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DEPOLARIZATION OF GROUP II MUSCLE AFFERENTS BY STIMULI APPLIED IN THE LOCUS COERULEUS AND RAPHE NUCLEI OF THE CAT

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SUMMARY

1. Electrical stimuli applied in the locus coeruleus/subcoeruleus (LC/SC) and raphe nuclei produce a profound depression of transmission in reflex pathways from group II muscle afferents. The present experiments were performed to determine whether presynaptic inhibitory mechanisms contribute to these effects.

2. Changes in the excitability of afferent terminals to electrical stimuli have been used as an indication of primary afferent depolarization (PAD) produced by conditioning stimuli applied within the LC/SC and raphe nuclei and, for comparison, in the nucleus ruber. Group II afferents originating from ankle flexor muscles and terminating in the midlumbar segments were used for testing.

3. Clear changes in excitability were observed in fourteen of nineteen group II fibres in which the effects of conditioning stimuli applied in the LC/SC were tested and in twelve of seventeen fibres in which the effects of stimuli applied within the raphe nuclei were tested. By comparison, only one of the twelve fibres tested with conditioning stimuli applied to the nucleus ruber was found to be influenced. These effects matched those of the same conditioning stimuli on field potentials evoked by group II afferents at the location at which the terminals of group II fibres were stimulated.

4. Stimuli applied in the LC/SC and in the raphe nuclei both produced a mean decrease in threshold stimulus current of 19%. These effects are comparable to those produced by the most effective volleys in peripheral afferents which, in the same fibres, produced a mean decrease in threshold stimulus current of 24%.

5. In all cases (twelve) in which the effects of stimuli applied in the LC/SC and raphe nuclei were tested on the same group II fibre, either both or neither were found to be effective. This strengthens previous indications that some populations of neurones might be activated by stimuli applied in each of these regions of the brain.

6. In contrast to group II afferents, group Ia afferents investigated in the same experiments were only exceptionally affected. Of seven fibres tested with stimuli applied in the LC/SC, six with stimuli applied in the raphe nuclei and seven with stimuli applied in the nucleus ruber, only one fibre showed any clear change in

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threshold and this was a single fibre which was similarly affected by stimuli in all three sites.

7. It is concluded that presynaptic inhibitory mechanisms contribute to the depression of transmission in spinal reflex pathways from group II muscle afferents produced by stimulation in the LC/SC and raphe nuclei.

INTRODUCTION

Neuronal systems activated by electrical stimuli applied in the locus coeruleus/subcoeruleus (LC/SC) and raphe nuclei induce a profound depression of transmission in group II reflex pathways (Noga, Bras & Jankowska, 1992; Jankowska, Riddell, Skoog & Noga, 1993). This depression is exerted on both the excitatory and inhibitory reflex actions of group II afferents and actions originating from group II afferents in a variety of hindlimb muscle nerves are depressed with similar effectiveness. Furthermore, it has been demonstrated that, when appropriately timed, the same conditioning stimuli that depress transmission from group II afferents have only a negligible effect on transmission from group I muscle afferents and that the inhibitory control of group II reflex actions is exerted at a premotoneuronal level. There is evidence that monoaminergic neurones may be involved in this selective control of group II pathways (Bras, Jankowska, Noga & Skoog, 1990; Skoog & Noga, 1991) but since electrical stimulation within brain structures may result in co-activation of a number of descending systems, the identity of those involved remains to be resolved.

A variety of descending systems are known to influence the excitability of the terminals of group Ia and group Ib muscle afferent fibres (see Rudomin, 1990 for review), while in contrast only the action of the corticospinal tract on the presynaptic control of transmission from group II muscle afferents has so far been investigated (Carpenter, Lundberg & Norrsell, 1963). The aim of this study was therefore to determine whether stimulation in the LC/SC and/or raphe nuclei leads to the activation of neuronal systems involved in the presynaptic control of transmission from group II afferent fibres and could thereby contribute to the depression of group II reflex actions evoked by such stimuli. This possibility has been investigated by using a refinement of the technique in which changes in the excitability of the terminals of sensory fibres are used as an indication of primary afferent depolarization (Wall, 1958). In the present experiments changes in the intensity of threshold intraspinal stimuli evoking action potentials in the terminals of single group II muscle afferent fibres were used as a measure of their excitability (see Madrid, Alvarado, Dutton & Rudomin, 1977; Curtis, 1977) and the effects of conditioning stimuli applied in the LC/SC and raphe nuclei investigated.

METHODS

Preparation

The experiments were performed on five cats deeply anaesthetized with chloralose. Details of anaesthesia and other general procedures for preparing and maintaining the animal in a good physiological condition were as described in the companion paper (Jankowska *et al.* 1992). The spinal cord was exposed between the second lumbar and sacral segments and at the level of the

Th13 segment. Craniotomies were performed over the parietal lobe and over the middle part of the cerebellum.

Recording and stimulation procedures

A diagram of the experimental arrangement showing recording and stimulating locations is shown in Fig. 1. Recordings were made from single fibres of the nerves to the ankle flexors tibialis anterior and extensor digitorum longus. The nerves were mounted on a common stimulating

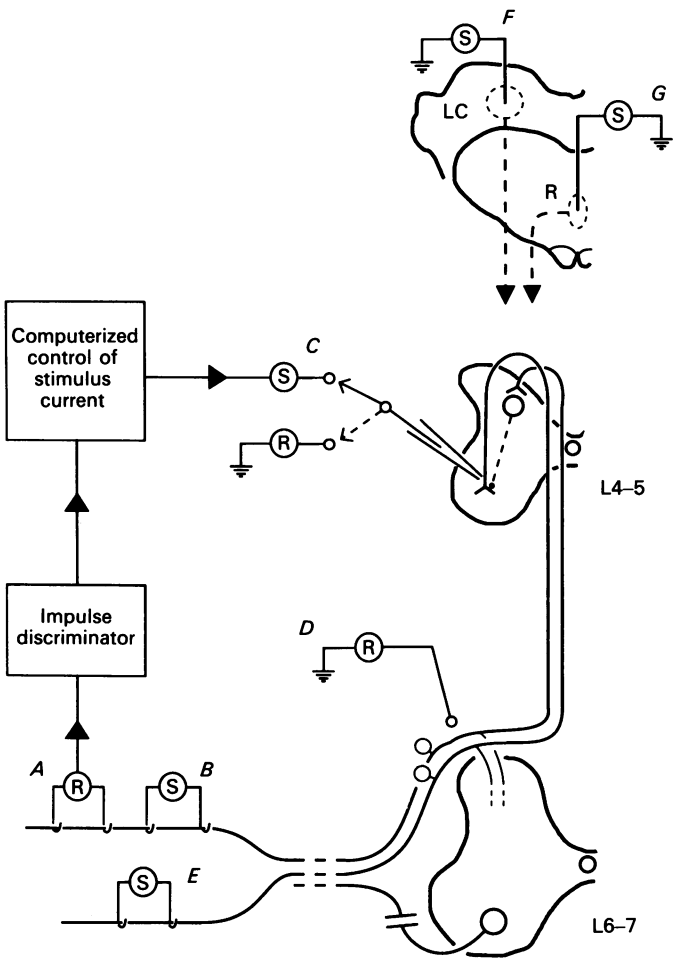

Fig. 1. Diagram of the experimental arrangement in which the sites of stimulating (S) and recording (R) electrodes are indicated. *A* and *B*, electrodes for recording and stimulation, respectively, of fibres in a natural filament of the deep peroneal nerve. *C*, microelectrode for recording of field potentials and stimulation of afferent fibre terminals. *D*, recording electrode on cord dorsum. *E*, stimulating electrode on peripheral nerves for producing conditioning volleys. *F* and *G*, stimulating electrodes in the locus coeruleus (LC) and raphe nuclei (R) respectively.

electrode proximally while naturally occurring filaments, separated as far distally as possible, were placed on individual recording electrodes. The L7 and S1 ventral roots were cut in order to prevent reflex discharges in motor axons from contaminating recordings of responses in sensory fibres. To

determine the region of the spinal cord within which the application of electrical stimuli would be most likely to excite the terminals of group II afferents of the deep peronei, field potentials (see Fig. 3*C*) evoked by electrical stimulation of the nerve were first located by recording with glass microelectrodes filled with 2 M NaCl (1·5–2·5 μm tip diameter, 1–3 MΩ resistance). The same electrode was then used to apply cathodal stimulus pulses (0·3 ms duration, 1–8 μA) within the spinal cord while monitoring the antidromic discharges in afferent fibres of the deep peroneal nerve recorded in the periphery. The latencies of antidromically conducted impulses in single fibres were measured and peripheral thresholds determined from the strength of stimuli required to evoke an orthodromic volley in the deep peroneal nerve that resulted in collision with the antidromically conducted impulses in the fibre. The stimulus strengths are expressed relative to the threshold (*T*) for the most excitable afferents in the nerve as detected by direct recording from the individual nerve branches.

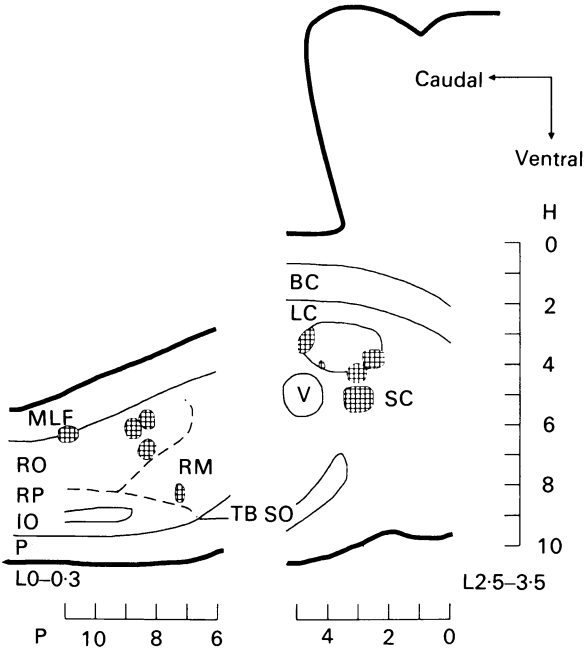

Fig. 2. Locations of stimulation sites in and around the locus coeruleus (right) and in the raphe nuclei (left). The shaded areas show the location of lesions made at the end of the experiments and are indicated on schematic outlines of sagittal sections through the medulla and pons. The lesions were located 0–0·3 and 2·5–3·5 mm lateral to the mid-line, respectively. The scales indicate antero-posterior (P) and horizontal (H) Horsley-Clarke co-ordinates. LC, locus coeruleus; SC, sub-coeruleus; BC, brachium conjunctivum; V, motor nucleus of the trigeminal nerve; SO, superior olive; MLF, medial longitudinal fascicle; IO, inferior olive; TB, trapezoid body; P, pyramid; RM, raphe magnus; RO, raphe obscurus; RP, raphe pallidus.

Conditioning stimuli

Conditioning stimuli were applied within the ipsilateral locus coeruleus/subcoeruleus and in the raphe obscurus or magnus nuclei in all five experiments and in the contralateral nucleus ruber in three experiments. Full details of the method of placement of stimulating electrodes are given in the companion paper (Jankowska *et al.* 1993). The location of all brain stimulation sites was verified histologically by relating the positions of electrolytic lesions made at the end of the experiment to clearly identifiable stained structures and are shown in Fig. 2. Conditioning stimuli consisted of a train of six to eight shocks, 333 Hz (100 μA or less, 200 μs) and normally preceded the test stimuli by intervals of around 90–100 ms.

Conditioning stimuli were also applied to a variety of hindlimb peripheral nerves in order to verify that changes in excitability could be reliably induced in those fibres in which brainstem conditioning had no effect and in order to compare the changes produced by volleys in peripheral nerves with those produced by descending volleys where these were effective. The standard conditioning volleys in peripheral nerves were evoked by a train of five stimuli (200 Hz, 100 μs) up to five times threshold (T) for the most excitable fibres in the nerve. The following hindlimb nerves were mounted for stimulation: quadriceps, sartorius, posterior biceps–semitendinosus, anterior biceps–semimembranosus, deep peronei (comprising nerve branches to tibialis anterior and extensor digitorum longus muscles), gastrocnemius–soleus, plantaris, flexor digitorum longus (including the branch to the interosseous membrane), caudal branch of sural, superficial peroneal, and the posterior nerve to the knee joint.

Automatic control of stimulus current

The intensity of the intraspinal stimulus was under automatic computer control such that its amplitude was continuously adjusted and maintained at threshold for discharging the fibre. Antidromic discharges in the fibre, recorded in the periphery, were filtered and gated using a discriminator with adjustable time and voltage windows. The output from the discriminator was fed to a computer which produced a step increase or decrease in stimulus strength according to the presence or absence of a pulse. Thus, stimuli discharging the fibre were followed by a step decrease in current, while stimuli too weak to excite the fibre were followed by a step increase in current. By this method a firing index of 50% was maintained with small fluctuations of stimulus current around the threshold level. Changes in excitability, evoked by conditioning stimuli, were reflected in a series of adjustments to a new threshold level. The resulting change in threshold stimulus strength (expressed as a percentage of the initial strength) was used as a measure of the primary afferent depolarization (PAD) evoked. The amplitude of the current steps could be varied but values of 0·1 μA or less generally produced the clearest records while still providing sufficiently rapid adjustment to new levels of excitability. The stimulating current was measured and displayed on the computer screen such that the values accumulated from successive stimulus tests formed a histogram of the stimulus current with time. A stimulation rate of 2 Hz was generally employed. Simultaneous records of the presence or absence of a discharge in the fibre, the voltage drop across the electrode, blood pressure and a marker indicating the duration of application of conditioning stimuli were displayed. When conditioning stimuli produced blood pressure changes, to verify that changes in fibre threshold were not the result of circulatory alterations, tests were repeated using conditioning–test intervals too short (< 30 ms) to induce changes in excitability (as determined in experiments without obvious circulatory changes).

RESULTS

The sample of afferent fibres

Since there is significant overlap in the peripheral thresholds of group I and group II fibres of muscle nerves, including those of the deep peroneal (DP) nerve (see Jack, 1978; see also MacLennan, 1971, for afferent fibres innervating tibialis anterior), and the division between the conduction velocities of group I and group II fibres is highly arbitrary (see Jankowska, 1992), no single criterion differentiates muscle spindle secondaries from primaries or tendon organ afferents. The present sample of group II fibres was therefore selected on the basis of both their peripheral thresholds ($\geqslant 2\ T$) and conduction time between the intraspinal stimulation site and the peripheral nerve recording electrode. Taking into account the slowing of conduction velocity of hind limb muscle afferents ascending in the dorsal columns to the L4/L5 level (Fern, Harrison & Riddell, 1988), impulses evoked with latencies of 3·7 ms or more were considered to originate in group II fibres (see Edgley & Jankowska, 1987*a*; Harrison & Jankowska, 1989).

A smaller sample of group Ia fibres were also investigated in order to provide a direct comparison of the effects of stimulation in the LC/SC and raphe nuclei on the excitability of group II fibres with that of Ia fibres in the same nerve. Since the earliest impulses encountered in the DP nerve were of 2·5 ms latency, impulses

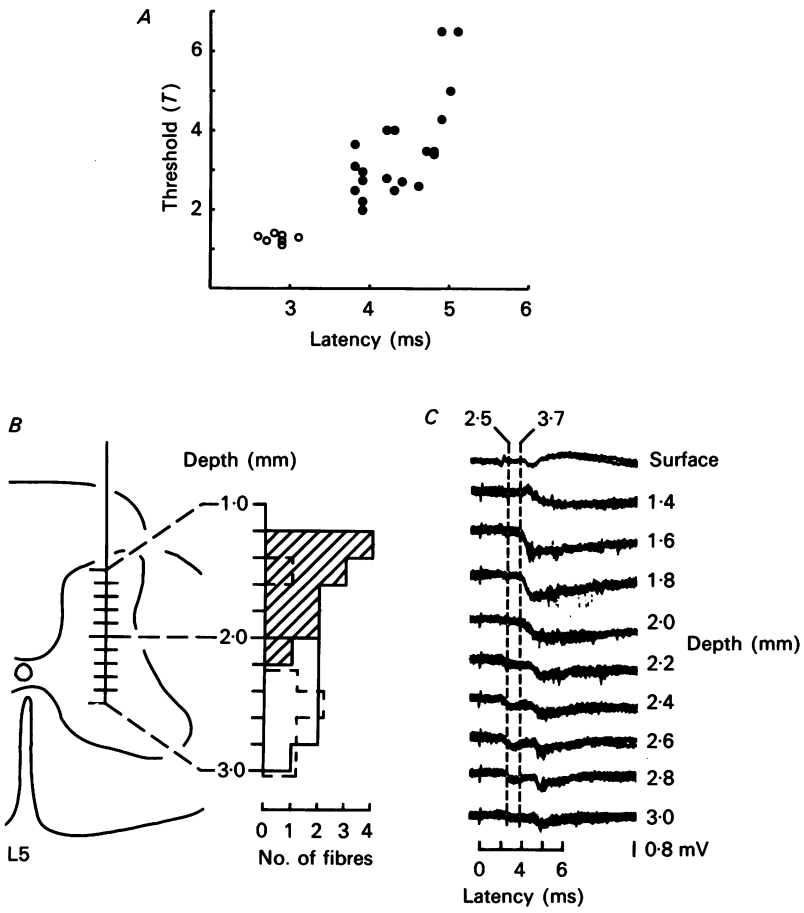

Fig. 3. *A*, plots of peripheral threshold *versus* latency of activation by intraspinal stimuli for twenty-six afferent fibres in the deep peroneal nerve. The filled circles represent those fibres ($n = 19$) classified as group II. A smaller sample ($n = 7$) of group Ia fibres studied for comparison are represented by the open circles. *B*, location of stimulation of afferent fibres. The locations in the grey matter corresponding to different depths in a typical electrode track are indicated on an outline of the spinal cord at the mid-L5 level. The adjacent histogram indicates the distribution of depths at which afferent fibres were antidromically activated. The shaded area represents group II fibres activated in the dorsal horn and the open area group II fibres activated in the intermediate zone. The area enclosed by the dashed line represents the sample of group Ia afferents. *C*, field potential recordings, at the depths indicated, in a typical electrode track through the grey matter. Dashed lines indicate the conduction time for the fastest conducting group Ia fibres (2·5 ms) and group II fibres (3·7 ms) included in the sample.

evoked with a delay of 2·9 ms or less and activated by stimuli 1·4 times threshold or less were considered to originate from group I afferents. In order to differentiate between Ia and Ib fibres, use was made of the established actions of conditioning volleys in cutaneous afferents on functionally identified muscle spindle Ia and tendon organ Ib afferents (see e.g. Jimenez, Rudomin & Solodkin, 1988). Since such volleys increase the excitability of some Ib but not Ia afferents, fibres influenced by conditioning stimuli applied to the sural or superficial peroneal nerve were excluded from the sample. In addition only fibres that were demonstrated to be in a good functional state, that is those fibres in which changes in excitability could be produced by conditioning volleys in hindlimb muscle and/or cutaneous nerves, were tested for the effects of stimulation in supraspinal sites.

Figure 3*A* shows a plot of peripheral threshold *versus* latency of activation by intraspinal stimuli for a sample of nineteen group II fibres (●) and seven group Ia fibres (○) selected according to the above criteria.

Sites of intraspinal stimulation of fibres

Following electrical stimulation of the deep peroneal nerve, monosynaptic field potentials evoked by group II fibres can be recorded in the midlumbar segments of the spinal cord in two distinct regions; in the dorsal horn and in the intermediate zone (Edgley & Jankowska, 1987*a*). To improve the chances of encountering group II fibres and of activating them close to their regions of termination, stimuli were applied in those locations in which such potentials were recorded. Examples of such records are shown in Fig. 3*C*, where it can be seen that the latencies of onset of group II field potentials increased with depth, typically ranging from around 3·7 to 4·7 ms and taking a further 0·5 ms or so to peak (see also Table 1 of Edgley & Jankowska, 1987*a*). If allowance is made for synaptic delay and the generation of synaptic current then their latencies match well with the latencies of activation of the sample of group II fibres which, as shown in Fig. 3*A*, were in the range of 3·7 to 5 ms, and could thus contribute to all but the earliest components of the dorsal horn field potentials.

The location within the spinal cord at which the fibres were activated was determined from recordings of field potentials made at the same location. The distribution of the stimulus sites in relation to the topography of the L5 spinal segment is shown in Fig. 3*B*. Twelve of the group II fibres were antidromically activated by stimuli in the dorsal horn (shaded area of histogram) while the remaining seven were activated from the intermediate zone (open area). All but one of the seven group Ia fibres were activated from within the intermediate zone (dashed lines).

Effects of supraspinal stimulation on the excitability of group II fibres

Locus coeruleus/subcoeruleus region

The effects of stimulation in this region were investigated in a total of nineteen group II fibres; reconstructions of the sites of stimulation are shown in Fig. 2. All fibres were tested in the first instance using trains of conditioning stimuli with standardized parameters (see Methods) including a strength of 100 μA and conditioning–test interval of 90–100 ms. Such stimuli when applied within the LC/SC

produced clear reductions in the threshold current required to activate fourteen of the nineteen group II fibres tested. Examples of these effects are illustrated in Figs 4*B* and 5*B*, together with the effects of the same conditioning stimuli on field potentials evoked in the same region of the spinal cord by stimulation of group II

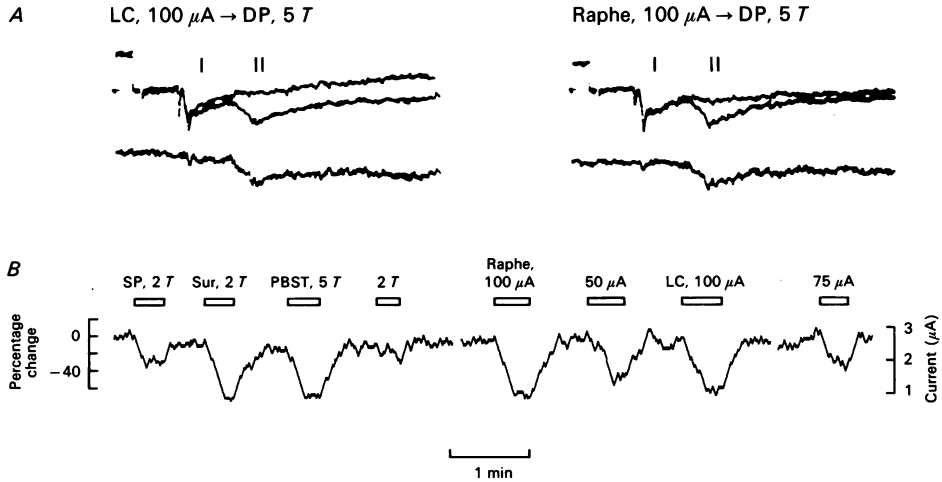

Fig. 4. Effects of stimulation in the LC/SC and raphe nuclei on field potentials evoked by group II afferents in the midlumbar segments and on the excitability of a group II fibre terminating in the same region. *A*, field potentials evoked by stimulation of the DP nerve at five times threshold. The upper records show superimposed control (larger amplitude) and conditioned (smaller amplitude) records (averages of sixteen sweeps each). The lower records show the difference between the two. I and II, components of the potentials evoked by group I and group II afferents, respectively. The records on the left illustrate the effect of conditioning stimuli applied in the LC and those on the right the effect of stimulation in the raphe nuclei. The conditioning–test interval was 80 ms. Calibration pulses are 200 μV and 1 ms. *B*, records of the strength of stimulus current just threshold for evoking an antidromic discharge in the terminal of a group II muscle afferent (peripheral threshold 3·65 *T*, conduction time 3·8 ms) of the DP nerve. The bars indicate the time period over which conditioning stimuli were applied to a peripheral nerve (conditioning–test interval 35 ms) or within a supraspinal structure (conditioning–test interval 95 ms) at the strengths indicated. SP, superficial peroneal; Sur, sural; PBST, posterior biceps–semitendinosus; LC, locus coeruleus. The records in *A* and *B* are from the same experiment.

afferents in the periphery (Figs 4*A* and 5*A*). Stimulation in the LC/SC reduced the threshold stimulus current by between 12 and 38% (mean $\pm$ S.D., 19·0 $\pm$ 9·2%).

Stimuli of 100 μA applied through electrodes with exposed tips of around 100 μm can be expected to activate neuronal elements some 0·5–1·0 mm distant, depending upon the excitability of the neuronal elements within the region of stimulation (cf. Roberts & Smith, 1973; Gustafsson & Jankowska, 1976). Two procedures were adopted in an attempt to assess the degree of localization of the neuronal elements responsible for the observed effects on fibre excitability. The first of these was to vary the strength of conditioning stimuli up to a maximum of 100 μA. In the six fibres in which this was examined, stimuli of 100 μA were in all cases the most effective.

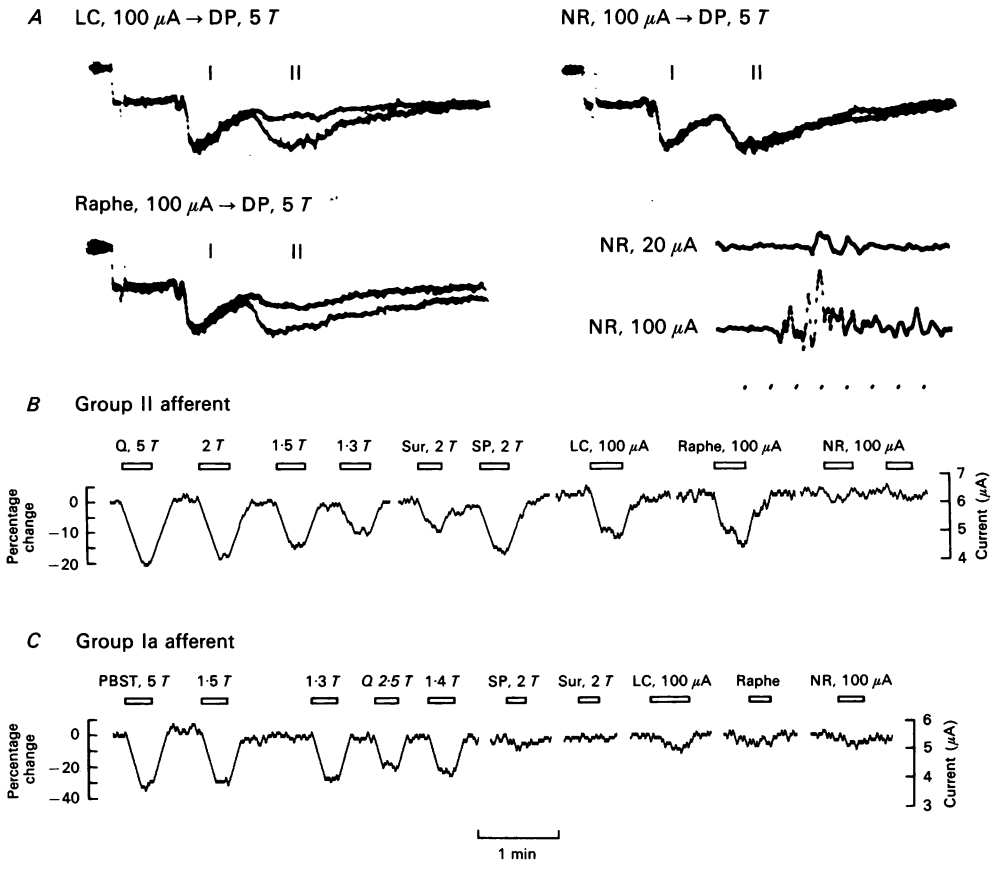

Fig. 5. Comparison of the effects of stimulation in the LC/SC and raphe nuclei with that of stimulation in the contralateral nucleus ruber. The records illustrate the effects on field potentials recorded in the midlumbar segments and on the excitability of a group II and a group Ia afferent fibre terminating in the same region. *A*, field potentials evoked by stimulation of the DP nerve at five times threshold. The records show superimposed control (larger amplitude) and conditioned (smaller amplitude) records (averages of sixteen sweeps each). I and II, components of the potentials evoked by group I and group II afferents, respectively. Conditioning stimuli were applied in the LC, raphe nuclei and nucleus ruber (NR) as indicated. Conditioning–test interval 80 ms. Records of the volley evoked by stimulation in the nucleus ruber and recorded from the lateral funiculus at the Th13 level are shown in the lower right of panel *A*. Calibration pulses on field potential records are 200 μV and 1 ms and the time calibration for volleys evoked from the nucleus ruber 1 ms. *B*, records of the strength of stimulus current just threshold for evoking an antidromic discharge in the terminal of a group II muscle afferent (peripheral threshold 3·5 *T*, conduction time 4·8 ms) of the extensor digitorum longus nerve. The bars indicate the time period over which conditioning stimuli were applied to a peripheral nerve (conditioning–test interval 35 ms) or within a supraspinal structure (conditioning–test interval 95 ms) at the strengths indicated. *C*, similar records as in *B* but for a group Ia fibre in the same nerve (peripheral threshold 1·35 *T*, conduction time 2·9 ms). Q, quadriceps; Sur, sural; SP, superficial peroneal; PBST, posterior biceps–semitendinosus; LC, locus coeruleus; NR, nucleus ruber. All records are from the same experiment.

Stimuli of 75 μA were also effective in all five cases in which they were tested and stimuli of 50 μA or less were effective in two of four fibres tested. Examples of the effects of conditioning stimuli of different strength are illustrated in Fig. 4*B*.

The second procedure used was to maintain a constant stimulus strength of 100 μA and to make stepped adjustments of the depth of the stimulus location in the vertical

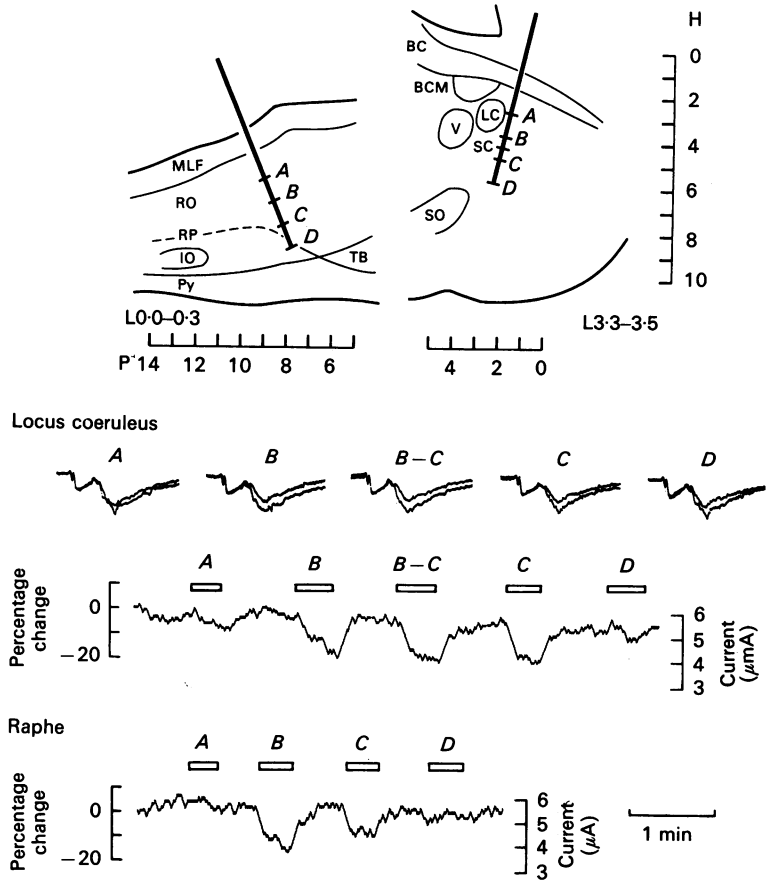

Fig. 6. Effects of changes in the depth of stimulating electrodes passing through the medulla and pons on the depression of field potentials evoked by group II afferents and on the excitability of group II muscle afferents. Top panel, semi-schematic diagrams of sagittal sections through the medulla and pons. The diagram on the right shows the position of an electrode track which passed slightly lateral and rostral to the LC and entered the SC region. The diagram on the left indicates the position of an electrode track passing through the raphe nuclei. *A*, *B*, *C* and *D* indicate the positions where conditioning stimuli were applied. Abbreviations as in Fig. 2, with addition of BCM, marginal nucleus of the brachium conjunctivum. The records beneath illustrate the effects of stimulating at different depths on field potentials evoked by stimulation of the DP nerve at 5 *T* and on the excitability of a group II muscle afferent.

plane. An example of the results obtained in one of the three fibres tested in this way is shown in Fig. 6 from which it can be seen that changes in excitability could be produced from sites separated by up to 1 mm along an electrode track. From

histological reconstructions this track was judged to pass slightly lateral ($\approx$ 0·3 mm) and rostral to the LC and enter the SC region (see Fig. 6, right diagrams). In this example effective actions were produced by stimulation in the SC region but not from more dorsal and ventral sites along the electrode track.

Raphe nuclei

The effects of conditioning stimuli applied within the raphe nuclei (see Fig. 2, for stimulus locations) were investigated in seventeen group II fibres. In twelve of the seventeen fibres tested the conditioning stimuli produced clear increases in terminal excitability as detected by a decrease of the stimulus current required to activate them. Examples of the effects of stimuli applied within the raphe nuclei are illustrated in Figs 4*B* and 5*B*. The threshold stimulus current was reduced by between 8 and 43% (mean $\pm$ S.D., 19·0 $\pm$ 10·5%), that is by a similar extent as following stimuli applied in the LC/SC. All of the fibres in which the effects of conditioning stimuli applied in the raphe nuclei were investigated were also tested for effects produced by stimuli applied in the LC/SC. Remarkably, in all cases either both proved to be effective or else neither were effective. Stimuli in the LC/SC produced greater changes in excitability than did stimuli in the raphe nuclei in six fibres, were similarly effective in two fibres and were less effective in four fibres.

The effects of changes in stimulus strength were investigated in three fibres and of step alterations of stimulus location in two fibres. As for stimulation in the LC/SC, stimuli of 100 μA were most effective but in all three cases stimuli of 50 μA produced smaller but nevertheless clear effects (see Fig. 4*B*). The effects of changes in stimulus site within the raphe nuclei are illustrated in Fig. 6. Here it can be seen that stimulation at sites up to 1 mm apart within the raphe nuclei was effective.

Time course of excitability changes

In three fibres the time course of the effects of stimuli applied in the LC/SC (two fibres) or raphe nuclei (one fibre) was investigated by varying the conditioning–test interval over a full range of intervals from 15 up to 250 ms. In each case no, or only marginal, changes were produced when short intervals of up to 25–30 ms were used, while clear reductions in threshold had developed by 40–50 ms and were maximal at between 60 and 100 ms. Thereafter, the effectiveness of the conditioning declined but in two of the three fibres small but clear effects persisted at 175 and 250 ms respectively.

Comparison with the effects of stimuli applied in the nucleus ruber

The effects of conditioning stimuli applied in the contralateral nucleus ruber were investigated as a control and comparison for the effects of stimulation in other brain sites. Such stimuli were without effect in all but one of the twelve group II fibres tested, results consistent with the lack of effect of the same conditioning stimuli on field potentials of group II origin recorded at the stimulus site in the midlumbar segments (see Fig. 5). These observations are also consistent with the lack of effect of stimulation in the nucleus ruber on postsynaptic potentials evoked in motoneurones by group II afferents (Jankowska *et al.* 1992). In addition, since seven of the twelve fibres tested were influenced by stimulation within the LC/SC and

raphe nuclei, the ineffectiveness of rubrospinal systems shows that the depolarization produced from the LC/SC was not secondary to the activation of rubrospinal neurones.

Comparison with segmental sources of PAD

Analysis of changes in excitability produced by stimulation in supraspinal sites was confined to those fibres in which changes in excitability could be produced by conditioning volleys in hindlimb muscle and/or cutaneous nerves. The results of these tests, together with results of similar investigations on other species of group II afferents, will be presented in greater detail elsewhere (J. S. Riddell, E. Jankowska & J. Huber, in preparation). In the present context they are used only for comparison with the efficacy of stimuli applied in supraspinal sites.

Conditioning volleys in peripheral nerves were evoked using stimuli of up to five times threshold applied to muscle nerves and two times threshold applied to cutaneous nerves since such stimuli have been found effective in producing PAD in group II fibres (Harrison & Jankowska, 1989). Examples of the effects of peripheral conditioning volleys on the excitability of group II fibres are shown in the records of Figs 4*B* and 5*B* alongside the effects of stimuli in supraspinal sites on the same fibre. While volleys in a variety of muscle and cutaneous nerves produced an increase in excitability, different nerves were found to vary in their effectiveness; volleys in peripheral nerves typically reduced the threshold stimulus current by between 5 and 25% with the most effective peripheral nerve (usually quadriceps or posterior biceps–semitendinosus) producing a mean decrease of 24%. In comparison, in the same group of fibres conditioning stimuli applied in both the LC/SC and raphe nuclei produced a mean decrease of the threshold stimulus current by 19%. Thus, while stimuli applied in supraspinal sites could be as, or more, effective than many peripheral sources, they were rarely more so than the most effective peripheral nerves.

Topographical differences in effects on group II fibres

Since the terminals of group II fibres in the dorsal horn and intermediate zone contact functionally different populations of spinal neurones (see Edgley & Jankowska, 1987*b*; Bras, Cavallari, Jankowska & Kubin, 1989) one might expect there to be differences in the origin of PAD of the terminals in these two regions that might be detectable using excitability testing. In the present results, although a limited sample of fibres is involved, there was some tendency for fibres activated by stimuli applied within the dorsal horn to be more frequently affected by conditioning stimuli applied in the LC/SC and raphe nuclei than fibres stimulated within the intermediate zone. Thus stimuli in supraspinal sites increased the excitability of ten of the eleven fibres stimulated at a location where dorsal horn field potentials were recorded (see Fig. 3) but produced similar changes in only four of eight fibres stimulated in the intermediate zone.

Effects of supraspinal stimulation on group Ia fibres

In contrast to the frequent effects evoked by stimuli in the LC/SC and raphe nuclei on the excitability of group II fibres of the deep peronei, group Ia fibres in the same nerve and terminating in the same location were largely unaffected. Seven Ia fibres

were tested using stimuli applied in the LC/SC; six of these were in addition tested with stimuli applied in the raphe nuclei and seven with stimuli applied in the nucleus ruber. Only one of the fibres tested showed any clear change in threshold and this was a single fibre which was similarly affected by stimuli in all three sites. In all other cases supraspinal stimuli were without any clear effect despite the fact that, as exemplified in Fig. 5*C*, all the fibres tested were strongly depolarized by volleys in peripheral nerves. The same stimulation parameters were used as in tests on group II fibres and the Ia fibres were all tested in experiments in which such stimuli produced changes in the excitability of at least some of the group II fibres investigated.

DISCUSSION

The results of this study show that activation of neuronal systems by stimuli applied in the LC/SC and raphe nuclei leads to depolarization of the terminals of group II muscle afferents as indicated by an increase in their excitability to electrical stimuli. To the extent that PAD is associated with presynaptic inhibition, the results suggest that this mechanism may contribute to the depression of transmission in reflex pathways from group II afferents produced by conditioning stimuli applied in these sites.

The identity of the group II fibres

In certain muscle nerves an unusually high proportion of group II fibres originate from non-muscle receptors. For example, in the branch to tibialis anterior, one of the nerves used in this study, non-spindle afferents account for about 40% of the fibres recruited by stimuli between two and five times threshold (MacLennan, 1971, 1972). This raises the question as to what extent the group II fibres of the present sample originated from receptors other than spindle secondaries. Information is lacking on the segmental sites of termination of non-spindle group II afferents but there is clear evidence that spindle secondaries of pretibial flexors terminate within the midlumbar segments. Activation of these receptors by muscle stretch (Edgley & Jankowska, 1987*a*) or by stimulation of fusimotor axons (Harrison, Jami & Jankowska, 1988), neither of which would be expected to activate non-spindle receptors (MacLennan, 1971, 1972), induces field potentials in the same locations where electrical stimulation of the nerve evokes monosynaptic field potentials of group II origin. At least a proportion of the fibres of the present sample of group II afferents are therefore likely to originate from the secondary endings of muscle spindles. Furthermore, the sample appears to be representative of the group II afferents responsible for evoking field potentials in the midlumbar segments, transmission from which is depressed by stimuli applied in the LC/SC and raphe nuclei. Thus the sample consists of fibres with a spread of peripheral thresholds within the group II range and fibres terminating both in the dorsal horn and intermediate zone with conduction times which match the time course of field potentials evoked in these regions by electrical stimulation of group II afferents.

Can presynaptic inhibition account for the depression of transmission in reflex pathways from group II afferents?

The aim of the present investigation was to look for evidence that the depressive actions of stimuli applied in the LC/SC and raphe nuclei on transmission from group II muscle afferents (Noga *et al.* 1992; Jankowska *et al.* 1993) might involve a presynaptic mechanism of action. Several observations from the present study are consistent with this possibility. (1) Stimuli in the LC/SC and raphe nuclei produced PAD of a large proportion of the terminals of group II muscle afferents terminating in the midlumbar segments. (2) These effects were produced by the same conditioning stimuli that produced depression of field potentials evoked by group II afferents in the same region of the spinal cord. (3) The conditioning–test intervals over which PAD was produced by stimuli in supraspinal sites are consistent with the time course of the depression of midlumbar field potentials and postsynaptic potentials in motoneurones evoked by group II afferents (Noga *et al.* 1992; Jankowska *et al.* 1993). (4) Stimuli in the nucleus ruber which have little effect on the midlumbar field potentials produced by group II afferents are also largely without effect on the excitability of the terminals of group II muscle afferents in this region. (5) Field potentials evoked by group I afferents in the intermediate zone of midlumbar segments are insignificantly affected by conditioning stimuli in the LC/SC and raphe nuclei at conditioning–test intervals in excess of 40 ms, or so, and such stimuli likewise have minimal effect on the excitability of group Ia muscle afferents terminating in this region.

Comparison with previous studies

There are several previous studies indicating that areas of both the pontine and medullary reticular formation may be involved in the presynaptic control of transmission from primary afferents. There are reports that dorsal root potentials (DRPs) are evoked by stimulation in the locus coeruleus (Apkarian, Hodge, Stevens & Franck, 1984; Mokha & Iggo, 1987; Fung & Barnes, 1987) and in the raphe nuclei (Carpenter, Engberg & Lundberg, 1966; Proudfit & Anderson, 1974; Mokha, McMillan & Iggo, 1986). Similarly, stimuli in the locus coeruleus are reported to lead to an increase in the excitability of laryngeal afferents (Lucier & Sessle, 1981) and of unspecified afferents of the inferior alveolar nerve (Sasa & Takaori, 1973) terminating in the medulla. To this information it may now be added that stimulation in the LC/SC region leads to depolarization of the terminals of group II but not Ia muscle afferents.

The apparent involvement of the raphe nuclei in the control of nociceptive pathways has aroused particular interest in the influence of stimuli applied within these nuclei on the excitability of nociceptive and cutaneous primary afferents. Thus stimulation of the nucleus raphe magnus has been reported to increase the excitability of tooth pulp and laryngeal afferents terminating in the brainstem (Dostrovsky, Sessle & Hu, 1981; Lovick, 1981: Lucier & Sessle, 1981) and the terminals of nociceptive and non-nociceptive cutaneous afferents both in the brainstem (Hu & Sessle, 1988) and in the spinal cord (Martin, Haber & Willis, 1979). With regard to muscle afferents, stimulation in the raphe nuclei has been reported to be without effect on group Ia afferents but to increase the excitability of Ib afferents

(Carpenter *et al.* 1966; Jimenez, Quevedo, Eguibar & Rudomin, 1989). We may now add that group II muscle afferents, which have not previously been studied, are also depolarized following stimulation in the raphe nuclei.

With respect to the nucleus ruber, it has been reported that stimulation in this site evokes PAD of group Ib and cutaneous afferents, and only weak and inconsistent effects on group Ia afferents (Hongo, Jankowska & Lundberg, 1972). From the present result, however, it appears that the contribution of the nucleus ruber to the presynaptic control of transmission from muscle afferents does not extend to group II afferents.

Which pathways are responsible for the PAD evoked by supraspinal stimuli?

Monoaminergic pathways originating within the LC/SC and raphe nuclei

Mapping of the sites within the brainstem from which depression of transmission from group II afferents can be evoked have identified the LC/SC and raphe nuclei as two of the locations where stimulation is most effective (Noga *et al.* 1992). These regions are among those providing the main source of monoaminergic innervation of the spinal cord (see Noga *et al.* 1992 for references) and there is evidence that depression of transmission from group II afferents involves monoamines (Bras *et al.* 1990; Skoog & Noga, 1991). There are therefore good reasons to consider the possibility that monoaminergic pathways originating in the LC/SC and raphe nuclei are involved in the changes in excitability of group II afferents produced by stimulation in these sites.

Investigation of the pharmacology of the PAD produced by stimulation in the reticular formation has been restricted to attempts to antagonize DRPs. These have provided evidence for the involvement of both serotonin and GABA in at least some of the effects on primary afferents produced by stimulation in the raphe nuclei (Proudfit & Anderson, 1974; Proudfit, Larson & Anderson, 1980; Lovick, 1981). There is however, little evidence that monoaminergic fibres act directly on the terminals of afferent fibres. Studies of catecholamine- and serotonin-containing boutons suggest they make few, if any, axo-axonic contacts (Ruda, Coffield & Steinbusch, 1982; Hagihira, Senba, Yoshida, Tohyama & Yoshiya, 1990; Doyle & Maxwell, 1991) though it might be argued that serotonin, at least, might act via diffusion (see for example Maxwell, Léránth & Verhofstad, 1985). Furthermore, noradrenaline and serotonin (Curtis, Leah & Peet, 1983) and the agonists tizanidine and 5-OH-DPAT (D. R. Curtis, E. Jankowska, G. R. Lacey & J. S. Riddell, unpublished observations) appear not to depolarize the terminals of group I muscle afferents in the motor nuclei of lower lumbar segments (but see Gharagozloo, Holohean, Hackman & Davidoff, 1990).

If monoamines do not act directly on the terminals of afferent fibres then an alternative possibility is that monoaminergic systems act via excitation of spinal neuronal systems producing presynaptic inhibition. While the actions of noradrenergic and serotonergic compounds on spinal neurones appear in the main to be inhibitory, there are reports of excitatory actions on neurones in the dorsal horn (Todd & Miller, 1983; El-Yassir, Fleetwood-Walker & Mitchel, 1988).

Other pathways

In view of the diverse interconnections of the LC and raphe nuclei with other brainstem structures the possibility that descending systems other than those originating in the LS/SC or raphe nuclei might be involved must be considered. For example, one source of afferents to the LC is the vestibular nuclei (Fung, Reddy, Bowker & Barnes, 1987). These are known to be involved in the presynaptic control of transmission from group I a and I b muscle afferents (Cook, Cangiano & Pompeiano, 1969; Rudomin, Solodkin & Jimenez, 1986), raising the possibility that activation of vestibulospinal neurones, as a result of stimulation in the LC/SC or activation of vestibulospinal fibres in the medial longitudinal fasciculus (Carpenter *et al.* 1966) by stimuli applied in the raphe nuclei, might be involved in the effects reported here. This seems unlikely, however, since in the present experiments, with the exception of a single fibre, no effects on the excitability of group I a afferents were observed.

Similarly, current spread to fibres in the brachium conjunctivum could result in synaptic activation of rubrospinal neurones which are also known to be involved in the presynaptic control of transmission from muscle afferents (Hongo *et al.* 1972). This can be ruled out, however, since stimulation directly in the nucleus ruber influenced neither field potentials evoked by group II afferents in the midlumbar segments nor the excitability of group II afferents.

Two further possibilities remain: firstly, that non-monominergic neurones within the LC/SC and raphe nuclei (see Noga *et al.* 1992 for references) may activate spinal interneurones which in turn induce PAD of group II afferents and secondly, that such interneurones may be activated via reticulo-spinal neurones as a result of current spread to the reticular formation.

Conclusions

The results of this study provide strong evidence that a presynaptic inhibitory mechanism contributes to the depression of transmission from group II muscle afferents produced by stimulation within the LC/SC and raphe nuclei. However, the extent to which this mechanism is responsible for the depression observed and the related question of the extent to which monoaminergic neurones are involved in evoking PAD of group II muscle afferents requires further investigation.

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