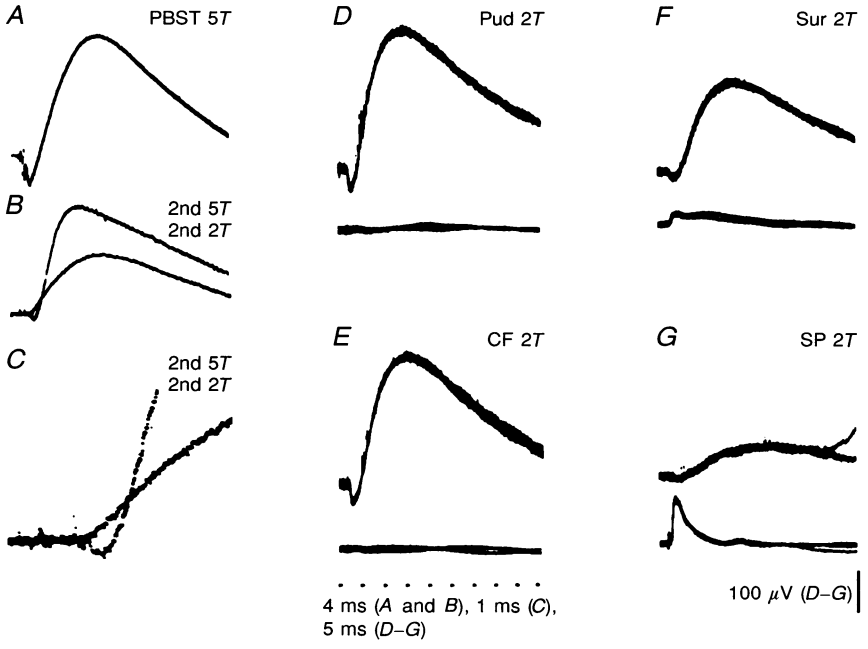

Figure 4. DRPs evoked by group II muscle afferents and cutaneous afferents in a sacral dorsal root filament

A–C, comparison of the latencies of DRPs evoked by stimuli near threshold (2*T*) and near maximal (5*T*) for group II afferents of the PBST nerve. The DRPs were evoked in the most caudal filament of the S1 dorsal root. Averages of 32 records. *A*, DRPs evoked by double stimuli of 5*T*. *B*, superimposed records of DRPs evoked by the second of two stimuli of 5*T* and the second of two stimuli of 2*T*. They were obtained using the procedure illustrated in Fig. 2*D*, i.e. by subtraction of DRPs evoked by single stimuli from DRPs evoked by double stimuli. *C*, expanded section of the records shown in *B*. *D–G*, comparison of DRPs evoked by single stimuli of 2*T* applied to cutaneous nerves: pudendal (Pud), cutaneous femoris (CF), sural (Sur) and superficial peroneal (SP). The DRPs (3 superimposed single records) were evoked in another filament of S1 dorsal root.

discharges (vertical lines) to those of longest latency (Fig. 5*G*) and, for a given interneurone, are ranged from those obtained by averaging the smallest number of sweeps to those obtained with larger numbers of sweeps. The DRPs were induced in a highly reproducible manner. When spike-triggered averaging was repeated (up to 7 times) for the same interneurone, then DRPs were either consistently present or absent following each successive averaging sequence. Examples of two to three such sequences are shown in Fig. 5*B*, *C*, *E* and *G*. DRPs were just as prominent following averaging of 1000–2000 potentials as of 500 ($n = 12$), or even less than 200 ($n = 6$). The amplitudes of DRPs varied between 0·6 and 6·7 μV (mean $\pm$ S.D., 2·7 $\pm$ 1·7 μV for 10 DRPs for which amplitudes were measured). These amplitudes were 8–167 times (mean, 55 times) smaller than the amplitudes of DRPs evoked in the same filament by electrical stimulation of group II afferents of the PBST nerve. These amplitudes are, however, comparable to the amplitudes of DRPs following activity of single group I-activated interneurones investigated by Rudomin *et al.* (1987; 2–5 μV in the illustrated records of their Figs 3 and 11), which were not preceded by cord dorsum potentials.

As for DRPs evoked by electrical stimuli, the onset of DRPs evoked by single interneurones was difficult to define to within less than 0·5–1·0 ms, both because of the noisy character of the records and because their onsets were not very sharp. However, the latencies of only four DRPs appeared to be close to 2 ms with respect to interneuronal spikes, while the latencies of the remaining potentials were close to 3 ms ($n = 2$), 4–5 ms ($n = 4$) or between 6 and 8 ms ($n = 8$). Judging from these latencies, it is probable that most of the interneurones tested were not directly responsible for depolarizing the dorsal root fibres.

The majority of DRPs rose from a flat baseline. Only three DRPs were preceded by small negative potentials, which started before the interneuronal spikes; one of these is illustrated in Fig. 5*D*. Eight DRPs were preceded by even smaller positive potentials, which started either before, or more or less synchronously with, the interneuronal spikes; an example of one of these is shown in Fig. 5*G*. Both types of potential were much smaller than the negative DRPs.

Characteristics of the effective interneurones

DRPs were found to follow spike potentials of eighteen out of thirty-eight interneurones located between 1 mm rostral

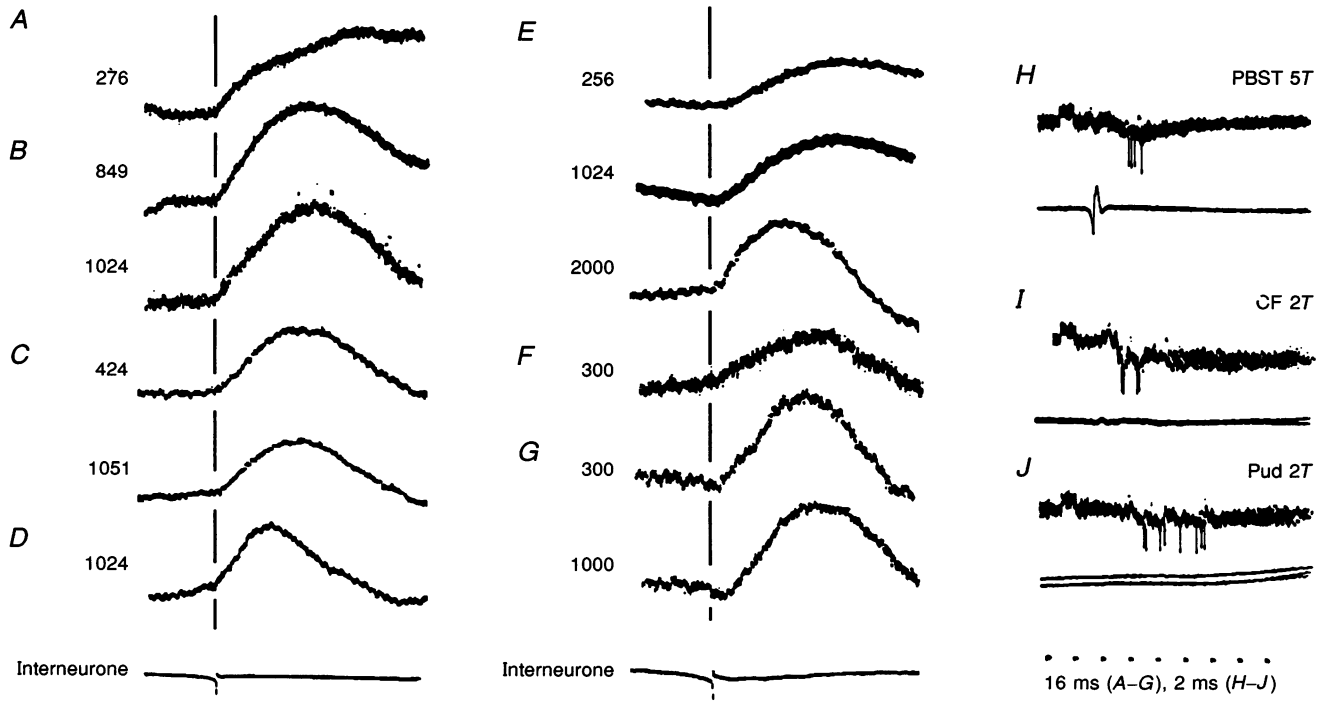

Figure 5. Examples of DRPs following spike activity of 7 sacral dorsal horn interneurones (*A–G*)

The DRP recordings were obtained from 3 sacral dorsal root filaments (*A* and *B*; *C–E* and *G*; and *F*) using spike-triggered averaging (40 μs per address, number of averages indicated to the left of each record). The records underneath the DRPs show spike potentials from one of the interneurones recorded simultaneously with the DRPs and used to trigger the averager. The vertical lines indicate the onset of the interneuronal spikes, the DRPs being aligned with respect to the spikes. *H–J*, records from one of the interneurones illustrating the short latency of activation by stimuli applied to both muscle (*H*) and cutaneous (*I* and *J*) nerves.

and 3 mm caudal to the rostral border of Onuf's nucleus (see Jankowska & Riddell, 1993). However, the proportions of interneurones effective in a given preparation varied from practically all in one of the experiments, through one-half in one experiment to one-quarter in two experiments. All of the interneurones tested were located within 1 mm rostral or caudal to the dorsal root filament from which the recordings to be averaged were obtained.

Distinct DRPs were evoked in all these filaments by single stimuli applied to group II afferents of the PBST nerve and the filaments contained considerable proportions of PBST and GS fibres, as judged from records from the distal ends of the filaments (see Methods). Finally, no major differences were seen between the input to interneurones contributing and those not apparently contributing to DRPs, and all of the interneurones were similarly located within the dorsal horn regions, where large focal field potentials were evoked by group II afferents. There are, therefore, no obvious factors to explain the varying proportions of interneurones the activity of which was followed by DRPs.

There were also no systematic differences between those interneurones the activity of which was followed at shorter ($\leqslant$ 2 ms) or at longer (3–8 ms) latencies by DRPs. Both kinds of interneurone were activated by group II afferents of PBST and by cutaneous afferents with similar ranges of latencies (1·9–2·5 and 1·5–2·9 ms from group I afferents of PBST; 0·7–2·4 and 0·7–1·8 ms from cutaneous volleys). The shorter latencies within these ranges – those of less than 2·5 ms for group II afferents (16 interneurones) and of less than 1·5 ms for cutaneous afferents (12 interneurones)

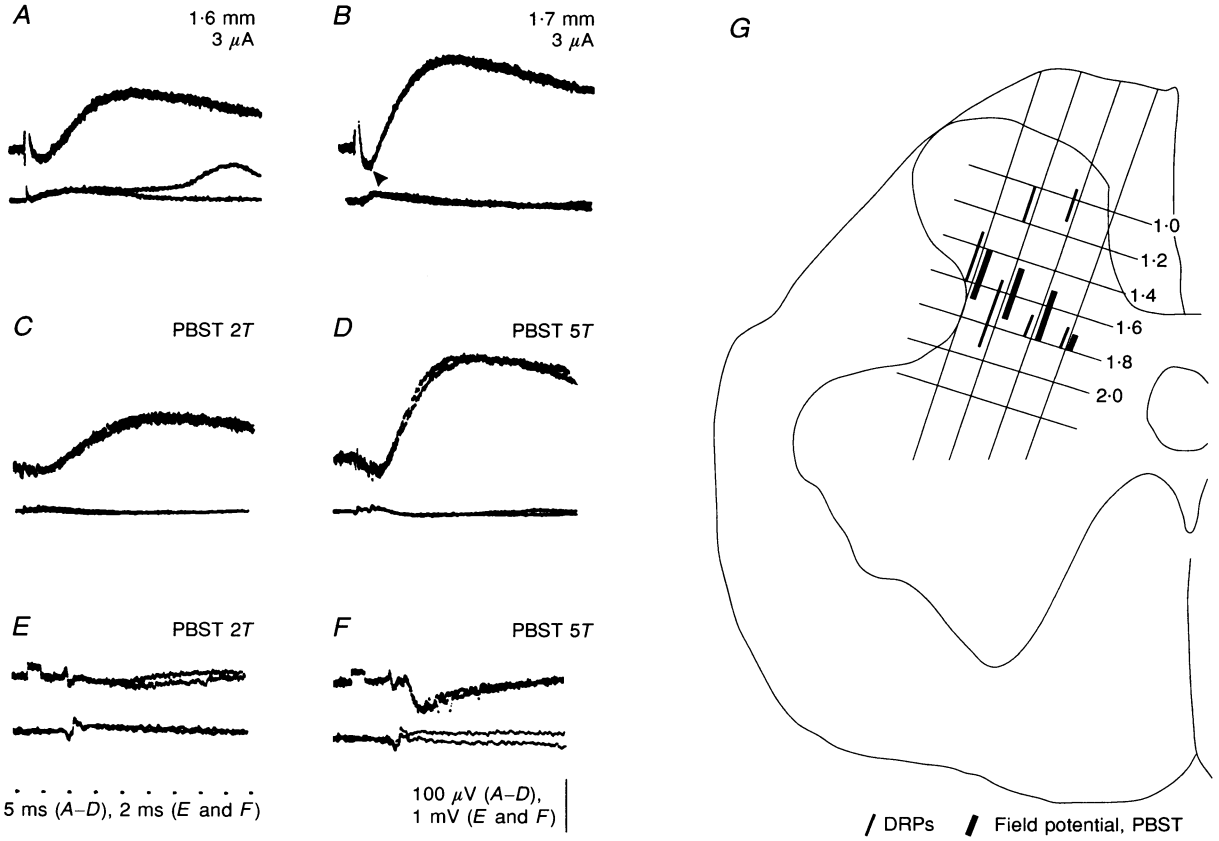

Figure 6. DRPs evoked by intraspinal stimuli

A and *B*, DRPs evoked in a middle S1 dorsal root filament by single 3 μA stimuli applied at depths 1·6 and 1·7 mm from the surface of the spinal cord along the third electrode track from the left in *G*. Note that, in addition to evoking a DRP, the stimuli applied at the deeper location also evoked a clear positive potential (arrowhead) and a corresponding cord dorsum potential. *C* and *D* show examples of DRPs evoked in the same dorsal root filament by stimulation of the PBST nerve. Note that the component of the DRPs attributable to group II afferents was of a size comparable to the DRPs evoked by the intraspinal stimuli. *E* and *F*, field potentials evoked by stimuli maximal for group I (*E*) and group II (*F*) afferents in the PBST nerve at the site of the intraspinal stimulation. *G*, transverse section through the spinal cord showing 4 electrode tracks along which the intraspinal stimuli were applied. Thin bars indicate those sites from which DRPs $\geqslant$ 60% of the maximum were evoked and thick bars those sites at which field potentials ($>$ 200 μV) of group II origin were recorded.

– are compatible with the monosynaptic actions of these afferents (cf. Jankowska & Riddell, 1994). The interneurones inducing the two shortest latency ($\leqslant$ 2 ms) DRPs were nevertheless activated by peripheral afferents with the longest latencies within these ranges (2·3 and 2·5 ms from PBST; 1·8 and 2·4 ms from Sur), which could also be compatible with disynaptically evoked actions of these afferents.

The great majority of interneurones found to induce DRPs were co-excited by group II afferents of more than one muscle nerve (11/18) and/or by both muscle group II and cutaneous afferents (15/18). The results of this study therefore indicate that depolarization of the terminals of group II afferents in sacral segments by muscle and skin afferents (Riddell *et al.* 1995) is induced by neurones co-excited by these afferents rather than via separate neuronal pathways.

DRPs evoked by intraspinal stimulation

In one cat electrical stimuli were applied within the grey matter, 1 mm caudal to the rostral border of Onuf's nucleus, in an attempt to localize the areas from which DRPs attributable to last-order PAD interneurones can be evoked. DRPs evoked by intraspinal stimuli are exemplified in Fig. 6*A* and *B*. The figure shows that even very weak intraspinal stimuli (3 μA) could evoke DRPs as large as those evoked by stimulation of peripheral nerves.

A systematic mapping of the areas from which DRPs are evoked by intraspinal stimuli revealed a considerable overlap between these areas and the areas over which the largest field potentials of group II origin were evoked. As shown in Fig. 6*G*, the stimuli were applied every 100 μm at depths 1–2 mm along four electrode tracks 200 μm apart. The stimuli were of 3 μA (0·3 ms duration) and were expected to stimulate fibres or neurones within about 50 μm. In agreement with this, stimuli applied 100 μm apart at the border of an effective area were found to induce distinctly different responses (i.e. large at one site, small at the other). The areas from which distinct DRPs were evoked could thus be fairly sharply delineated. Large DRPs were also induced by stimuli applied dorsal to the areas of field potentials of group II origin: within the dorsal part of the dorsal horn where field potentials are evoked by cutaneous afferents and/or at the border between the dorsal horn and the dorsal columns.

Unfortunately, the largest DRPs were always preceded by positive potentials, which masked their onset and precluded an accurate measurement of their latencies. At a best estimate, the DRPs were evoked at similar minimal latencies (2·5–3·0 ms, measured between the stimulus and the foot of the rising phase of the DRPs, indicated by the arrowhead in Fig. 6*B*) from all of the effective stimulation sites and were not much shorter than latencies of DRPs evoked by electrical stimulation of nerves, measured with respect to the incoming volleys. Since DRPs evoked by last-order PAD interneurones ought to appear within about 1 ms, records of this kind cannot be used reliably to localize such interneurones.

DISCUSSION

Coupling in pathways of presynaptic inhibition of group II afferents

From measurements of the latencies of DRPs evoked by electrical stimulation of muscle nerves, it has been postulated that the minimal linkage in the neuronal pathways responsible for presynaptic inhibition of group I afferents is trisynaptic. In the present experiments, DRPs of group II origin evoked by single stimuli appeared with latencies of up to 5–7 ms with respect to group I volleys, but when temporal facilitation (by pairs of stimuli) was used to increase the security of transmission through the interneurones, the DRPs appeared at much shorter latencies. The minimal latencies of such DRPs recalculated with respect to volleys in group II afferents (1·8–2·2, 2·2–3·2 and 2·1 ms when evoked from PBST, Q and DP nerves, respectively) were then similar to, or only slightly longer than, those of DRPs evoked from group I and cutaneous afferents (1·7–2·0 ms; see Eccles *et al.* 1962*b*; Carpenter, Engberg & Lundberg, 1966; Jankowska, McCrea, Rudomin & Sykova, 1981; Brink, Jankowska & Skoog, 1984). Since allowance must be made for the longer time required for conduction along the intraspinal collaterals of group II compared with group I afferents (0·3–0·5 ms, cf. Fu, Santini & Schomburg, 1974), the evidence suggests that the latencies of group II-evoked DRPs are also compatible with a trisynaptic pathway.

Role of sacral dorsal horn group II interneurones

The results of this study indicate that a fairly large proportion of sacral dorsal horn interneurones with group II input might be involved in the presynaptic control of transmission from group II afferents. Since, as discussed above, the minimal connectivity in pathways of PAD is likely to be trisynaptic, a further question that arises is whether the interneurones studied here represent first- and/or last-order interneurones in pathways of PAD.

Two criteria may be used to differentiate between those interneurones that are likely to be first-order and those likely to be last-order interneurones in these pathways. These are, firstly, the latency of activation of the interneurones by peripheral afferents and, secondly, the delay with which spike potentials in the interneurones are followed by DRPs. In the present sample of sacral dorsal horn interneurones the activity of which was followed by DRPs, all but two responded to stimulation of muscle nerves within 1·5–2·5 ms of group I volleys, i.e. within less than 1·0–1·5 ms of group II volleys (which are delayed by 0·5–1·0 ms with respect to group I volleys; see Fu *et al.*

1974; Jankowska & Riddell, 1994). Considering that action potentials in such neurones usually arise some 0·5 ms after the onset of EPSPs (see Jankowska & Riddell, 1994), the latencies of activation of the majority of the interneurones can be considered compatible with monosynaptic input from group II afferents. On the other hand, the earliest disynaptically evoked activation of the interneurones by group II afferents might be evoked with latencies as short as 1·5 ms from group II volleys or 2·0 ms from group I volleys, i.e. with latencies overlapping those of monosynaptically evoked actions. Similarly, the earliest disynaptically evoked actions of cutaneous afferents might be induced at latencies of less than 1·5 ms. On the basis of these considerations, six of the eighteen interneurones the activity of which was associated with DRPs might have been activated disynaptically and represent last-order PAD interneurones.

However, when the second of the two criteria (that relating to the delay between interneuronal spike potentials and the appearance of DRPs) is applied, then only two interneurones qualify for consideration as last-order PAD interneurones, since the spike activity of only two interneurones was followed by DRPs with a delay of about 2 ms. In a previous study in which spike-triggered averaging was used to identify interneurones mediating presynaptic inhibition of group I afferents, the minimal latencies of DRPs concluded to be evoked monosynaptically were 1·7–1·8 ms (Rudomin *et al.* 1987). These values are at the longer end of the range of latencies of PSPs evoked in motoneurones by direct actions of interneurones in disynaptic pathways of Ia reciprocal inhibition (0·7–1·7 ms), recurrent inhibition (0·5–2·0 ms) and group I non-reciprocal inhibition (1·1–2·1 ms; see Brink, Harrison, Jankowska, McCrea & Skoog, 1983). The latencies of the earliest DRPs induced by two interneurones tested in this study (about 2 ms) fall within the same ranges but might also be considered borderline cases. The latencies of the synaptic activation of these same two interneurones (2·3 and 2·5 ms from the afferent volleys by group II afferents and 1·8 and 2·4 ms by cutaneous afferents) were also at the border between those classifiable as evoked mono- or disynaptically. Both criteria are therefore consistent with the possibility that these interneurones represent last-order PAD interneurones, but do not provide an unequivocal identification of the interneurones.

The longer latencies (3–4 ms) of DRPs associated with the discharges of other interneurones would be more in keeping with the disynaptically evoked actions of first-order interneurones in trisynaptic PAD pathways. It might nevertheless be argued that such latencies are too long even for disynaptic actions and that two or more interneurones might be interposed between the tested interneurones and the terminals in which the DRPs were induced, i.e. that the DRPs were evoked tri- or polysynaptically. Although this possibility cannot be refuted, we consider a disynaptic coupling more likely. One reason for this is that DRPs evoked with latencies of 3–4 ms were apparent after only a small number of interneuronal discharges (i.e. averages of less than 100 records) and such effective spike-triggered averaging has so far been demonstrated for monosynaptically (e.g. Jankowska & Roberts, 1972) and disynaptically (e.g. Watt, Stauffer, Taylor, Reinking & Stuart, 1976) but not for polysynaptically evoked actions. Furthermore, when DRPs were evoked under optimal conditions of electrical stimulation of peripheral nerves (by the second stimulus of a pair) they appeared with a segmental delay of some 3·0 ms and not much more than one-half of this delay (i.e. 1·5 ms) should occur between the action potentials of first-order interneurones and the DRPs. Under much less favourable conditions, i.e. when DRPs are evoked by less synchronous actions of nerve impulses in axon collaterals of a first-order interneurone and these collaterals are long, the second-order interneurones must be activated much less effectively and a doubling or even tripling of the delay in the apparent onset of DRPs might be accounted for.

The much longer latencies (up to 6–8 ms) with which some DRPs followed the activity of interneurones are less readily explained, especially since these DRPs did not require the averaging of larger numbers of records than the shorter latency DRPs. If these latencies were not overestimated, then the longer latency DRPs might have been evoked via pathways involving additional interneurones, or they might have been caused by a synchronization of the activity of interneurones responsible for PAD of group II afferents with the activity of interneurones in other group II pathways.

Observations on the actions of single interneurones were made only for dorsal horn interneurones in the sacral segments, but it seems justified to generalize the conclusions of this study to group II-activated interneurones in the dorsal horn of midlumbar segments. Firstly, DRPs evoked by group II afferents in sacral and midlumbar dorsal root filaments were induced by single stimuli with similar effectiveness and at similar latencies with respect to group II volleys. Secondly, the sources of input to dorsal horn interneurones at the sacral and midlumbar levels (see Jankowska & Riddell, 1994) closely match the sources of DRPs evoked in the corresponding dorsal roots. Thirdly, there is evidence that transmission from group II muscle afferents to both sets of neurones is under the inhibitory control of serotonergic descending pathways (Bras, Jankowska, Noga & Skoog, 1990; Jankowska, Szabo-Läckberg & Dyrehag, 1994*b*). Finally, dorsal horn midlumbar and sacral interneurones share the same morphological properties (see Bras, Cavallari, Jankowska & Kubin, 1989; Jankowska, Riddell, Szabo-Läckberg & Hammar, 1994*a*). Neither first- nor last-order PAD interneurones have as yet been morphologically identified, but some dorsal horn interneurones monosynaptically activated by group II afferents in both sacral and

midlumbar segments have extensively branching initial collaterals with a high density of terminal swellings within about half a millimetre of the soma (Bras *et al.* 1989; Jankowska *et al.* 1994*a*). Furthermore, axons of some sacral interneurones appear to have projections restricted to the segments containing Onuf's nucleus. Such neurones are likely to exert their strongest actions upon neurones located in the same segments and thus have a morphology appropriate to first-order group II PAD neurones (see Discussion of Riddell *et al.* 1995).

The results of this study suggest that presynaptic control of group II afferents is mediated by interneurones co-excited by cutaneous and muscle afferents rather than by separate populations of interneurones with input from only muscle or only cutaneous receptors. This conclusion is based on the study of input to sacral dorsal horn interneurones; those found to induce DRPs (in the present study) as well as the population as a whole (Jankowska & Riddell, 1994). However, since PAD of group II afferents in midlumbar segments is evoked by the same kinds of fibre (although from different nerves) and since midlumbar dorsal horn interneurones are similarly co-excited by muscle group II and cutaneous afferents (Edgley & Jankowska, 1987), the conclusion may be extended to the midlumbar PAD interneurones.

Topographical specialization of neurones contributing to the presynaptic control of transmission from group II muscle afferents

Previous investigations of DRPs have generally failed to show any major contribution of group II afferents to the generation of DRPs recorded from dorsal root filaments at the border between the L6 and L7 segments (see e.g. Fig. 1 of Eccles, Schmidt & Willis, 1963). The only exceptions are the records of DRPs shown by Eccles *et al.* (1962*b*; their Fig. 19) and by Eccles *et al.* (1962*a*; their Fig. 2), in which a considerable contribution of muscle afferents activated by stimuli in the range $2T$–$5T$ is evident. In contrast, group II muscle afferents were systematically found to be one of the main sources of DRPs evoked in dorsal root filaments of the sacral and midlumbar segments. Whatever the reason for the differences in the contribution of group II afferents to DRPs evoked in dorsal roots of different segments, the sources of such DRPs concur with the sources of PAD of group II afferents established using other methods. The present study shows that DRPs of group II origin in filaments from the sacral and midlumbar segments are most effectively evoked by afferents of different nerves; the same nerves which are most effective in increasing the excitability of single group II afferents and in depressing monosynaptic field potentials of group II origin (Riddell *et al.* 1995) within the sacral and midlumbar segments. The present results therefore provide further evidence that the terminals of group II afferents in different segments are under the presynaptic control of largely different populations of neurones.

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Acknowledgements

We wish to thank S. Dolk and R. Larsson for their invaluable assistance in this study. The study was supported by the Swedish Medical Research Council (grant 5648). J.S.R. was supported by a Wellcome Travelling Research Fellowship.

Received 4 November 1993; accepted 24 August 1994.