Raphe magnus and reticulospinal actions on primary afferent depolarization of group I muscle afferents in the cat

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1. In the anaesthetized cat, electrical stimulation of the bulbar reticular formation produced a short latency (2·1 ± 0·3 ms) positive potential in the cord dorsum. In contrast, stimulation of the nucleus raphe magnus with strengths below 50 μA evoked a slow negative potential with a mean latency of 5·5 ± 0·6 ms that persisted after sectioning the contralateral pyramid and was abolished by sectioning the ipsilateral dorsolateral funiculus.

2. The field potentials evoked by stimulation of the bulbar reticular formation and of the nucleus raphe magnus had a different intraspinal distribution, suggesting activation of different sets of segmental interneurones.

3. Stimulation of these two supraspinal nuclei produced primary afferent depolarization (PAD) in single Ib fibres and inhibited the PAD elicited by group I volleys in single Ia fibres. The inhibition of the PAD of Ia fibres produced by reticulospinal and raphespinal inputs appears to be exerted on different interneurones along the PAD pathway.

4. It is concluded that, although reticulospinal and raphespinal pathways have similar inhibitory effects on PAD of Ia fibres, and similar excitatory effects on the PAD of Ib fibres, their actions are conveyed by partly independent pathways. This would allow their separate involvement in the control of posture and movement.

Studies on the possible role of the nucleus raphe magnus (NRM) and of the adjacent brainstem reticular formation (RF) in the control of sensory information, including nociception, have established that electrical stimulation of the RF and NRM reduces the spontaneous and evoked activity of dorsal horn and spinothalamic neurones that receive nociceptive and non-nociceptive inputs (McCreery & Bloedel, 1975; Mokha, McMillan & Iggo, 1985; see Gebhart & Randich, 1990). On the basis of the similarity of the effects exerted by the stimulation of the NRM and of the RF, it has been suggested that both nuclei are part of a common functional system that regulates nociceptive and non-nociceptive information (Anderson, 1984; Hammond & Yaksh, 1984; Willis, 1984).

Activation of the RF and of the NRM also affects motor activity (Peterson, 1984). It produces mono- and polysynaptic excitatory and inhibitory potentials in spinal motoneurones (Jankowska, Lund, Lundberg & Pompeiano, 1968; Peterson, 1984; Fung & Barnes, 1989; Takakusaki, Ohta & Mori, 1989), as well as dorsal root potentials (DRPs), the latter suggesting presynaptic inhibitory actions (Lundberg & Vyklicky, 1966; Proudfit & Anderson, 1974; Jiménez, Rudomin & Solodkin, 1987).

At the time when this research was started, the available evidence indicated that electrical stimulation of the bulbar RF produced primary afferent depolarization (PAD) of tendon organ and of cutaneous afferents, and inhibited the PAD generated in muscle spindle afferents (Rudomin, Jiménez, Solodkin & Dueñas, 1983; Rudomin, Solodkin & Jiménez, 1986; Jiménez *et al.* 1987; Jiménez, Rudomin & Solodkin, 1988; Rudomin, 1991). It was also known that stimulation of the NRM depolarized low threshold cutaneous fibres (Martin, Haber & Willis, 1979). However, there was no information on the effects of the NRM on muscle afferents.

In the present study, we analysed in detail the effects produced by electrical stimulation of the NRM and of the RF on the PAD evoked in single group Ia and Ib muscle afferents by segmental inputs. Particular emphasis was placed on ensuring selective stimulation of these brainstem nuclei. Preliminary aspects of this study have been published (Jiménez, Quevedo, Eguibar & Rudomin, 1989).

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METHODS

General procedures

The experiments were performed in twenty-eight adult cats anaesthetized with sodium pentobarbitone (Smith Kline, México; initial dose, 35 mg kg^{-1} I.P.), supplemented during the dissection with additional doses of 10 mg kg^{-1} I.V. as necessary to maintain deep anaesthesia. The left carotid artery was dissected, tied and its central end cannulated to record blood pressure. The left radial vein was cannulated to allow injection of fluids. The lumbosacral and lower thoracic spinal cord was exposed by laminectomy and the left L6 to S1 ventral roots cut. Both dorsal columns were sectioned at a low thoracic level to avoid antidromic activation of afferent fibres following brainstem stimulation. The right hemicord was transected at the same level to restrict descending effects to ipsilateral spinal pathways. The sural (SU) and superficial peroneus (SP) nerves in the left leg were dissected free and prepared for stimulation. The posterior biceps and semitendinosus (PBSt) and gastrocnemius–soleus (GS) nerves were dissected, sectioned and separated into several fine filaments to record antidromic action potentials of single muscle afferents (see below). The adequacy of anaesthesia was verified by assessing that withdrawal reflexes were absent, that the pupils were constricted and that blood pressure was between 100 and 120 mmHg during noxious stimulation.

After the main dissection, the animal was transferred to a rigid metal frame allowing fixation of the head and of the spinal vertebrae. The cerebellum was laid open and parts of it were removed to expose the floor of the fourth ventricle. Fine tungsten stimulating electrodes (about 10 μm tip diameter) were placed in the medial brainstem NRM and in the adjacent RF, ipsilateral to the dissected leg nerves, using the obex as reference. Pools filled with mineral oil were constructed around the spinal cord and hindlimb nerves. The temperature of the pools was automatically kept between 37 and 38 °C by means of radiant heat.

The animal was paralysed with a single dose of pancuronium bromide (Pavulon, 0·3 mg kg^{-1}; Organon Mexicana, México City, México) and maintained under artificial respiration. A bilateral pneumothorax was made to prevent transmission of respiratory movements, and tidal volume was adjusted to maintain expiratory CO_2 at about 4%. Additional doses of pancuronium bromide (0·1 mg kg^{-1} h^{-1}) were given to maintain paralysis. Mean blood pressure was continuously monitored and was usually between 100 and 120 mmHg. When necessary, a solution of etilefrine (Effortil; Boehringer-Ingelheim, México City, México), diluted with isotonic saline (1 : 10) or dextran (10%) was infused intravenously at a rate of 3 ml h^{-1} to maintain the blood pressure within this range.

During paralysis, deep anaesthesia was maintained by I.V. injections of pentobarbitone (3 mg kg^{-1} h^{-1}). Blood pressure and heart rate were monitored to ensure they were stable and did not alter in response to noxious stimulation. The pupils were also checked to ensure they were completely constricted and that there was no dilatation in response to noxious stimulation. These tests were made every 30 min.

At the end of the experiment, the stimulated brainstem sites were marked by passing anodic direct current (50 μA, 20–30 s) through the tungsten electrodes and the animal was killed with a overdose of anaesthetic and perfused with a 10% solution of formalin. The brainstem and thoracic spinal cord were prepared for histological verification of the stimulating sites and of the lesions (see Rudomin *et al.* 1983).

Recordings

Cord dorsum potentials (CDPs) produced by stimulation of sensory nerves and by stimulation of the NRM and of the RF were recorded at lumbar segments L6–L7 by means of a silver ball electrode placed on the cord dorsum against an indifferent electrode inserted in the back muscles. These potentials were amplified with a low noise–high gain differential amplifier (bandpass filters 0·3 Hz–10 kHz). To determine the spinal projection of sensory fibres for the excitability testing (see below), a glass micropipette filled with 2 M sodium chloride (1·2–1·8 MΩ) was introduced in the intermediate nucleus, using as a guide the extracellular field potentials (EFPs) produced by stimulation of peripheral nerves. In some experiments, EFPs were also recorded following stimulation of the NRM and RF. Dorsal root potentials (DRPs) were recorded from the central end of a L6–L7 dorsal rootlet placed on two silver Ag–AgCl hook electrodes, one placed very close to the spinal cord and the other placed distally on the severed end of the rootlet. Care was taken to avoid accumulation of cerebrospinal fluid close to the spinal cord. During the experiment, descending and segmental CDPs and DRPs were averaged on-line (32 samples at 1 Hz) and subsequently stored.

Intraspinal threshold measurements

Alterations in the membrane potential of single afferent fibres ending in the intermediate nucleus were estimated from changes in their intraspinal threshold (Rudomin *et al.* 1983). To this end, we used the 'threshold hunting method' described in detail elsewhere (Madrid, Alvarado, Dutton & Rudomin, 1979; Curtis, 1979; Rudomin *et al.* 1983, 1986) and summarized here. The glass micropipette was initially used to record the EFPs produced in the intermediate nucleus by stimulation with single pulses of the PBSt and GS nerves. Once in the optimal position (see Fig. 4*A* and *B*) the recording micropipette was connected to a pulse generator controlled by the computer. Constant current pulses (0·4 ms duration, 1·5–10 μA) were applied through the micropipette while recording from a fine PBSt or GS nerve filament. The position of the stimulating micropipette was further adjusted to produce, with minimum strengths, antidromic responses of a single fibre. Subsequently, the magnitude of the stimulating current was varied automatically in order to activate antidromically the fibre with a constant firing probability of 0·5. The stimulating current pulses were integrated. The values obtained were maintained until the next trial (usually at 1 s^{-1}) and recorded with a penwriter as a continuous trace (see Fig. 5). Using this method, PAD could be detected as a reduction in the intraspinal threshold of the fibre, and a relative hyperpolarization as a threshold increase (Rudomin *et al.* 1983).

The changes in the intraspinal threshold of a single fibre produced by different stimulation protocols were calculated as follows. It is assumed that the fibre's resting threshold was 5 μA (T_R) and that PBSt conditioning stimulation reduced this threshold to 3 μA (T_{PBSt}). The threshold attained during PBSt stimulation would then be:

$$(T_{PBSt}/T_R) \times 100 = (3\ \mu A/5\ \mu A) \times 100 = 60\%\ T_R \text{ (control)}.$$

As shown in Results, stimulation of the SU and SP nerves, as well as stimulation of the NRM and RF, had no effect on the intraspinal threshold of many Ia fibres, but was able to inhibit

the PAD produced in them by stimulation of PBSt nerve afferents. The assumption was made that the threshold of the fibre, already lowered by PBSt stimulation from 5 to 3 μA, was raised to 4 μA by the conditioning stimulation of the SU nerve. That is, in control conditions PBSt stimulation reduced the intraspinal threshold of the fibre by 2 μA. During conditioning stimulation of the SU nerve, PBSt stimulation reduced the threshold by 1 μA, which means that the effects of PBSt were reduced by 50%. A 100% effect would mean complete removal of the PAD induced by PBSt stimulation. For convenience, a relative increase in the intraspinal threshold due to removal of background PAD (inhibition of PAD) is expressed with a negative sign. A decrease in the intraspinal threshold below the level attained during the background PAD is expressed with a positive sign.

Changes in PAD of single fibres were produced by stimulation of the PBSt nerve with a train of three to four pulses at 300 Hz, and that of cutaneous nerves (SP and SU) with a single pulse, applied 25–35 ms before the intraspinal threshold testing pulse. Stimulation of the NRM and of the RF with single pulses had little or no effect on the intraspinal excitability of the afferent fibres, but stimulation with trains was more effective, probably because of the temporal summation of synaptic actions at spinal level (see Eccles, Magni & Willis, 1962; Lundberg, 1964). We used trains of thirteen to twenty pulses at 400–500 Hz, applied 65–75 ms before the excitability testing pulse. As shown in Fig. 6*F*, at 65–75 ms the DRPs produced by stimulation of the NRM attain their maximal amplitude. The DRPs produced by RF stimulation have a shorter onset latency and attain their maximum about 20 ms earlier, but are still 70–80% of maximum (see Fig. 6*F* and *H*).

Electrical stimulation of the brainstem frequently evokes cardiovascular changes and these could potentially disturb the excitability testing procedure. Blood pressure was monitored continuously and it was always verified that the excitability changes produced by brainstem stimulation occurred independently of the blood pressure changes (see also Rudomin *et al.* 1983).

PAD patterns

In the anaesthetized cat, group Ia fibres are depolarized by stimulation of group I muscle afferents from flexors and also by vestibulospinal pathways. Stimulation of cutaneous nerves, of the RF, the red nucleus and of the pyramidal tract fails to produce PAD in most Ia fibres, but may, however, inhibit the PAD elicited in these fibres by group I PBSt or vestibulospinal fibres (type A PAD pattern; see Rudomin *et al.* 1986). Group Ib fibres have two different PAD patterns. A fraction of these fibres is depolarized by stimulation of group I flexors and of cutaneous afferents as well as by stimulation of the RF, contralateral red nucleus and the pyramidal tract (type B PAD pattern; see Eccles, Schmidt & Willis, 1963; Rudomin *et al.* 1983, 1986; Jiménez *et al.* 1988). Another fraction of the group Ib fibres is depolarized by stimulation of group I fibres and descending pathways, but not by cutaneous afferents which instead inhibit the PAD produced by group I flexors (type C PAD pattern; Rudomin *et al.* 1986; Jiménez *et al.* 1988).

RESULTS

CDPs evoked by brainstem stimulation

A systematic analysis was made of the cord dorsum responses (recorded at L6–L7) produced by stimulation of the brainstem either at the mid-line, comprising the NRM or, more laterally, in the bulbar RF. Figure 1 shows data obtained in one experiment where the brainstem was stimulated with single pulses with a strength of 130 μA at three different rostrocaudal levels (6, 4 and 2 mm rostral to the obex). Stimulation of the RF 1·5 mm from the mid-line and between 2 and 5 mm below the dorsal surface of the brainstem produced in the cord dorsum, at all rostrocaudal levels, a brief positive potential seldom followed by negativity (Fig. 1 *B*, *E* and *H*). This positive potential had two components (see Fig. 2*A*).

Positive CDPs with two components were recorded in fourteen experiments from segments L6–L7 following stimulation of the brainstem between 0 and 8 mm rostral to the obex, 1·5–2·0 mm lateral from the mid-line and at depth of 1–5 mm. The onset latency of the first component, measured from the stimulus artifact, was of 2·1 $\pm$ 0·3 ms (mean $\pm$ S.D.). The calculated conduction velocities varied between 109 and 130 m s^{-1} (mean $\pm$ S.D., 120 $\pm$ 8·5 m s^{-1}; $n = 7$). The second component had a onset latency of 4·5 $\pm$ 0·3 ms. This component is probably due to postsynaptic activation of spinal interneurones, although there may be some contribution of slower conducting fibres (Peterson *et al.* 1984). Similar brief positive potentials with two components were obtained when stimulating along the mid-line, at a depth of between 2 and 3 mm (Fig. 1 *A*, *D* and *G*), in the region corresponding to the medial longitudinal fasciculus (MLF; see also Floeter, Sholomenko, Gossard & Burke, 1993).

In contrast to what was observed following stimulation to the RF or to the MLF, single current pulses applied at a depth of between 4 and 6 mm on the mid-line produced at all rostrocaudal levels a slow negative potential, usually with two components (Fig. 1*A–G*; see also Fig. 2*C*). The sites producing these negative responses appear to be located within the NRM (▲ in Fig. 1*C*, *F* and *I*). In fourteen experiments, the onset latency of the fastest negative component produced by stimulation of the NRM was of 5·5 $\pm$ 0·6 ms, suggesting activation of descending fibres conducting between 35 and 58 m s^{-1} (mean $\pm$ S.D., 47 $\pm$ 9·2 m s^{-1}; $n = 6$), in agreement with the observations of West & Wolstencroft (1977) and Wessendorf, Proudfit & Anderson (1981).

In order to establish appropriate stimulus parameters for independent activation of the NRM and of the RF, in three experiments we analysed the CDPs evoked by graded electrical stimulation of these nuclei. Figure 2 illustrates data obtained in one experiment. A relatively weak stimulus (10–35 μA) applied to the NRM (mid-line, 4 mm rostral to obex and 6 mm below the dorsal surface) evoked only negative CDPs (Fig. 2*C* and *E*, ●). However, at higher intensities (above 40 μA) an additional early positive response similar to that obtained by stimulation of the RF was evoked (Fig. 2*E*, ■). On the other hand, stimulation of the RF produced only positive CDPs throughout the whole explored range of intensities (Fig. 2*A* and *D*, ▲).

The maximal intensities of stimulation of the NRM with single pulses that produced negative CDPs not preceded by positive potentials *varied in different experiments*. These values could be as low as 25 μA and as high as 180 μA (mean $\pm$ S.D., 99 $\pm$ 38 μA; $n = 37$) and were between 2·5 and 8 times those required to produce a detectable negativity in the cord dorsum. The RF was stimulated with intensities varying between 30 and 200 μA (mean $\pm$ S.D., 128 $\pm$ 65 μA). This intensity was between 1·2 and 4·2 times the threshold of the positive CDP.

Effects of the transection of the pyramidal tract and of the dorsolateral funiculus on the CDPs produced by stimulation of the NRM

Lindblom & Ottosson (1957) have shown that stimulation of the motor cortex or of the pyramidal tract produces long-lasting negative potentials in the cord dorsum which resemble the negative responses produced in our experiments by stimulation of the NRM. In order to exclude possible coactivation of corticospinal fibres close to the NRM, in three experiments the brainstem was exposed from the ventral side and the pyramid contralateral to the recording site was completely sectioned caudal to the site of stimulation of the NRM. Data obtained in one experiment are illustrated in Fig. 3*A* and *B*. The extent of the pyramidal tract lesion is shown in Fig. 3*A a*. Under these conditions, electrical stimulation of the NRM (Fig. 3*A b*) with a single pulse (80 μA) applied at various depths along the mid-line still produced negative CDPs (Fig. 3*B*). This suggests that the negative CDPs produced by stimulation of the NRM with low strengths may be evoked independently of pyramidal activation. Similar results were obtained in the other two experiments in which pulses of 100–120 μA were used.

Available information shows that in the cat, descending fibres from the NRM (as well as corticospinal fibres) are conveyed through the dorsolateral funiculus (DLF; see Willis, 1984) whereas reticulospinal and MLF fibres descend mostly through the ventrolateral funiculus (Peterson, 1984; Floeter *et al.* 1993). In order to assess further whether stimulation of the NRM with low strengths activates descending fibres without significant coactivation of RF or MLF fibres, we analysed the effects of transection

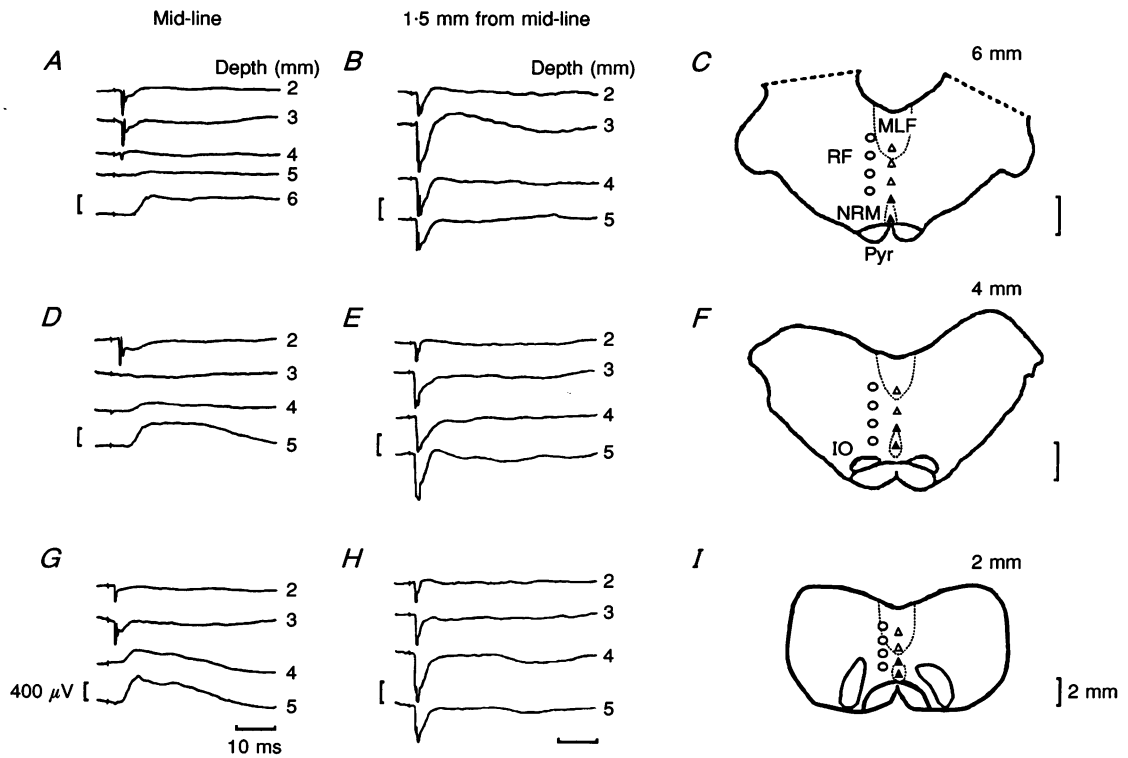

Figure 1. CDPs elicited by brainstem stimulation

A, *D* and *G*, cord dorsum responses produced by mid-line stimulation at various depths, 6, 4 and 2 mm rostral to the obex, as indicated in *C*, *F* and *I*. Stimuli were single cathodal pulses of 130 μA. *B*, *E* and *H*, as for *A*, *D* and *G*, but with another stimulating electrode placed 1·5 mm lateral from the mid-line, ipsilateral to the recording site. Negativity is upwards. Each trace is the average of 32 responses elicited at 1 Hz. Calibrations in *G* apply to other traces. *C*, *F* and *I*, histological reconstructions showing the stimulation sites. Both dorsal columns and the contralateral half of the spinal cord were sectioned at a low thoracic level. ○, stimulation with the lateral electrode. Triangles, stimulation with the middle electrode. ▲, sites producing a negative CDP. IO, inferior olive; MLF, medial longitudinal fasciculus; NRM, nucleus raphe magnus; RF, reticular formation; Pyr, pyramidal tract. Calibration in *I* applies to *C* and *F*.

of the ipsilateral DLF in the low thoracic cord on the spinal potentials produced by stimulation of the NRM and RF in five experiments.

Figure 3*C–D* shows data obtained in one experiment, but similar results were obtained in the other four (see also Fig. 6). The control cord dorsum responses produced by stimulation of the NRM and RF with 100 μA are shown in Fig. 3*Da*. After the additional transection of the DLF (Fig. 3*Cb*) the responses evoked by stimulation of the NRM were completely abolished, leaving the RF responses practically unaffected (Fig. 3*Db*). Stimulation of the NRM with a higher strength (400 μA) produced a positive response which was very similar to that produced by stimulation of the RF (Fig. 3*Dc*). Thus, in this experiment, stimuli up to 100 μA applied to the NRM did not coactivate fibres from the RF or MLF.

Stimulation of the NRM with trains of pulses with strengths usually below 100 μA generated a slow negative CDP due to the merging of the individual negative responses (see Fig. 5*C*, *F* and *I*, Fig. 6*B* and *F* and Fig. 10*D*). This potential was also abolished following transection of the dorsolateral funiculus (Fig. 6*D* and *H*). In contrast, repetitive stimulation of the RF produced a summated slow positive potential that was not affected by transection of the DLF (Fig. 6*D* and *H*). It thus seems that recording of a slow negative CDP following stimulation of the NRM with trains of pulses may be used as an additional criterion to exclude a significant coactivation of the RF and MLF.

Intraspinal distribution of field potentials produced by stimulation of NRM and RF

It was shown above that stimulation of the NRM and of the RF with relatively low strengths produces CDPs with opposite signs and different time courses. The question arose whether fibres from the NRM or from the RF activate the same or different sets of spinal interneurones. To this end, we analysed the intraspinal distribution of the field potentials produced by stimulation of these two descending systems.

Figure 4 illustrates the CDPs and the EFPs produced in one experiment by single stimuli (2*T*) applied to the PBSt and SU nerves (Fig. 4*A*) and by single 50 μA stimuli applied to the RF and NRM (Fig. 4*C*). Like the CDP, the EFPs recorded dorsally were negative. They became maximal at a depth of about 1·4 mm, close to the region in which the field potentials produced by stimulation of the SU nerve

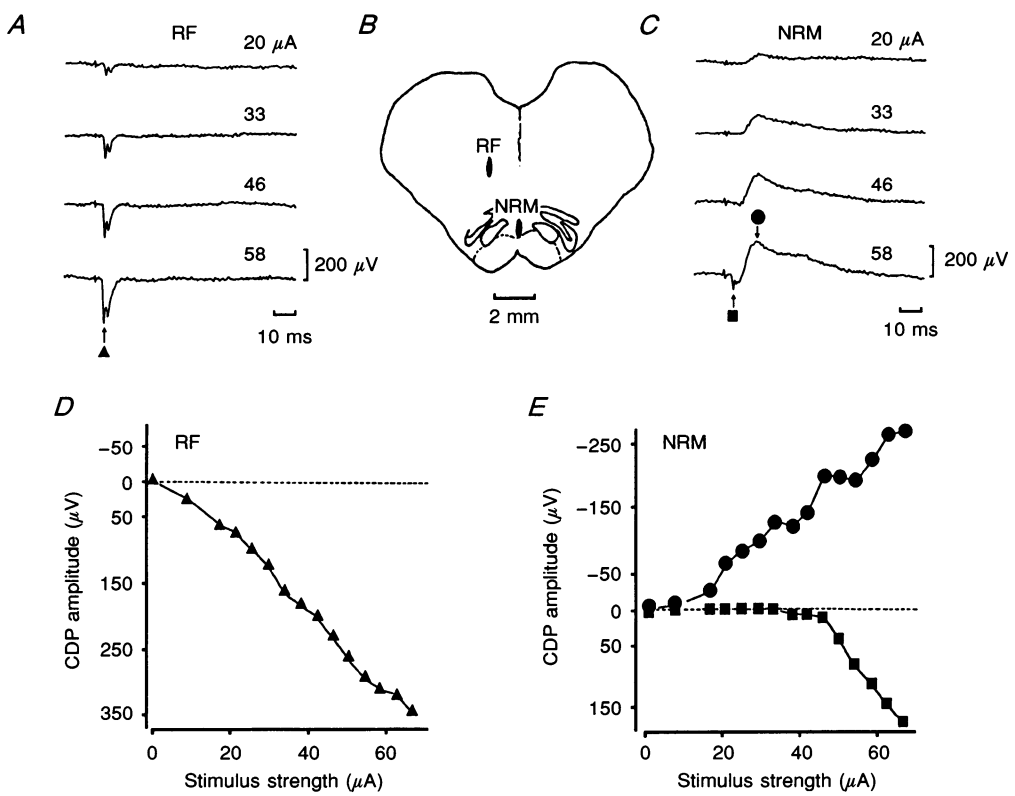

Figure 2. Effects of graded stimulation of the RF and NRM on CDPs

A and *C*, CDPs evoked by single pulses with increasing strength as indicated, applied to the bulbar RF (1·5 mm lateral to mid-line, 4 mm rostral to obex and 4 mm deep) and to the NRM (4 mm rostral to obex and 6 mm deep, mid-line). *B*, histological section showing the stimulation sites in the brainstem (dark areas are reconstructions of the lesions). *D* and *E*, amplitude of the CDPs produced by stimulation with single pulses of increasing strengths applied to the RF (▲) and to the NRM (● and ■), as indicated. Negativity upward. Arrows show the time at which amplitude measurements were made. All responses are means of 32 potentials elicited at 1 Hz.

were largest, and reversed sign deeper in the spinal cord (2·8–3·0 mm in Fig. 4*C*). In contrast, the intraspinal field potentials produced by stimulation of the RF were positive dorsally, reversed to negativity at a depth of between 2·2 and 2·4 mm, and acquired maximal amplitude at a depth of 3·4 mm. Figure 4*B* and *D* summarizes data obtained from ten different experiments. To facilitate comparison of the data the recording depth is expressed relative to the depth at which the field potentials produced by stimulation of the PBSt nerve were largest (▲). As shown in Fig. 4*D*, the region of maximum negativity evoked by stimulation of the NRM was about 0·4 mm dorsal to the site at which the PBSt-evoked field potentials were largest. The negative intraspinal field potentials produced by stimulation of the RF were maximal about 1·4 to 1·6 mm ventrally, somewhat medial to the region where these fibres make synaptic contacts with motoneurones (see Peterson, 1984). The negative field potentials produced by stimulation of the RF and NRM had mean onset latencies of 2·3 ± 0·9 and of 5·6 ± 0·6 ms, respectively ($n = 10$). These latencies were not significantly different (Student's t test) from the mean onset latencies of the CDPs (see also Fig. 5*C*).

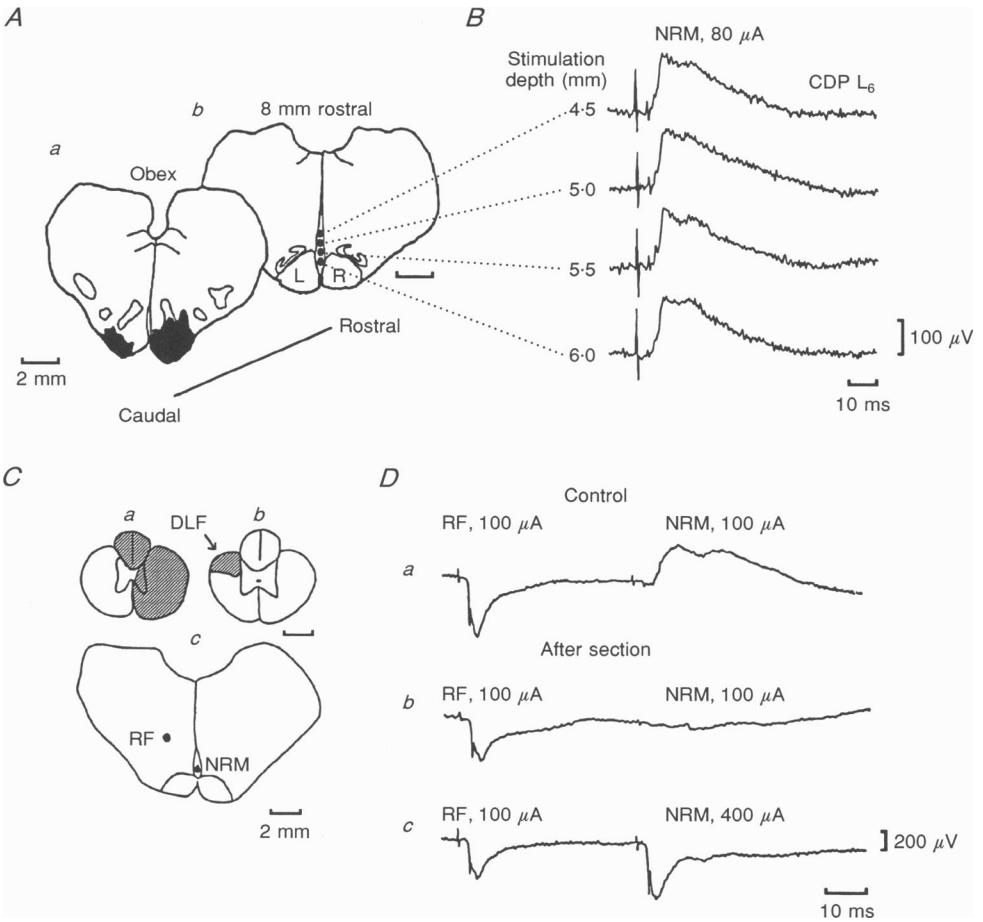

Figure 3. Effects of transection of the contralateral pyramidal tract and of the thoracic dorsolateral funiculus on the CDPs evoked by NRM stimulation

A, histological reconstruction of the pyramidal tract lesions made at the level of the obex (*A a*) and of the stimulation sites in the brainstem (*A b*, 8 mm rostral to obex). *B*, CDPs produced by single pulses (strength, 80 μA) applied at different depths within the brainstem, as indicated. The contralateral spinal cord and the dorsal columns were sectioned at the thoracic level. *C*, histological reconstruction of the thoracic spinal cord lesions in another experiment. *Ca*, section of the contralateral spinal cord and of the dorsal columns made at the beginning of the experiment. *Cb*, additional lesion of the dorsolateral funiculus. *Cc*, location of the stimulated brainstem sites. RF was stimulated 2 mm lateral to mid-line, 4 mm rostral to obex and at a depth of 4·5 mm and NRM was stimulated at the mid-line, 4 mm rostral to obex and at a depth of 6 mm depth. *D*, effects of sectioning the dorsolateral funiculus on the CDPs produced by RF and NRM stimulation. Trace *a* was obtained before and traces *b* and c after transecting the ipsilateral dorsolateral funiculus (DLF). After DLF section, stimulation of NRM with higher stimulus strengths (400 μA) produced an early positive response resembling that produced by RF stimulation (trace *c*). Each record is the average of 32 cord dorsum responses. Negativity upwards.

Effects of stimulation of NRM and RF on the intraspinal threshold of single group I afferent fibres

In this section we describe the effects produced by stimulation of the NRM and the RF on the intraspinal threshold of single muscle afferent fibres. This has allowed disclosure of the target fibres the PAD of which is affected by these supraspinal inputs.

Figure 5 illustrates the effects of segmental and descending stimulation on the intraspinal threshold of three single group I PBSt afferent fibres with different PAD patterns. The upper traces in Fig. 5*A*, *D* and *G* show the control antidromic responses of the afferent fibres (indicated by arrows) and the lower traces illustrate the collision of the antidromic responses with the orthodromic responses

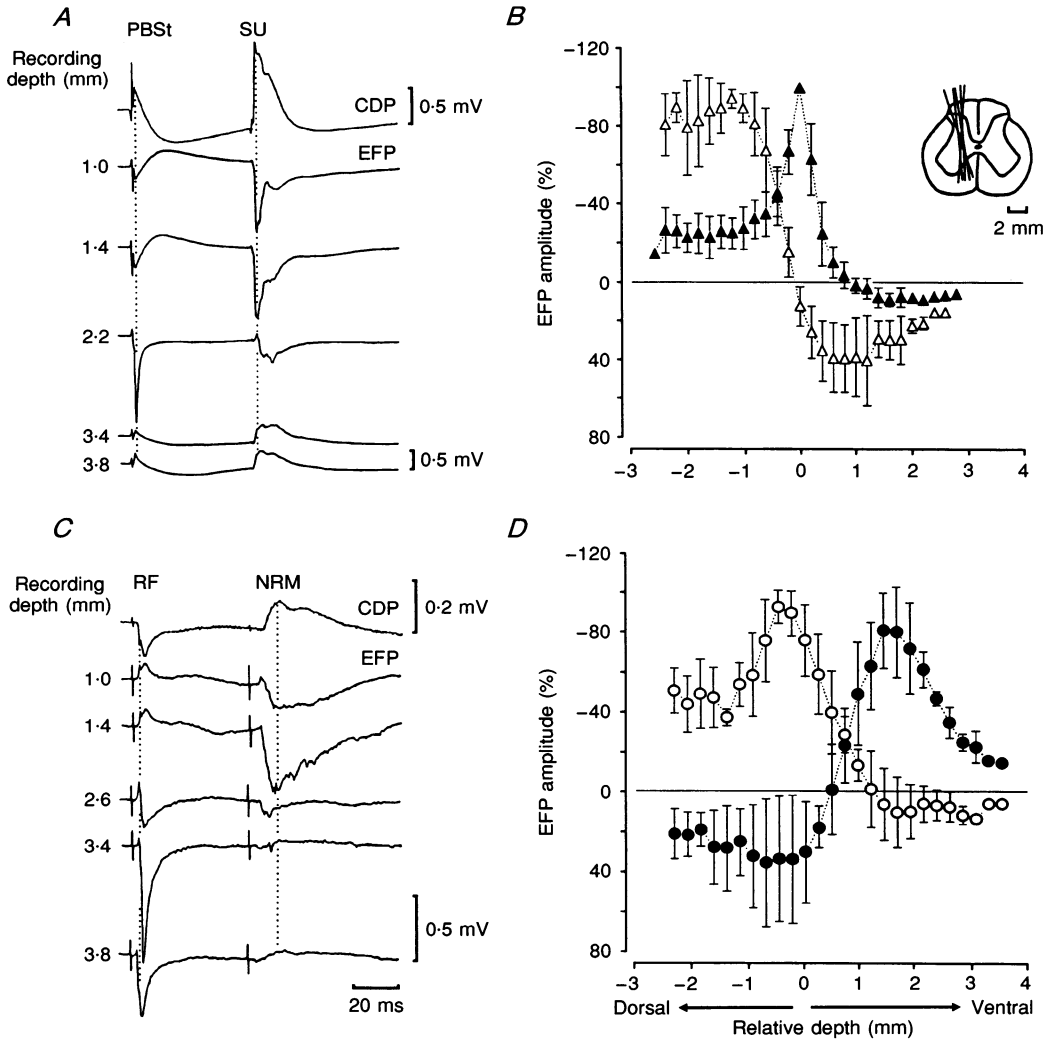

Figure 4. Intraspinal distribution of the extracellular field potentials evoked by activation of segmental and descending pathways

A, CDP (upper trace) and extracellular field potentials (EFPs) recorded at different depths in the spinal cord by stimulation of the PBSt and SU with single pulses (2*T*). CDP, negativity up; EFPs, negativity down. *B*, plot of amplitude *versus* depth of the EFPs obtained by stimulation of the SU (△) and PBSt (▲) nerve in 10 different experiments. Abscissa shows depth of recording relative to the depth of maximal amplitude of the EFPs produced by stimulation of the PBSt nerve (taken as zero). Positive and negative values denote recordings made ventral and dorsal to the reference point. Ordinate, amplitude of the responses (mean ± S.D., $n = 10$) expressed as percentage of the largest EFP produced by stimulation of the PBSt nerve.The inset shows the histological reconstruction of five recording microelectrode tracks. *C*, EFPs evoked, at the indicated depths, by stimulation of the NRM and of the RF (1 pulse, 50 μA). NRM was stimulated at the mid-line, 4 mm rostral to obex at a depth of 6 mm. RF was stimulated 2 mm lateral to mid-line, 4 mm rostral to obex at a depth of 4 mm. *D*, intraspinal distribution of the NRM (○) and RF (●) responses. Abscissa, as in *B*. In *B* and *D*, negative values are plotted upward. Vertical dotted lines in *A* and *C* show times at which amplitude measurements were made.

produced by stimulation of the whole PBSt nerve (applied 0·5 ms before the test intraspinal stimulus) at the indicated strengths. This test gives the upper estimate of the fibres peripheral threshold (Rudomin *et al.* 1986).

The afferent fibre of Fig. 5*B* had a type A PAD pattern. Conditioning stimulation of the NRM and of the RF with trains of pulses (75 and 90 μA, respectively), or of the SU and SP nerves (strength of pulses 5*T*), had practically no effect on the resting intraspinal threshold of the fibre (see also Riddell, Jankowska & Eide, 1992). However, these same conditioning stimuli were partly able to reverse the effects produced in the fibre by PBSt nerve stimulation. The intraspinal threshold changes produced by supraspinal and segmental inputs on a single PBSt afferent fibre with a type B PAD pattern are shown in Fig. 5*E*. Stimulation of cutaneous nerves (SU and SP) and of the NRM and RF with a train of pulses (100 μA) decreased the intraspinal threshold of the fibre. It is to be noted that the PAD produced by the cutaneous and descending inputs was completely occluded by the PBSt-induced PAD, suggesting activation of common interneurones along the PAD pathway (see Rudomin *et al.* 1986). Figure 5*H* illustrates the responses of a fibre with a type C PAD pattern. The resting intraspinal threshold of this fibre was unaffected by stimulation of the SU and SP, but was clearly reduced by stimulation of the NRM and RF. When tested against the background PAD evoked by stimulation of the PBSt nerve (1·12*T*), conditioning stimulation of the SP and SU *inhibited* the

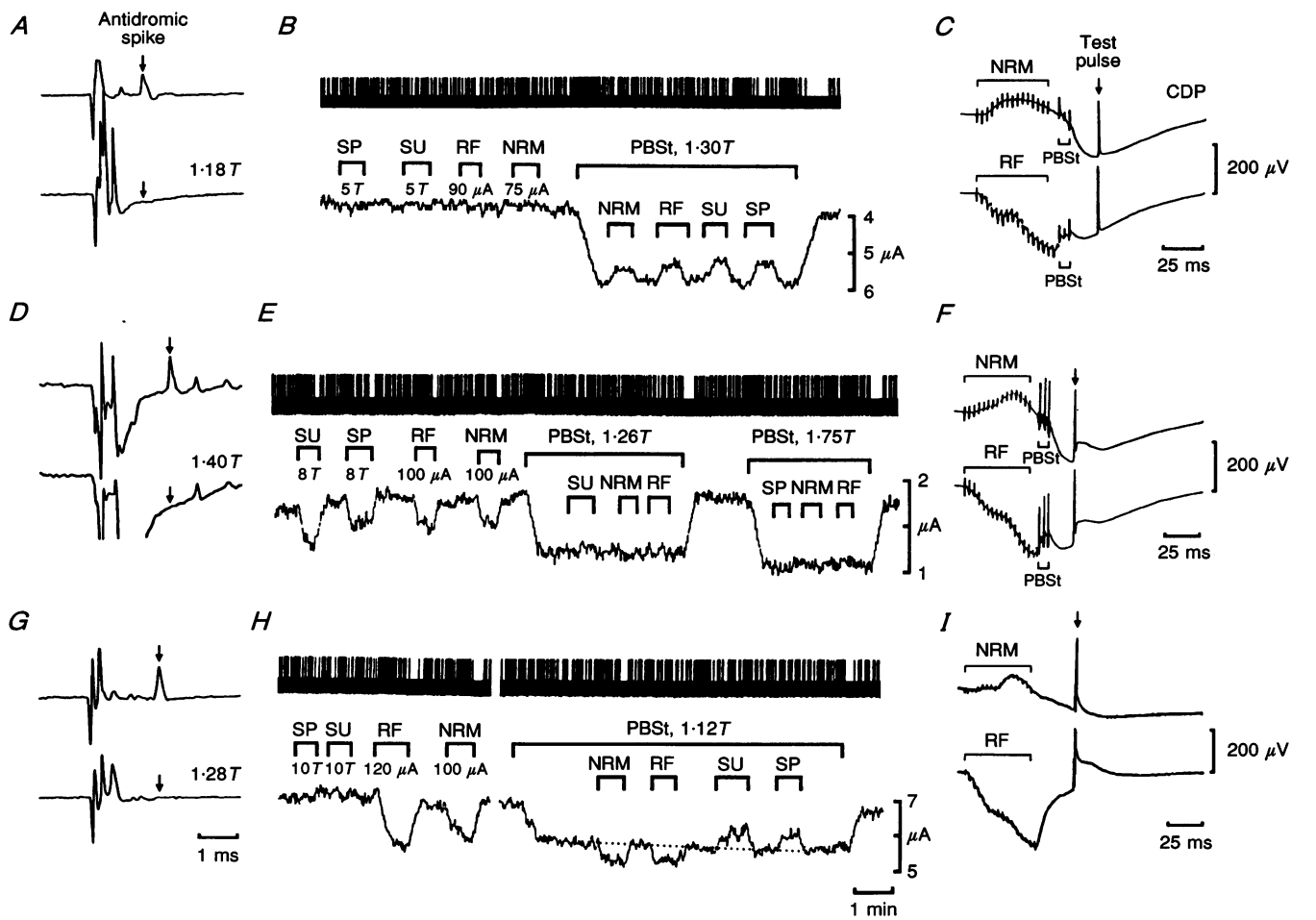

Figure 5. Effects of NRM and RF stimulation on the intraspinal threshold of single afferent fibres with different types of PAD patterns

A, *D* and *G*, upper trace, antidromic responses of 3 single PBSt afferent fibres (arrow) produced by intraspinal stimulation. Lower trace, collision of the antidromic response with the orthodromic responses produced by stimulation of the whole PBSt nerve, at the indicated strength. *B*, *E* and *H*, upper trace antidromic firing and lower trace, continuous estimate of the intraspinal threshold of the fibre. The PBSt nerve was stimulated with 3 pulses at 300 Hz, applied 35 ms before the intraspinal threshold testing pulse. SU and SP stimulation was one pulse and NRM and RF stimulation a train of 15 pulses at 400 Hz, all applied 75 ms before the excitability testing pulse. *C*, *F* and *I*, CDPs recorded during the intraspinal threshold testing procedure. Arrows show artifact produced by the threshold testing pulse. Conduction velocities of fibres in *A*, *D* and *G* were 93·6, 82·3 and 76·1 m s^{-1}, respectively. Further explanations in text.

PAD. In contrast, the PAD produced by stimulation of the NRM and RF *was added* to the background PAD.

In the experiments shown in Fig. 5 the NRM was stimulated with strengths in which each individual pulse in the train produced a negative response not preceded by positivity. This led to a slow negative summated response (upper traces in Fig. 5*C*, *F* and *I*). To assess further whether under these conditions stimulation of the NRM with trains of pulses was not significantly coactivating the adjacent RF, we tested, in several experiments ($n = 5$), the effect of transecting the dorsolateral funiculus while examining the intraspinal threshold changes produced by these descending inputs on the same afferent fibre.

Figure 6*A* and *C* shows the effects of stimulation of the NRM and RF on the PAD of a group I fibre with a type A PAD pattern. Stimulation of the NRM and of the RF had no effect on the intraspinal threshold of the fibre (not illustrated), but reduced very clearly the PAD produced by PBSt conditioning stimulation (Fig. 6*A*). Transecting the DLF eliminated almost completely the NRM-induced inhibition of the background PAD, practically without affecting the inhibition produced by stimulation of the RF (Fig. 6*C*). The negative CDP produced by stimulation of the NRM (Fig. 6*B*) was also eliminated, and only a small, slow positivity was recorded (Fig. 6*D*). On the other hand, the positive CDP produced by stimulation of the RF remained about the same (Fig. 6*B* and *D*).

The effects of stimulation of the NRM and RF on the intraspinal threshold of a fibre with a type C PAD pattern are shown in Fig. 6*E* and *G*. In this experiment we recorded DRPs in addition to the CDPs. Stimulation of the NRM and of the RF with trains of pulses (100 and 120 μA, respectively) produced a negative DRP, even though the CDPs produced by these two descending pathways were of opposite sign (Fig. 6*F*). Stimulation of the NRM reduced the intraspinal threshold of the afferent fibre to 86% of control values, and RF stimulation to 82% (Fig. 6*E*). Transection of the DLF eliminated the PAD produced by stimulation of the NRM (Fig. 6*G*) and also the negative CDPs and the DRPs (Fig. 6*H*). In contrast, it had practically no influence on the PAD produced by the RF (Fig. 6*G*). The RF-induced CDPs and DRPs were also unaffected (Fig. 6*H*).

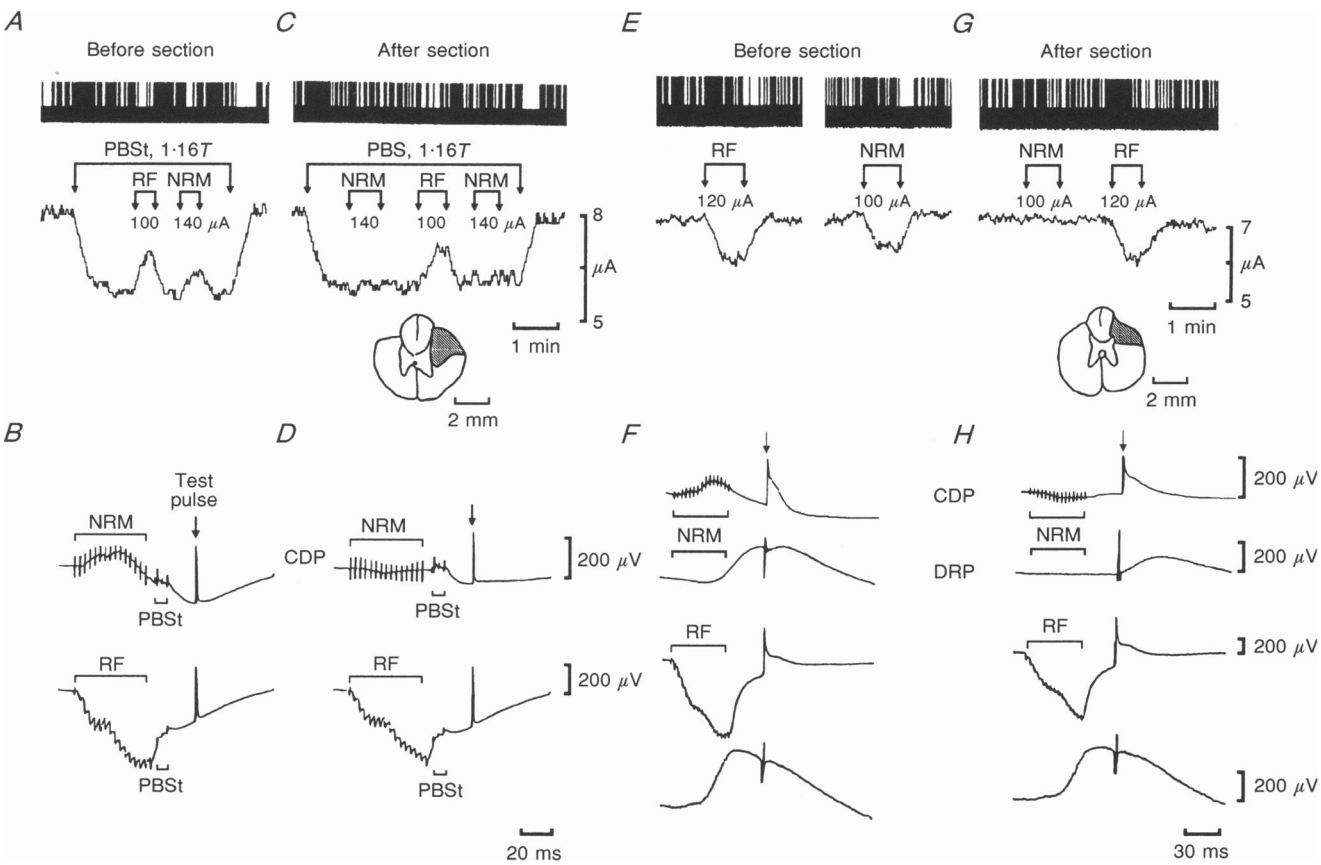

Figure 6. Section of the DLF abolishes effects produced by NRM stimulation

A and *C*, antidromic firing and intraspinal threshold changes of a single fibre with a type A PAD pattern. *B* and *D*, CDPs recorded during the intraspinal threshold testing. Stimuli as indicated. The arrows show the artifacts produced by the threshold testing pulse. *A* and *B* before DFL section; *C* and *D*, after DLF section. Insets show histological reconstruction of the lesion. *E–H*, same format but for a fibre with a type C PAD pattern. Data from another experiment. *F* and *H* show CDPs as well as DRPs. *E* and *F* were taken before the DLF section and *G* and *H* after the DLF section.

Figure 7 summarizes data from all fibres with a type A PAD pattern that were studied in detail. The distribution of the intraspinal threshold changes (relative to control threshold) produced by stimulation of cutaneous nerves (SU and SP) is shown in Fig. 7*A*. In 30/37 fibres (81%), conditioning stimulation of the SU nerve modified the intraspinal threshold of the fibres by less than 5% relative to control threshold, suggesting that in these fibres cutaneous volleys produced no significant PAD, in confirmation of earlier studies (Rudomin *et al.* 1983, 1986; Jiménez *et al.* 1988). Similar results were obtained when testing the effects of SP conditioning stimulation where changes were within 5% of control threshold in 22/35 fibres (63%).

Figure 7*B* and *C* shows the effects produced by stimulation of the NRM and RF. When the NRM was stimulated with trains of pulses in the low intensity range (between 25–50 μA; Fig. 7*B*, ○), the intraspinal threshold of 8/10 fibres (80%) changed by no more than 5% of control values.

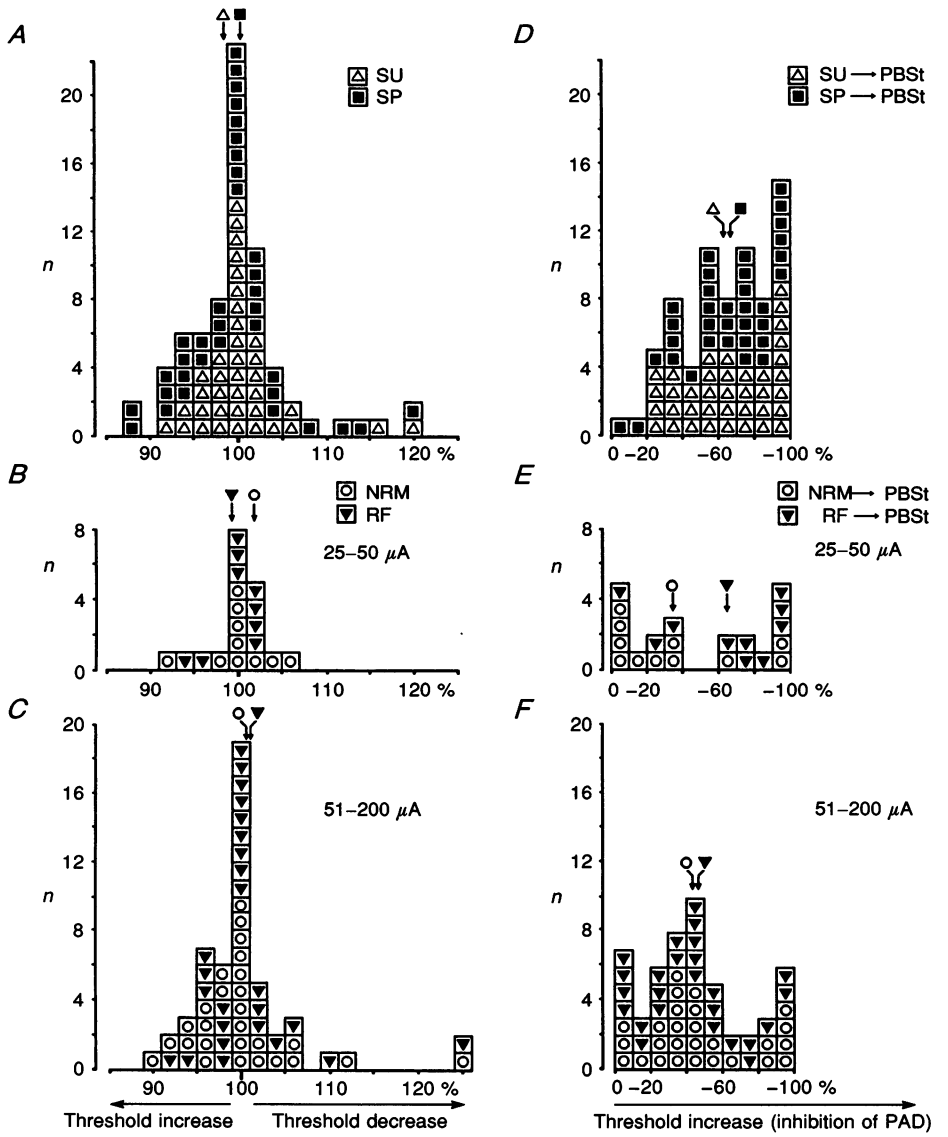

Figure 7. Summary of intraspinal threshold changes produced by segmental and descending stimulation on single muscle afferent fibres with type A PAD pattern

A, percentage changes of the intraspinal threshold of individual fibres relative to control threshold produced by stimulation of SU and SP. *B–C*, intraspinal threshold changes produced by stimulation of the NRM and RF with different strengths, as indicated. *D–F*, percentage increment of the intraspinal threshold, already lowered by stimulation of the PBSt nerve, following cutaneous (*D*) and supraspinal (*E–F*) stimulation. Vertical arrows indicate corresponding means. The PBSt nerve was stimulated with 3 pulses, 300 Hz, 1·15–2*T*, applied 25–35 ms before excitability test pulse; the SU and SP nerves were stimulated with 1 pulse, 5–10*T*, and the NRM and RF with a train of 13–20 pulses, 400–500 Hz, all applied 65–75 ms before the test pulse, at the indicated strengths.

That is, stimulation of the NRM produced practically no changes in the resting intraspinal threshold of most fibres. Similar results were obtained when the NRM was stimulated with trains of 51–200 μA (Fig. 7*C*, ○). At this strength, stimulation of the NRM had no effects on the intraspinal threshold of 19/27 fibres (70%), increased the intraspinal threshold of 4/27 fibres and decreased the intraspinal threshold of 4/27 fibres. The intraspinal threshold changes produced by stimulation of the RF on the same set of afferent fibres resembled quite closely those obtained by stimulation of the NRM (see Fig. 7*B* and *C*, ▼). Pooling data obtained at all intensities showed that the intraspinal threshold of 28/34 fibres (82%) was unaffected by conditioning stimulation of the RF beyond the 5% range.

The effects of conditioning stimulation of the SU and SP on the PBSt-induced PAD are shown in Fig. 7*D*. In all the thirty-eight fibres tested, stimulation of the SU nerve inhibited the PAD by more than 20%, while the SP nerve inhibited the PAD in 32/34 fibres (94%). As illustrated in Fig. 7*E* and *F*, stimulation of the NRM and RF also inhibited the PBSt-induced PAD. The magnitude of the inhibition varied among the different fibres and could be as large as 100%, even when the central stimuli were in the low intensity range (25–50 μA; Fig. 7*E*). It should be noted that increasing the strength of stimulation of the NRM, changed from 55 to 86% the number of fibres in which PAD was inhibited more than 20%. That is, the inhibition of the PAD produced by stimulation of the NRM and RF in the low intensity range (25–50 μA) was nearly two-thirds of maximum.

The data depicted in Fig. 8 summarize the effects of segmental and descending stimuli on afferent fibres with a type B and C PAD pattern. In fibres with a type B PAD pattern, stimulation of cutaneous nerves and of the RF produced PAD (Fig. 8*A–C*, filled symbols), or facilitated the background PAD elicited by stimulation of the PBSt nerve (Fig. 8*D–F*, filled symbols). Fibres with a type C PAD pattern were also depolarized by stimulation of the RF (Fig. 8*B* and *C*, ▽), but stimulation of cutaneous nerves inhibited their background PAD (Fig. 8*D*, open symbols). The differences in the action of cutaneous inputs on the background PAD of type B and type C fibres was statistically significant ($P < 0{\cdot}05$, *t* test).

Stimulation of the NRM with low as well as with high strengths reduced the intraspinal threshold of type B and type C fibres (Fig. 8*B* and *C*, circles). In most fibres, the PAD produced by stimulation of the NRM occluded with the background PAD or was facilitated.

Possible differences in the action of supraspinal pathways on PAD

The ratio (*R*) obtained by dividing the percentage threshold changes produced in individual fibres by stimulation of the NRM and of the RF gives an estimate of the relative effectiveness of these two descending inputs. The histograms of Fig. 9*A* and *B* show the distribution of the *R* values determined for fibres with a type A PAD pattern. In this case supraspinal stimulation inhibited the background PAD elicited by PBSt stimulation (see Fig. 7*E* and *F*). The *R* values obtained during supraspinal stimulation with low (25–50 μA) and high strengths (51–200 μA) varied between 0·01 and 2·3, suggesting differences in the relative effectiveness of the descending inputs. However, no statistical differences (*t* test) were found between the mean *R* values of both distributions (arrows).

The individual *R* values obtained from type B and C fibres tested without a background PAD appeared grouped around a mean close to 1, with a relatively small dispersion (Fig. 9*C* and *D*, filled symbols). In contrast, individual *R* values determined in the presence of a background PAD showed wider variations (Fig. 9*C* and *D*, open symbols). However, no significant differences were found between the mean *R* values (*t* test). There were also no statistical differences between the *R* values of fibres with type A and type B or C PAD patterns. The wide variations in the effectiveness of the reticulospinal and raphespinal stimulation on the background PAD of group I fibres could be due to variability in the strength and/or the location of the brainstem stimulation, but could also be due to differences in the interactions of the descending pathways with the segmental interneurones mediating the PAD. Nevertheless, it must be stressed that in each fibre tested, the effects produced by stimulation of the NRM were always in the *same* direction as those produced by RF stimulation.

Conduction velocity and peripheral threshold of the fibres

The data depicted in Fig. 9*E* show the relationship between the peripheral threshold of the fibres, determined by collision with the antidromic spike (as in Fig. 5*A*, *D* and *G*) and the estimated conduction velocity. Different symbols have been used to indicate the PAD patterns of the fibres, determined using the tests described in Fig. 5*B*, *E* and *H*. It may be seen that 38/66 fibres (58%) had a type A PAD pattern and peripheral thresholds between 1·01 and 1·75*T*, that is, within the group I range. The conduction velocities of these fibres varied from 62 to 101 m s^{-1}. There were 10/66 fibres with a type B PAD pattern and 10/66 fibres had a type C PAD pattern. These fibres had peripheral thresholds between 1·1 and 2·9*T* and their conduction velocities varied from 48 to 87 m s^{-1}. Correlation tests were performed with the data depicted in Fig. 9*E*. The calculated coefficients of correlation (*r*) obtained from sets of fibres with the same PAD pattern or from the whole sample were *negative* and varied between −0·44 and −0·61 (see inset). That is, in all cases there was an inverse relation between the conduction velocity and the peripheral threshold of the fibres.

Nine out of sixty-six fibres (13%) could not be classified according to their PAD patterns. Interestingly, all of them showed a clear reduction of their intraspinal threshold (to 84·3 ± 17·4% of control) following stimulation of the PBSt

nerve at stimulus strengths ranging between 1·2 and 2T. However, stimulation of the SU or SP nerves (2–10T), of the RF (40–165 μA) and of the NRM (40–120 μA) had no effect on the resting intraspinal threshold of the fibres beyond 5% of control. Stimulation of cutaneous and descending fibres was also unable to change significantly the background PAD produced by stimulation of the PBSt nerve. These fibres had peripheral thresholds between 1·4 and 3·0T and conduction velocities of 50–88 m s^{-1}. The reasons for the lack of effects of the cutaneous and descending inputs on the PAD of these fibres are not clear, but may reveal some selectivity in the connections of the PAD-mediating interneurones because stimulation of group I afferents was indeed a very effective means of producing PAD (see also Riddell *et al.* 1992).

Effects of stimulation of the NRM and RF on the monosynaptic PAD produced in Ia fibres by intraspinal microstimulation

Previous studies have shown that under the appropriate conditions, microstimulation within the intermediate nucleus in the spinal cord may directly activate interneurones that produce PAD of group I fibres (Jankowska, McCrea, Rudomin & Sykova, 1981; Rudomin *et al.* 1983, 1986). The short onset latency of this PAD (0·6–0·8 ms) suggests a monosynaptic linkage (Jankowska *et al.* 1981; Rudomin *et al.* 1983, 1986; Quevedo, Eguibar, Jiménez & Rudomin, 1992).

Figure 10 illustrates the results from one experiment designed to test the effects of stimulation of the NRM on the monosynaptic PAD produced by intraspinal microstimulation. Stimulation of cutaneous nerves, of the NRM and of the RF, produced no appreciable changes in the resting threshold of the fibre (Fig. 10A), but was able to inhibit the background PAD evoked by PBSt stimulation with trains of three pulses 2T in strength (Fig. 10B), suggesting that this was a group Ia fibre.

In order to produce a monosynaptic PAD by intraspinal microstimulation applied through the same microelectrode that is being used for the excitability measurements, it is necessary to place the stimulating micropipette relatively far from the tested afferent fibre (see Rudomin *et al.* 1983). In the observations illustrated in Fig. 10C the resting intraspinal threshold of the fibre was 5 μA, suggesting that the tip of the stimulating micropipette was about 200 μm from the tested fibre (see Jankowska & Roberts, 1972). This

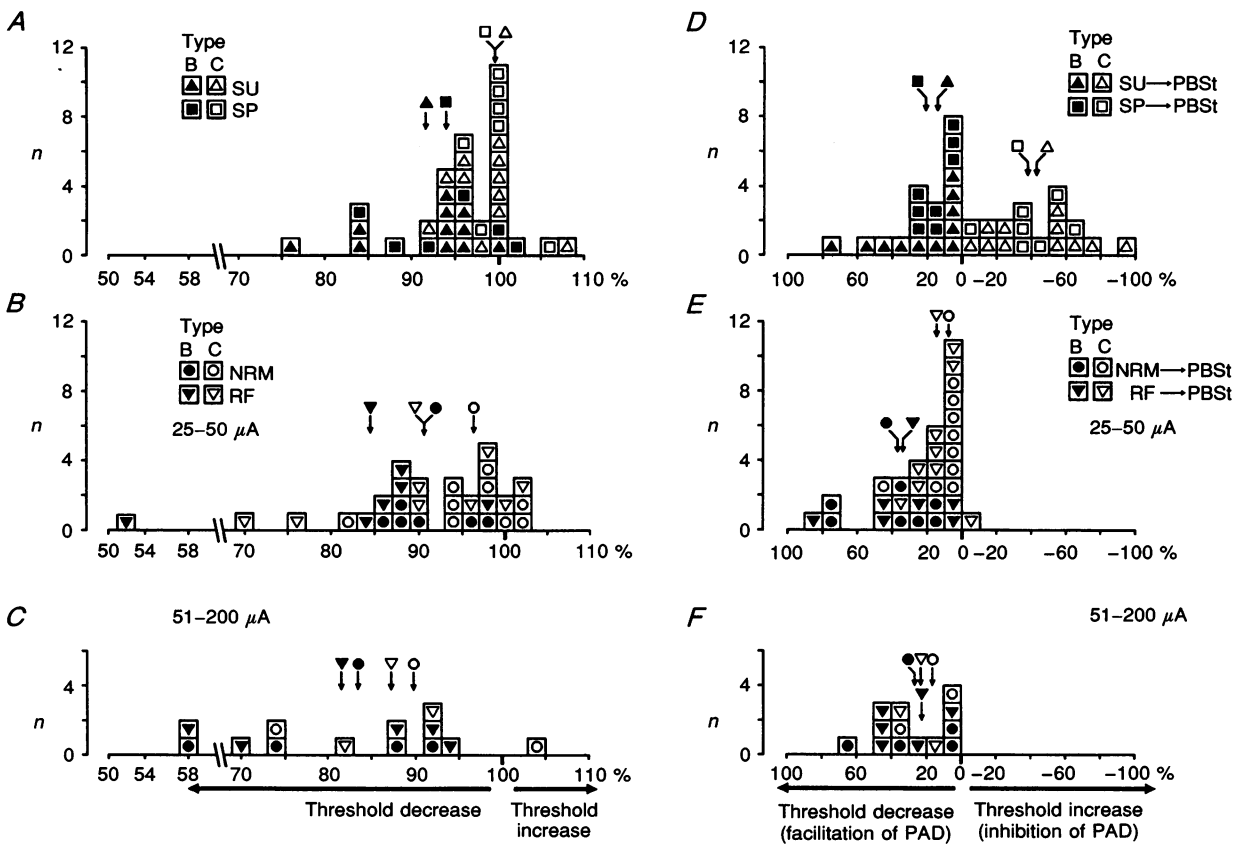

Figure 8. Summary of intraspinal threshold changes produced by segmental and descending stimulation on single muscle afferent fibres with type B and C PAD patterns

Same format and stimulation parameters as in Fig. 7. In *D–F*, positive values indicate a relative threshold increase above the background threshold (already lowered by conditioning stimulation of the PBSt nerve) and negative values indicate a relative threshold reduction (below the threshold attained during stimulation of the PBSt nerve). Further explanations in text.

allowed application of conditioning pulses which were strong enough to produce PAD, *without activating* the fibre whose excitability was being tested (Rudomin *et al.* 1983). The intraspinal conditioning stimulus was applied 3 ms before the excitability testing pulse, and it is very likely that even at this interval, the evoked PAD was most, if not all, monosynaptic (Jankowska *et al.* 1981; Rudomin *et al.* 1983; Quevedo *et al.* 1992). Conditioning stimulation of the RF produced a very clear inhibition of the monosynaptic PAD evoked by intraspinal microstimulation. However, stimulation of the NRM and of the SU and SP nerves was *completely ineffective*, suggesting that fibres from the raphe nucleus and from cutaneous afferents, unlike fibres from the reticular formation, have no inhibitory connections with the last-order interneurones mediating PAD of Ia fibres.

Using the same stimulation protocol as that of Fig. 10, we found that conditioning stimulation of the SU, SP, RF and NRM affected the resting intraspinal threshold of seven GS fibres with a type A PAD pattern less than 5% (the conduction velocity varied between 74 and 92 m s^{-1} and the peripheral threshold between 1·01 and 1·6T). Stimulation of the PBSt nerve (1·5–2T) reduced the intraspinal threshold of these fibres to a mean value of 80·6 ± 7·4% of control. Stimulation of the NRM and RF (pulse strengths 50–150 μA) inhibited the PAD produced by PBSt stimulation: the intraspinal thresholds, already lowered by stimulation of the PBSt nerve, were increased by 35·7 ± 24·7 and 63·4 ± 15·9%, respectively. Intraspinal microstimulation, applied 2–3 ms before the excitability testing pulse to produce a monosynaptic PAD, decreased the intraspinal threshold to 74·2 ± 12·5 of control, and stimulation of the RF reversed this effect by 40·2 ± 9·7%. In contrast, stimulation of the NRM and of cutaneous nerves appeared not to have any effect on the monosynaptic PAD.

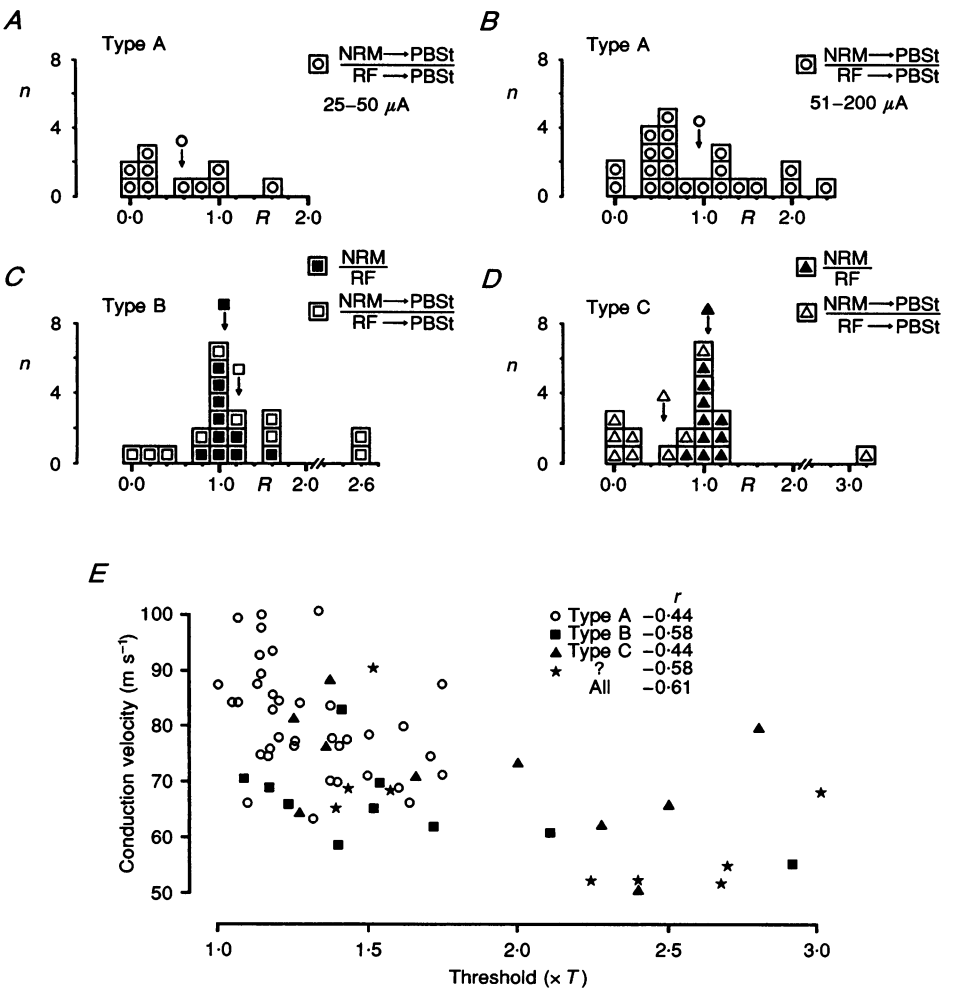

Figure 9. Comparative effects of NRM and RF on the PAD elicited in the same afferent fibre
A–D, abcissa, ratio (R) obtained by dividing the intraspinal threshold changes produced in the same afferent fibre by stimulation of the NRM and of the RF. Ordinates, number of fibres (n). Histograms were constructed with data depicted in Figs 7*D–F* and 8*D–F*, where stimulation parameters are indicated. *E*, relation between peripheral threshold and conduction velocity. The inset shows the coefficient of correlation (r) for each set of data and for the whole sample, as indicated. ★, fibres in which it was not possible to define a specific type of PAD pattern. Further explanations in text.

DISCUSSION

Selectivity of the stimulation of the NRM and RF

There are several studies in which electrical stimulation has been used to activate raphespinal and reticulospinal pathways (for references see Willis, 1984; Gebhart & Randich, 1990). However, few have considered the possible coactivation of reticulospinal or MLF pathways during stimulation of the NRM. We have shown here that stimulation of the RF produces brief positive potentials in the cord dorsum that are not conveyed through the DLF but more likely through the ventromedial funiculus. In contrast, stimulation of the NRM with single pulses, particularly with relatively low strengths (usually below 100 μA), produces negative CDPs. Stimulation of the NRM with higher strengths may also produce a short latency positive potential, but only the negative potential appears to be generated by fibres coursing through the dorsolateral funiculus. Similar results have been obtained with repetitive stimulation of the NRM. With low strengths, stimulation of the NRM produces a slow negative CDP due to summation of individual responses. This potential is also eliminated by sectioning the DLF.

It thus appears that stimulation of the NRM with strengths producing only the negative CDPs does not coactivate nearby reticulospinal or MLF pathways. The finding that sectioning the contralateral pyramidal tract did not abolish the negative cord dorsum responses produced by stimulation of the NRM with pulses of between 80 and 120 μA (Fig. 3*A* and *B*) suggests further that electrical stimulation of the NRM below this upper limit is very likely to activate fibre systems confined mainly within this nucleus, without significant coactivation of fibres in the pyramidal tract.

In two experiments we found that stimulation of the NRM with trains of pulses of 100 and 140 μA coactivated other fibres besides those descending through the DLF. However, the number of coactivated fibres appeared to be relatively small, because after section of the DLF these stimuli produced rather small positive CDPs and failed to change the intraspinal threshold of afferent fibres (Fig. 6). Stimulation of the NRM with higher strengths (above 200 μA) clearly coactivated other descending pathways, among them the reticulospinal, MLF–spinal or corticospinal fibres (Fig. 3*D*).

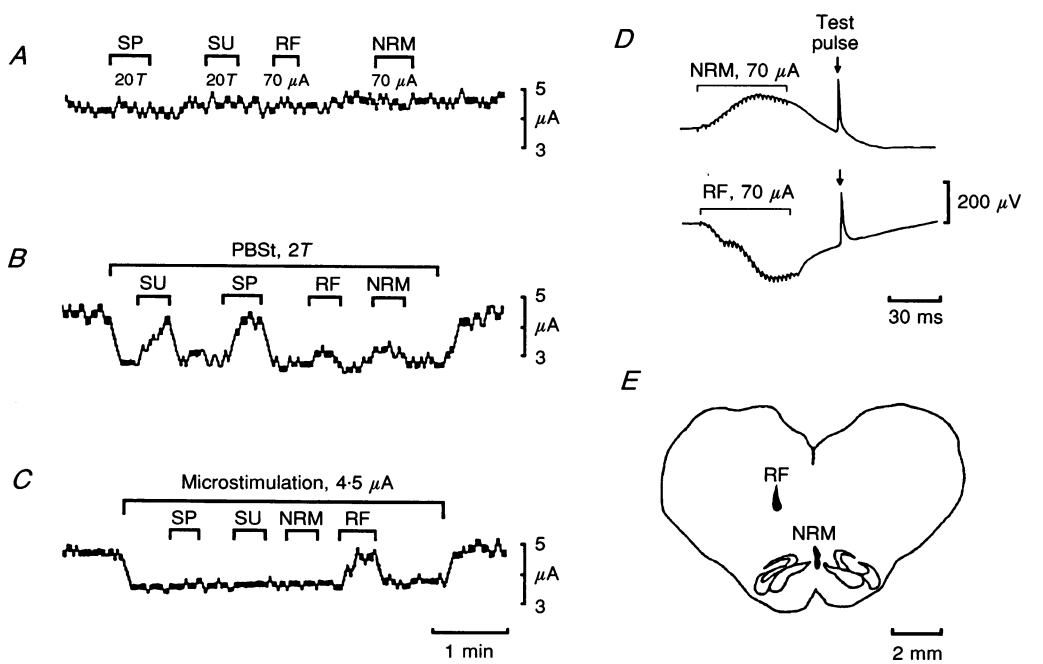

Figure 10. Differential effects produced by stimulation of the NRM and the RF on the monosynaptic PAD produced by intraspinal microstimulation

A, effects produced by stimulation of cutaneous nerves (SU and SP) and of supraspinal nuclei (NRM and RF) on the resting intraspinal threshold of a single GS fibre with a peripheral threshold of 1·23*T* and conduction velocity of 92 m s^{-1}. *B*, inhibition of the PBSt-induced PAD by stimulation of cutaneous and descending fibres. *C*, effects of these same stimuli on the background PAD produced by intraspinal microstimulation (1 pulse, 4·5 μA applied 3 ms before the excitability testing pulse to produce monosynaptic PAD; see Rudomin *et al.* 1983). Note inhibition of PAD by stimulation of the RF and lack of effects by the NRM and the cutaneous nerves. *D*, averaged CDPs evoked by stimulation of the NRM and the RF at 0·5 Hz taken during the threshold testing procedure. Arrows show artifact produced by threshold testing pulse. The PBSt nerve was stimulated with 3 pulses, 300 Hz, applied 25 ms before excitability test pulse; the SU and SP nerves were stimulated with 1 pulse and the NRM and RF with a train of 25 pulses, 500 Hz applied 55 ms before the test pulse. Stimulus strengths are indicated. *E*, histological section of the brainstem showing stimulation sites.

It thus seems possible to stimulate the NRM without a significant coactivation of the RF, MLF or pyramidal tract. The highest stimulus strengths allowing this selective stimulation will vary from one experiment to another, probably because of the position of the stimulating electrodes. From the experience gained in this investigation it seems that stimulus intensity below 100 μA is reasonably selective, but even so it is essential to control stimulus spread, either by using the CDPs as indicators, or by lesioning the DLF and pyramidal tract. The need of such controls is of importance because there are several pharmacological and electrophysiological studies where the NRM was stimulated with relative high stimulus strengths (0·1–2·0 mA; see Belcher, Ryall & Schaffner, 1978; Mokha, McMillan & Iggo, 1986). This could imply that some of the actions attributed in those studies to NRM stimulation probably involved coactivation of other descending systems.

Neuroanatomical tracing studies have shown that in the cat and in the rat, NRM neurones send axonal projections via the gigantocellular reticular formation to the lateral reticular nucleus (Nicholas & Hancock, 1990). Therefore, it is possible that electrical stimulation of the RF may antidromically activate fibres in the raphe nuclei. However, our observations indicate that stimulation of the RF did not evoke discernible negative CDPs resembling those produced by activation of the NRM, even with relative high stimulus strengths (Fig. 2*A* and *D*). This suggests that at the stimulated sites in the RF there were few, if any, collaterals of NRM neurones.

Intraspinal distribution of the field potentials

We found that the intraspinal field potentials produced by stimulation of the NRM are negative in the dorsal regions of the spinal cord and positive in more ventral regions, suggesting that the current sink generated by interneuronal activation is in the superficial layers. In contrast, stimulation of the RF is associated with currents flowing from a dorsal source to a ventral sink, suggesting activation of a population of ventrally located neurones (many of them motoneurones). It may be concluded, therefore, that low threshold fibres from the NRM excite a population of spinal interneurones that are mainly located in the dorsal horn, whereas stimulation of the RF activates a different population of spinal neurones, more ventrally located. This may explain why an electrode placed in the cord dorsum records a positive response after stimulation of the RF and a negative response following stimulation of the NRM.

The above considerations are based on the assumption that the fastest RF and NRM fibres have direct excitatory connections with spinal neurones. This seems to be the case for a significant fraction of RF fibres the activation of which produces monosynaptic EPSPs in spinal motoneurones (Peterson, 1984). However, this is not so clear with the fastest NRM fibres. There is practically no information on the kind of synaptic contacts they made with spinal neurones and detailed analysis of the central latency of the NRM actions is not available. Nevertheless, anatomical data indicate that NRM fibres end mostly in the dorsal horn (laminae I, II and V; see Basbaum, Clanton & Fields, 1978; Kuypers, 1981), while RF fibres end mostly in laminae VII, VIII and IX (Basbaum, *et al.* 1978; Peterson 1984). This means that, whatever synaptic actions they may have on other neurones, their first synaptic contacts must be established in that region.

Effects of stimulation of the NRM and RF on PAD of single muscle afferent fibres

The main purpose of the present investigation was to determine the effects produced by stimulation of the NRM on the PAD of single group Ia and Ib muscle afferents. One of the limitations of the experimental approach used in this and in related studies is that the origin of the afferents the intraspinal threshold changes of which are being measured cannot be established with certainty (see Rudomin *et al.* 1983, 1986; Riddell *et al.* 1992). An alternative approach would have been to record PAD intracellularly from fibres left in continuity with the muscle receptors as it was done in a previous study. In such a study Jiménez *et al.* (1988) classified group I muscle afferents as being from muscle spindles or from tendon organs because of their responses to muscle contraction, and it was concluded that most muscle spindle afferents show no PAD following stimulation of cutaneous nerves. Studies on the PAD patterns of functionally identified muscle afferents have now been expanded by using more rigorous tests for receptor characterization, namely ramp stretches and vibration (Enríquez, Hernández, Jiménez & Rudomin 1991; M. Enríquez, I. Jiménez & P. Rudomin, unpublished observations). Of fifty-two fibres that were clearly identified as from muscle spindle primaries, 80% had a type A PAD pattern, 10% a type B PAD pattern and 10% a type C PAD pattern. Of fourteen fibres that were classified as from tendon organs 35% had a type B and 58% a type C PAD pattern, only one fibre had a type A pattern.

The data obtained in the present investigation show that conditioning stimulation of the SU nerve had practically no effect on the intraspinal threshold of 81% of the fibres with a type A PAD pattern and produced PAD in 8% of the fibres. Stimulation of the NRM (with strengths 25–100 μA) was ineffective in 73% of the fibres and produced PAD in 14% of the fibres, while stimulation of the RF with similar strengths was ineffective in the 82% of the fibres and produced PAD in 9%. It thus seems reasonable to conclude that the majority of group I fibres with type A PAD pattern were from muscle spindles. There were, however, a small number of fibres in which stimulation of cutaneous nerves produced a relatively small PAD but which were nevertheless classified as type A, because stimulation of cutaneous nerves and of the RF inhibited the PBSt-induced PAD. These fibres could have been either from muscle spindles or from tendon organs. Most probably the majority of fibres classified with a type B or type C

PAD pattern were from tendon organs, but we cannot exclude the possibility that a fraction of those fibres in which stimulation of the RF produced very little PAD were from muscle spindles, even though RF and NRM stimulation facilitated the PBSt-induced PAD.

In view of the above uncertainties in the classification of the afferent fibres either as from muscle spindles or tendon organs, it is important to stress the finding that the effects produced by stimulation of the NRM were always in the *same direction* as those evoked by stimulation of the RF *independently* of the type of PAD pattern of the target afferent fibre (type A, B or C). This was observed even when the NRM was stimulated with relatively low strengths (25–50 μA), where the possibilities of a significant coactivation of reticulospinal fibres were minimal (Figs 7 and 8). It thus seems reasonable to conclude that, even though raphespinal and reticulospinal fibres activate different sets of spinal interneurones, these interneurones converge on the same pathways that mediate the PAD of group I muscle afferents, which are different for Ia and Ib fibres (Rudomin *et al.* 1983).

It is possible that fibres with type B and C PAD patterns, with peripheral thresholds above $2T$ and conduction velocities below 60 m s^{-1}, were not group I but rather group II (Riddell *et al.* 1992). This group of fibres showed PAD following stimulation of the NRM and of the RF, in agreement with the recent observations of Riddell *et al.* (1992) made on group II fibres at midlumbar levels. However, in contrast with what has been reported by these investigators, stimulation of cutaneous nerves produced very little PAD in these fibres, but was able to inhibit the PAD elicited by PBSt stimulation. This agrees well with a previous report (see Jiménez *et al.* 1988) and suggests that the PAD patterns of group II fibres ending at the L6–L7 segment are probably different from those fibres ending at midlumbar or sacral segments (Riddell, Jankowska & Huber, 1993), but this may require studies made on functionally identified fibres as was done for group I fibres (see Jiménez *et al.* 1988).

Sites of action of NRM and RF fibres on the segmental pathways mediating PAD

Under appropriate conditions, microstimulation within the intermediate nucleus may directly activate the last-order interneurones in the pathway mediating PAD (Jankowska *et al.* 1981; Rudomin *et al.* 1983, 1986; Quevedo *et al.* 1992). We have shown in this study that conditioning stimulation of the bulbar RF formation may inhibit the monosynaptic PAD of Ia GS fibres that is evoked by intraspinal microstimulation (Fig. 10*C*; see Jankowska *et al.* 1981 and Rudomin *et al.* 1983 for a discussion on the monosynaptic origin of this PAD). However, stimulation of the NRM and of cutaneous afferents was unable to inhibit the monosynaptic PAD, but very effectively inhibited the PAD produced by PBSt stimulation.

Assuming that the shortest pathway producing PAD of Ia fibres has at least two interposed interneurones (Jankowska *et al.* 1981; Rudomin *et al.* 1983) and that the PAD produced by intraspinal microstimulation elicited at short time intervals before the test stimulus (1·5–3·0 ms) is basically monosynaptic, the simplest explanation is that RF stimulation inhibits the last-order interneurones (i.e. those making axo-axonic GABAergic synapses with the afferent fibres) and that stimulation of cutaneous nerves and/or the NRM inhibits other (first-order) interneurones in the same pathway. It must be pointed out, however, that with the method employed in this study it is not possible to determine if, in addition to the inhibition produced by reticulospinal fibres on the last-order interneurones, there is also an inhibitory action exerted on previous-order interneurones (see Fig. 11 of Rudomin *et al.* 1986). In the case of Ib fibres it is not possible with the available data to decide whether RF and NRM fibres act on the first- or on the second-order interneurones mediating the PAD (see also Rudomin *et al.* 1986).

The action of NRM on PAD of group I fibres appears not to be mediated by serotonergic pathways

The NRM is one of the major serotonin-containing nuclei in the brainstem with neurones that project to the spinal cord (Anderson 1984; for references see Noga, Bras & Jankowska, 1992). Anatomical and physiological evidence supports the existence of a direct serotonergic input to spinal interneurones and motoneurones with predominantly inhibitory influences (Willis, 1984; however, see Fung & Barnes, 1989). There is also some evidence supporting the involvement of serotonin in the generation of the late DRPs evoked by bulbospinal stimulation (Proudfit & Anderson, 1974).

Since serotonergic pathways can be activated following stimulation of the NRM, it is not unreasonable to ask whether or not the effects produced by stimulation of the NRM on Ia and Ib fibres described here are due to activation of serotonergic pathways. Curtis, Leah & Peet (1983) have shown that iontophoretic application of noradrenaline and serotonin does not change the intraspinal threshold of group I fibres, and Bras, Jankowska, Noga & Skoog (1990) have reported that the postsynaptic field potentials elicited by group I fibres are not modified following application of monoaminergic agonists or antagonists. This would probably exclude a direct action of serotonergic descending fibres on group I intraspinal terminals following stimulation of the NRM.

In addition, Noga *et al.* (1992) have recently reported that stimulation of the NRM depresses the postsynaptic field potentials evoked by stimulation of group I muscle afferents at relatively short conditioning–testing stimulus time intervals (between 10 and 70 ms), suggesting involvement of raphespinal fibres with conduction velocities in the range of 8 to 20 m s^{-1}. According to Wessendorf *et al.* (1981), these fibres are predominantly non-serotonergic. In this study, conditioning stimulation of the NRM was applied

65–75 ms before the excitability testing pulse and it seems unlikely that at these time intervals the serotonergic actions are fully established (Noga *et al.* 1992). Nevertheless, further work seems necessary to rule out the contribution of serotonergic fibres. It is also likely that NRM actions on group I fibres involve activation of a separate system than that involved in the production of PAD of group II fibres, in agreement with the recent proposal made by Riddell *et al.* (1992).

The system of fast-conducting fibres from the raphe nucleus appears to have similar actions to the fibres from the reticular formation on the PAD of Ia and Ib fibres. However, the finding that fibres from the NRM and from the RF may inhibit the PAD of Ia fibres by acting on different interneurones further supports the notion that these two descending systems are nevertheless able to play independent roles in the control of the synaptic efficacy of group I muscle afferents. The inhibition exerted by the NRM on the first-order interneurones in the Ia PAD pathway would be counteracted by group I and vestibulospinal excitatory inputs converging on these same interneurones (Rudomin *et al.* 1983) without affecting the inhibition exerted on the second-order neurones by reticulospinal inputs. At the present time, the functional role of this separate presynaptic control is difficult to envisage because there is very little information on the connectivity of the fast raphespinal fibres with other spinal interneurones, besides those mediating the PAD of Ia fibres (the connections with pathways mediating PAD of Ib fibres remain unsettled as discussed above). Also, there is very little information on the inputs received by the fast raphespinal neurones (however, see Yates, Goto & Bolton, 1992) and on how these are activated during motor activity.

ANDERSON, E. G. (1984). The serotonin system of the spinal cord. In *Handbook of the Spinal Cord*, vol. I, *Pharmacology*, ed. DAVIDOFF, R. A., pp. 241–274. Dekker, New York.

BASBAUM, A. I., CLANTON, C. H. & FIELDS, H. L. (1978). Three bulbospinal pathways from the rostral medulla of the cat: an autoradiographic study of pain modulating systems. *Journal of Comparative Neurology* **178**, 209–224.

BELCHER, G., RYALL, R. W. & SCHAFFNER, R. (1978). The differential effects of 5-hydroxy-tryptamine, noradrenaline and raphe stimulation on nociceptive and non-nociceptive dorsal horn interneurones in the cat. *Brain Research* **151**, 307–321.

BRAS, H., JANKOWSKA, E., NOGA, B. R. & SKOOG, B. (1990). Comparison of effects of various types of NA and 5-HT agonists on transmission from group II muscle afferents in the cat spinal cord. *Journal of Neuroscience* **2**, 1029–1039.

CURTIS, D. R. (1979). A method for continuously monitoring the electrical threshold of single intraspinal fibres. *Electroencephalography and Clinical Neurophysiology* **47**, 503–506.

CURTIS, D. R., LEAH, J. D. & PEET, M. J. (1983). Effects of noradrenaline and 5-hydroxy-tryptamine on spinal Ia afferent terminations. *Brain Research* **258**, 328–332.

ECCLES, J. C., MAGNI, F. & WILLIS, W. D. (1962). Depolarization of central terminals of group I afferent fibres from muscle. *Journal of Physiology* **160**, 62–93.

ECCLES, J. C., SCHMIDT, R. F. & WILLIS, W. D. (1963). Depolarization of central terminals of group Ib afferent fibres of muscle. *Journal of Neurophysiology* **26**, 1–27.

ENRÍQUEZ, M., HERNÁNDEZ, O., JIMÉNEZ, I. & RUDOMIN, P. (1991). Is the PAD evoked in Ia fibres related to their responses to stretch? *Society for Neuroscience Abstracts* 17, 1024.

FLOETER, M. K., SHOLOMENKO, G. N., GOSSARD, J.-P. & BURKE, R. E. (1993). Disynaptic excitation from the medial longitudinal fasciculus to lumbosacral motoneurones: modulation by repetitive activation, descending pathways, and locomotion. *Experimental Brain Research* **92**, 407–419.

FUNG, S. J. & BARNES, CH. D. (1989). Raphe-produced excitation of spinal cord motoneurons in the cat. *Neuroscience Letters* **103**, 185–190.

GEBHART, G. F. & RANDICH, A. (1990). Brainstem modulation of nociception. In *Brainstem Mechanisms of Behavior*, ed. KLEMM, W. R. & VERTES, R. P., pp. 315–352. Wiley, New York.

HAMMOND, D. L. & YAKSH, T. L. (1984). Antagonism of stimulation-produced antinociception by intrathecal administration of methysergide or phentolamine. *Brain Research* **298**, 329–337.

JANKOWSKA, E., LUND, S., LUNDBERG, A. & POMPEIANO, O. (1968). Inhibitory effects evoked through ventral reticulospinal pathways. *Archives Italiennes de Biologie* **106**, 124–140.

JANKOWSKA, E., MCCREA, D., RUDOMIN, P. & SYKOVA, E. (1981). Observations on neuronal pathways subserving primary afferent depolarization. *Journal of Neurophysiology* **46**, 506–516.

JANKOWSKA, E. & ROBERTS, W. R. (1972). An electrophysiological demonstration of the axonal projections of single spinal interneurones in the cat. *Journal of Physiology* **222**, 597–622.

JIMÉNEZ, I., QUEVEDO, J. N., EGUIBAR, J. R. & RUDOMIN, P. (1989). Effects of selective stimulation of nucleus raphe magnus and adjacent reticular formation on the intraspinal excitability of Ia and Ib muscle afferents. *Society for Neuroscience Abstracts* **15**, 921.

JIMÉNEZ, I., RUDOMIN, P. & SOLODKIN, M. (1987). Mechanisms involved in the depolarization of cutaneous afferents produced by segmental and descending inputs in the cat spinal cord. *Experimental Brain Research* **69**, 195–207.

JIMÉNEZ, I., RUDOMIN, P. & SOLODKIN, M. (1988). PAD patterns of physiologically identified afferent fibres from the medial gastrocnemius muscle. *Experimental Brain Research* **71**, 643–657.

KUYPERS, H. G. J. M. (1981). Anatomy of the descending pathways. In *Handbook of Physiology*, vol. II, *Motor Control*, part I, ed. BROOKS V. B., pp. 597–666. American Physiological Society, Bethesda, MD, USA.

LINDBLOM, U. F. & OTTOSSON, J. O. (1957). Influence of pyramidal stimulation upon the relay of coarse cutaneous afferents in the dorsal horn. *Acta Physiologica Scandinavica* **38**, 309–318.

LUNDBERG, A. (1964). Supraspinal control of transmission in reflex paths to motoneurones and primary afferents. In *Progress in Brain Research*, vol. 12, *Physiology of Spinal Neurons*, ed. ECCLES, J. C. & SCHADÉ, J. P., pp. 197–221. Elsevier, Amsterdam.

LUNDBERG, A. & VYKLICKY, L. (1966). Inhibition of transmission to primary afferents by electrical stimulation of the brainstem. *Archives Italiennes de Biologie* **104**, 86–96.

McCreery, D. B. & Bloedel, J. R. (1975). Reduction of the response of cat spinothalamic neurons to graded mechanical stimuli by electrical stimulation of the lower brainstem. *Brain Research* **97**, 151–156.

Madrid, J., Alvarado, J., Dutton, H. & Rudomin, P. (1979). A method for the dynamic continuous stimation of excitability changes of single fibre terminals in the central nervous system. *Neuroscience Letters* **11**, 253–258.

Martin, R. F., Haber, L. H. & Willis, W. D. (1979). Primary afferent depolarization of identified cutaneous fibres following stimulation in medial brainstem. *Journal of Neurophysiology* **42**, 779–790.

Mokha, S. S., McMillan, J. A. & Iggo, A. (1985). Descending control of spinal nociceptive transmission. Actions produced on spinal multireceptive neurons from the nuclei locus coeruleus (LC) and raphe magnus (NRM). *Experimental Brain Research* **58**, 213–226.

Mokha, S. S., McMillan, J. A. & Iggo, A. (1986). Pathways mediating descending control of spinal nociceptive transmission from the nuclei locus coeruleus (LC) and raphe magnus (NRM) in the cat. *Experimental Brain Research* **61**, 597–606.

Nicholas, A. P. & Hancock, M. B. (1990). Evidence for projections from the rostral medullary raphe onto medullary catecholamine neurons in the rat. *Neuroscience Letters* **108**, 22–28.

Noga, B. R., Bras, H. & Jankowska, E. (1992). Transmission from group II muscle afferents is depressed by stimulation of locus coeruleus/subcoeruleus, Kölliker–Fuse and raphe nuclei in the cat. *Experimental Brain Research* **88**, 502–516.

Peterson, B. W. (1984). The reticulospinal system and its role in the control of movement. In *Brain Stem Control of Spinal Cord Function*, ed. Barnes, C. D., pp. 141–214. Academic Press, Orlando, FL, USA.

Proudfit, H. K. & Anderson, E. G. (1974). New long latency bulbospinal evoked potential blocked by serotonin antagonists. *Brain Research* **65**, 542–546.

Quevedo, J., Eguibar, J. R., Jiménez, I. & Rudomin, P. (1992). Differential action of (−)-baclofen on the primary afferent depolarization produced by segmental and descending inputs. *Experimental Brain Research* **91**, 29–45.

Riddell, J. S., Jankowska, E. & Eide, E. (1992). Depolarization of group II muscle afferents by stimuli applied in the locus coeruleus and raphe nuclei of the cat. *Journal of Physiology* **461**, 723–741.

Riddell, J. S., Jankowska, E. & Huber, J. (1993). Organization of neuronal systems mediating depolarization of group II muscle afferents in the spinal cord of the anaesthetized cat. *Journal of Physiology* **459**, 499*P*.

Rudomin, P. (1991). Presynaptic inhibition of muscle spindle and tendon organ afferents in mammalian spinal cord. *Trends in Neurosciences* **13**, 499–505.

Rudomin, P., Jiménez, I., Solodkin, M. & Dueñas, S. (1983). Sites of action of segmental and descending control of transmission on pathways mediating PAD of Ia- and Ib-afferent fibres in cat spinal cord. *Journal of Neurophysiology* **50**, 743–769.

Rudomin, P., Solodkin, M. & Jiménez, I. (1986). PAD and PAH response patterns of group Ia- and Ib-fibres to cutaneous and descending inputs in the cat spinal cord. *Journal of Neurophysiology* **56**, 987–1006.

Takakusaki, K., Ohta, Y. & Mori, S. (1989). Single medullary reticulospinal neurons exert postsynaptic inhibitory effects via inhibitory interneurons upon alpha-motoneurons innervating cat hindlimb muscles. *Experimental Brain Research* **74**, 11–23.

Wessendorf, M. W., Proudfit, H. K. & Anderson, E. G. (1981). The identification of serotonergic neurons in the nucleus raphe magnus by conduction velocity. *Brain Research* **214**, 168–173.

West, D. C. & Wolstencroft, J. H. (1977). Location and conduction velocity of raphespinal neurones in nucleus raphe magnus and raphe pallidus in the cat. *Neuroscience Letters* **5**, 147–151.

Willis, W. D. (1984). The raphe–spinal system. In *Brain Stem Control of Spinal Cord Function*, ed. Barnes, C. D., pp. 141–214. Academic Press, Orlando, FL, USA.

Yates, B. J., Goto, T. & Bolton, P. S. (1992). Responses of neurons in the caudal medullary raphe nuclei of the cat to stimulation of the vestibular nerve. *Experimental Brain Research* **89**, 323–332.

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