Journal of Physiology (1988), **403**, *pp.* 57–71
With 8 text-figures
Printed in Great Britain

EVIDENCE THAT MID-LUMBAR NEURONES IN REFLEX PATHWAYS FROM GROUP II AFFERENTS ARE INVOLVED IN LOCOMOTION IN THE CAT

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(*Received 13 August 1987*)

SUMMARY

1. A group of interneurones in the mid-lumbar segments of the cat spinal cord which mediate disynaptic excitation or inhibition of motoneurones from group II muscle afferents have recently been described. To test the possibility that the activity of these interneurones is related to the activity in the neuronal networks which subserve locomotion we have investigated whether they are influenced by two procedures which can induce locomotion. These procedures were electrical stimulation within the cuneiform nucleus (the 'mesencephalic locomotor region') in anaesthetized preparations and systemic administration of 3,4-dihydroxyphenylalanine (DOPA) in decerebrate, spinalized, unanaesthetized preparations. The interneurones we have tested were located in the fourth lumbar (L4) segment and were excited by group II muscle afferents; more than half of them were antidromically activated from the hindlimb motor nuclei.

2. Stimuli applied in the cuneiform nucleus evoked excitatory postsynaptic potentials (EPSPs) in a high proportion of these interneurones. The stimuli also evoked distinct extracellular field potentials in the ventral horn of the L4 segment. The properties and latencies of both the intra- and extracellularly recorded potentials show that they were evoked disynaptically, via supraspinally located relay neurones and a fast-conducting descending tract.

3. Stimulation of the cortico- and rubrospinal tracts excited or inhibited some of the L4 neurones, often at latencies suggesting mono- or disynaptic coupling. The neurones which appeared to be monosynaptically excited from the cortico- and rubrospinal tracts tended to be located dorsal to the neurones which were activated from the cuneiform nucleus.

4. Systemic administration of DOPA depressed the responses evoked by stimulation of group II afferents of L4 interneurones which projected to motor nuclei. DOPA also depressed extracellular field potentials evoked by group II afferents in the intermediate zone and in the ventral horn (at the location of the interneurones) but hardly affected those in the dorsal horn.

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5. By showing that both stimulation in the cuneiform nucleus and the administration of DOPA influence activity of L4 interneurones which are excited by group II afferents and which project to motor nuclei, the results of this study support the hypothesis that these neurones are in some way involved in locomotion. However, the opposing effects of DOPA administration and of stimulation in the cuneiform nucleus make the interpretation of their role in locomotion rather difficult before it is known to what extent they are active throughout the step cycle.

INTRODUCTION

This study is a continuation of an investigation of interneurones of the 4th lumbar segment of the cat spinal cord. Many interneurones in this segment were found to have particularly powerful input from group II muscle afferents (Edgley & Jankowska, 1987*b*) and to have direct excitatory or inhibitory actions upon hindlimb motoneurones (Cavallari, Edgley & Jankowska, 1987). The particular pattern of convergence of muscle, skin and joint afferents onto these neurones suggested to us that they might play an important role in movements determined by the position of the hip. Hip position is known to be of crucial importance in the control of locomotion, especially in termination of the stance phase and initiation of the swing phase of the step, but also for adjustment of spinal reflexes during the step cycle (e.g. for reflex reversal of placing and flexion reflexes, see Grillner, 1981, and the discussion in Edgley & Jankowska, 1987*b*). We have therefore considered that the activity of this particular population of L4 neurones with group II input is involved in locomotion, in agreement with the conclusions of previous studies on the role of unspecified interneurones in the L4 segment in locomotor and scratching movements (Berkinblit, Deliagina, Feldman, Gelfand & Orlovsky, 1978; Deliagina, Orlovsky & Pavlova, 1983).

To investigate the relationship between L4 interneurones with group II input and the neuronal networks which subserve locomotion we have examined how these interneurones are influenced by two procedures which can induce locomotor-like activity. Firstly, we have examined whether they are activated by electrical stimulation of a brain stem site from which locomotion can be induced in decerebrate cats. Stimuli were applied in the cuneiform nucleus, which is the main anatomical correlate of the 'mesencephalic locomotor region' (Shik, Severin & Orlovsky, 1967; for recent references see Armstrong, 1986, and Jordan, 1986). These experiments were performed in anaesthetized cats. Secondly, we have examined the effects of systemic administration of DOPA on the activity of these interneurones in decerebrate, spinalized and unanaesthetized cats. In such preparations DOPA can induce alternating activation of flexor and extensor muscles, as in stepping, and this finding led to the hypothesis that DOPA affects neuronal networks used during locomotion (Jankowska, Jukes, Lund & Lundberg, 1967*b*; see Lundberg, 1969, and Grillner, 1981). DOPA influences spinal reflex pathways by depressing short-latency flexor and some other reflex responses while facilitating the appearance of long-latency, long-duration flexor reflex responses (Anden, Jukes, Lundberg & Vyklicky, 1966*a*, *b*; Jankowska, Jukes, Lund & Lundberg, 1967*a*). Either the depression of short-latency responses, or the enhancement of long-latency responses of a neurone

to appropriate peripheral stimuli following the administration of DOPA, might thus indicate the involvement of this neurone in locomotion.

METHODS

The experiments involving stimulation in the cuneiform nucleus were carried out on seven intact cats under chloralose anaesthesia (60–80 mg kg^{-1} initial dose after preparation under ether, supplemented up to a total dose of 5–7 mg kg^{-1} h^{-1}), paralysed with gallamine triethiodide and artificially ventilated. The above doses of chloralose resulted in deep anaesthesia in ten non-paralysed cats kept under observation for more than 36 h. Furthermore, the level of anaesthesia was controlled by checking that noxious stimuli did not cause any pupil dilatation or increases in blood pressure or in the heart rate. The effects of administration of DOPA were investigated in six other experiments on cats which were decerebrated under ether anaesthesia, spinalized at a low thoracic level and similarly paralysed. Four of the cats in which the effects of DOPA were tested were pre-treated with the monoamine oxidase inhibitor nialamide (Sigma, catalogue No. 9502) in doses 60, 20, 10 and 5 mg kg^{-1} I.V. (in a solution of 10 mg ml^{-1} at pH 2·5–3·5). Nialamide was given just after the decerebration and the spinalization were completed. Some 20–50 mg kg^{-1} of L-DOPA (L-3-(3,4-dihydroxyphenyl)-alanin; Fluka, catalogue No. 37830; 10 mg ml^{-1} I.V.) was administered in these cats. In the two experiments without nialamide, 100 mg kg^{-1} of DOPA was given.

The main procedures used in all the experiments were those described previously (Edgley & Jankowska, 1987*a*, *b*). Additional procedures involved the placement of stimulating electrodes in the brain stem (tungsten electrodes insulated with varnish except for about 100 μm at the tip). These were placed in the ipsilateral (left) cuneiform nucleus and in the contralateral (right) red nucleus and pyramidal tract. The electrode to be placed in the cuneiform nucleus was introduced through a hole in the parietal bone. In order to avoid the tentorium, it was angled by 20 or 30 deg (the tip being directed caudally) to reach a point with Horsley–Clarke co-ordinates P1·0–1·5, L4 and H-1·0 (Shik *et al.* 1967). The position of the electrode relative to the brachium conjunctivum was then determined by stimulating through this electrode and recording from the contralateral red nucleus. The electrode was left at a depth about 0·5 mm above the depth from which stimuli of 200 μA evoked antidromic activation of neurones in the red nucleus. Stimuli of 250 μA applied at this location did not affect these neurones. Single stimuli or trains of two to six stimuli (0·1 ms, at 300 Hz, 20–200 μA) were applied in the cuneiform nucleus at a low repetition rate (about 0·5 Hz). For the description of the placement of the electrodes in the red nucleus and in the medullary pyramids see Hongo, Jankowska & Lundberg (1969) and Harrison & Jankowska (1985). Stimuli of up to 100 μA were applied through these electrodes. Subsequent to the experiments the correct placement of all the electrodes was verified histologically.

RESULTS

Supraspinal actions on L4 interneurones

Extracellular field potentials from the cuneiform nucleus

Trains of stimuli applied in the cuneiform nucleus evoked distinct synaptic field potentials in the ventral horn of the L4 segment. Field potentials recorded in one track and their distribution in the transverse plane are illustrated in Fig. 1*A* and *C*. The amplitudes of these potentials were maximal just ventral to the area where maximal field potentials were evoked from group II afferents in the quadriceps nerve (Fig. 1*B* and *C*). Single stimuli in the cuneiform nucleus were ineffective, but field potentials with sharp onsets (Fig. 1*A*, arrows) appeared following the 2nd and 3rd stimuli of a train; each of these stimuli was followed by a larger but equally distinct field potential. This kind of temporal facilitation which has previously been found in several disynaptic but not polysynaptic pathways (e.g. Hongo *et al.* 1969; Hongo, Jankowska & Lundberg, 1972) suggests that a pathway involving more than one, but

not more than two synapses, was responsible for these potentials (see also below). The intervening neurones would be expected to be located at a supraspinal level in view of the anatomical evidence that in the cat there are no direct projections from the cuneiform nucleus to the spinal cord (see Steeves & Jordan, 1984; Armstrong, 1986).

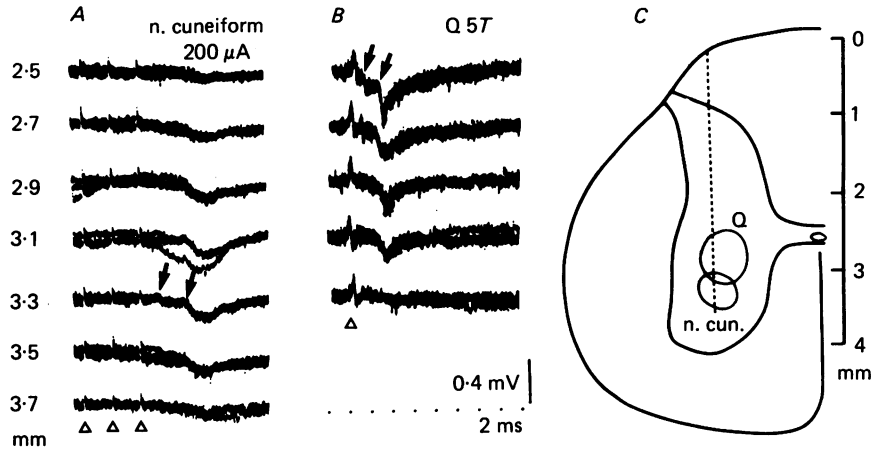

Fig. 1. Extracellular field potentials evoked from the cuneiform nucleus (*A*) and by group II afferents of quadriceps (*B*) along an electrode track shown in *C* (dotted line). The depths from the cord dorsum at which these potentials were evoked are shown to the left of the records in *A*. The arrows in *A* indicate field potentials evoked by the 2nd and the 3rd stimuli, the 1st stimulus having been ineffective. The stimuli (all 200 μA) are indicated by arrow-heads. The field potentials in *B* were evoked by single stimuli (arrow-head) at 5·0 times threshold (*T*) for the most excitable fibres to the quadriceps nerve. The arrows point out onsets of the field potentials evoked from group I afferents and from group II afferents, respectively; note that only the latter appeared below 2·7 mm depth. The areas encircled in *B* show the areas within which the largest field potentials were evoked from the cuneiform nucleus (n. cun.) and from the quadriceps nerve (Q).

Effects of stimulation in the cuneiform nucleus on L4 interneurones

The effects of stimulation in the cuneiform nucleus were tested on fifty interneurones recorded from intracellularly which were excited by group II afferents of at least one of the muscle nerves tested. Thirty-four of these interneurones were excited from the cuneiform nucleus. Before penetration the neurones were usually discharged only after the 4–6th stimulus of a train but intracellular records revealed distinct short-latency EPSPs following the second and each successive stimulus (Figs 2*G*–I, 3*J*–*K*, and 4*G*) in most (73%) of these neurones. These EPSPs appeared in parallel with the extracellular field potentials, showed similar temporal facilitation and had the same latencies. Since these EPSPs did not appear in response to single stimuli they were not evoked monosynaptically, but their latencies were only 0·5 ms longer than the latencies of EPSPs which were monosynaptically evoked from the red nucleus. This implies that they were relayed via a single interposed neurone and a very fast descending pathway. A major target of the cuneiform nucleus is the medullary mid-line reticular formation (see Steeves & Jordan, 1984) from which

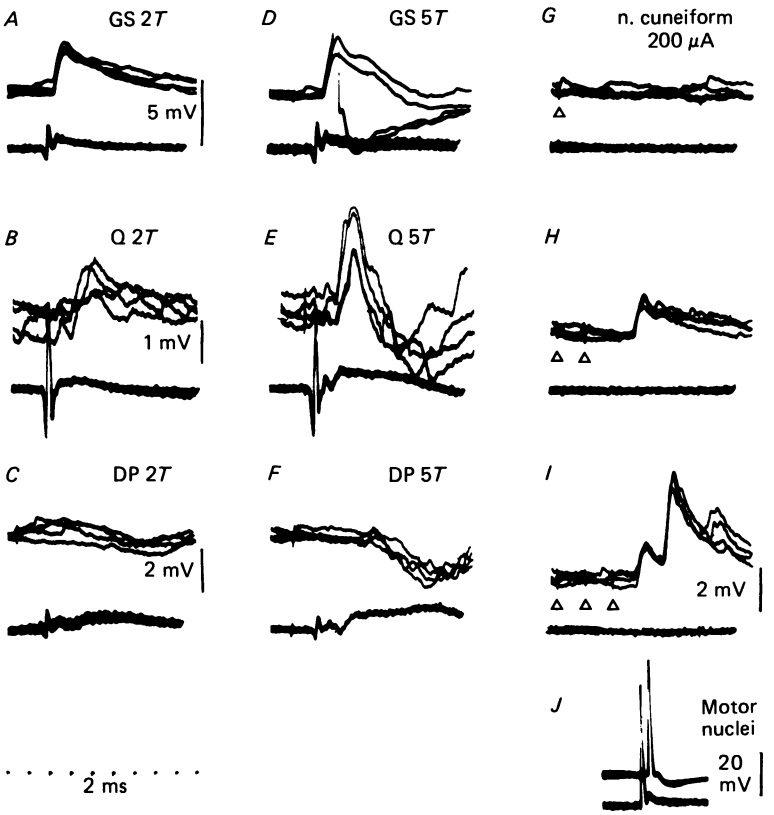

Fig. 2. Intracellular records from an L4 interneurone which was co-excited by group I and II afferents and from the cuneiform nucleus (upper traces) and the simultaneously recorded cord dorsum potentials (lower traces). *A–C*, PSPs evoked by stimuli near-maximal for group I afferents and near-threshold for group II afferents (2*T*). *D* and *E*, PSPs evoked by stimuli near-maximal for group II afferents (5*T*). *G–I*, PSPs evoked by stimuli applied in the cuneiform nucleus (one to three stimuli as indicated by the arrow-heads). *J*, antidromic action potential following stimuli applied in the posterior biceps–semitendinosus motor nuclei. Note the short-latency EPSPs from group I afferents of gastrocnemius–soleus (GS), longer-latency EPSPs from group II afferents of quadriceps (Q) and GS (the later components of the EPSPs in *D*) and long-latency IPSPs from group II afferents of both deep peroneal nerve (DP) and quadriceps nerve (the IPSPs following the EPSPs). Note also that EPSPs from the cuneiform nucleus followed the 2nd and 3rd (*H* and *I*) but not the 1st stimulus (*G*), showing clear temporal facilitation.

rapidly conducting reticulospinal axons (Lloyd, 1941) originate. This pathway thus seems likely to be the one responsible for the EPSPs.

Thirty-three interneurones were located at depths between 2·6 and 3·6 mm from the dorsal surface of the spinal cord where the extracellular field potentials were evoked from the cuneiform nucleus. This location corresponds to the ventral part of lamina VII and the dorsal part of lamina VIII of Rexed. Seventy per cent (twenty-three of thirty-three) of these interneurones were excited from the cuneiform nucleus, all at latencies of less than 6 ms. More dorsally, at depths 1·45–2·5 mm, only 10% of cells were excited at such short latencies with another 20% excited at longer latencies (up to 11 ms).

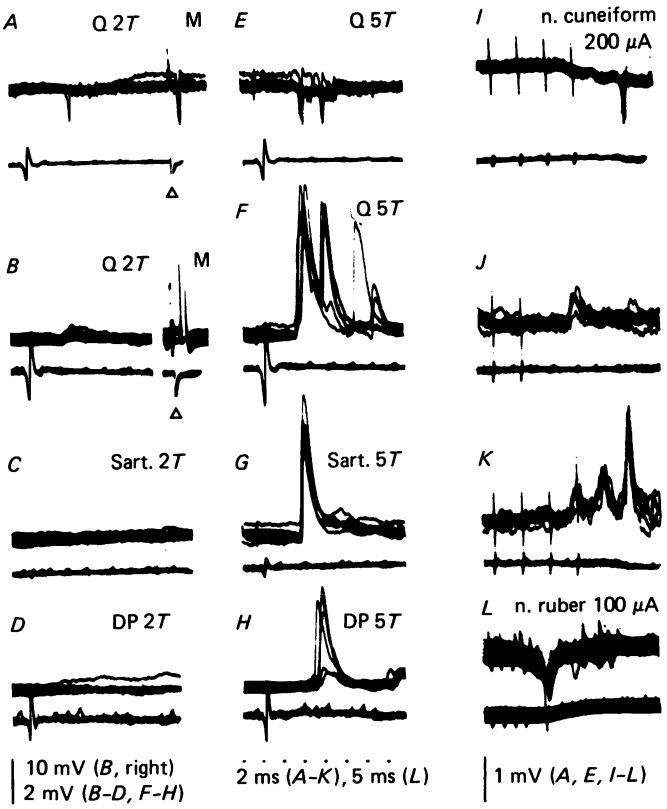

Fig. 3. Extracellular (*A*, *E* and *I*) and intracellular (*B–D*, *F–H* and *J–L*) records from an L4 interneurone which was excited by group II afferents and from the cuneiform nucleus (upper traces); lower traces are cord dorsum potentials. The format of the figure is as for Fig. 2. Note that stimuli applied at the border between triceps surae and hamstring motor nuclei (M; arrow-heads indicate stimulus artifacts) evoked an all-or-nothing antidromic action potential and that 2 times threshold stimuli were either ineffective (*C* and *D*) or just at threshold (*A* and *B*) for evoking EPSPs or action potentials. With stimuli (5*T*) which were near-maximal for group II afferents, large EPSPs (*F–H*) were evoked at the same latency; large unitary EPSPs from group II afferents are characteristic for this group of neurones as described previously (Lundberg, Malmgren & Schomburg, 1987*a*; Edgley *et al.* 1987*b*). Note also the temporal facilitation of effects of stimuli applied in the cuneiform nucleus and opposite effects of stimulation of the red nucleus (similarly evoked by four shocks).

Short-latency (less than 6 ms) excitation from the cuneiform nucleus was seen in a high proportion of the interneurones which were found to project to the motor nuclei (62%, sixteen of twenty-six), or were co-excited by group I and group II afferents (54%, fifteen of twenty-eight). Ten neurones belonged to both of these categories and records from one of them are illustrated in Fig. 2. In the remaining cells only input from group II afferents, excitatory or inhibitory, was found; two of these are illustrated in Figs 3 and 4. Additional input from afferents in joint and cutaneous nerves and from unspecified group III afferents was found in practically all of the neurones which were influenced from the cuneiform nucleus.

Only in three of the neurones tested were IPSPs seen following stimulation in the cuneiform nucleus. Since all of these were recorded in one experiment it is possible that they were evoked by current spread to different neurones or fibres than those which were responsible for the EPSPs.

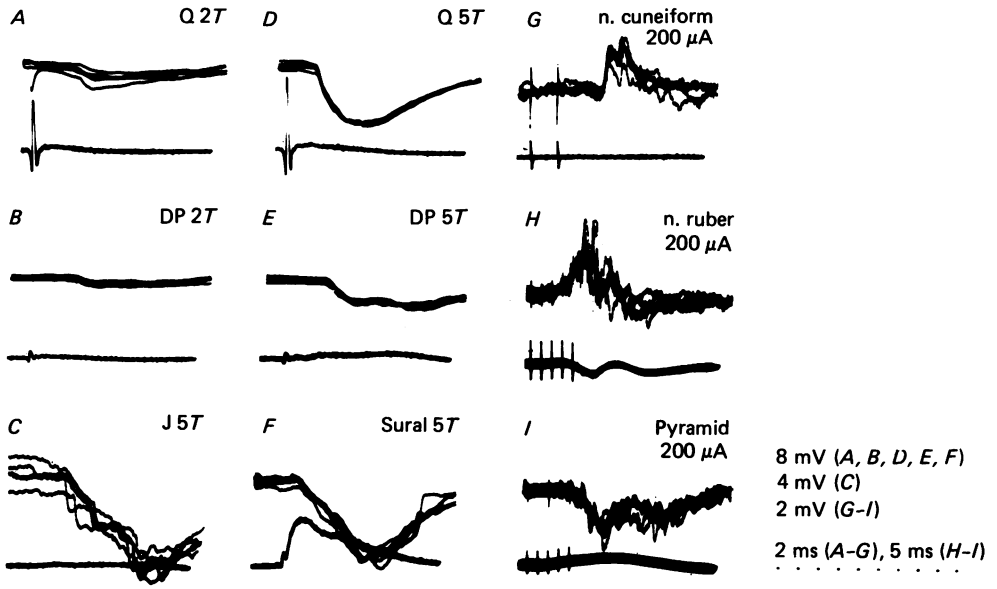

Fig. 4. Intracellular records from an interneurone which was inhibited by group II afferents of the tested muscle nerves: quadriceps (Q) and deep peroneal (DP), as well as by afferents in joint (J) and cutaneous (sural) nerves, and from the contralateral pyramid, but was excited from the cuneiform nucleus (most likely disynaptically) and from the red nucleus (polysynaptically).

Effects of stimuli applied in the medullary pyramid and the red nucleus

The effects of stimuli applied in the cuneiform nucleus were compared to those evoked from the pyramidal tract and the red nucleus. This was done partly to determine how specific the effects of stimuli in the cuneiform nucleus were and partly to investigate the relationship between the L4 interneurones with group II input and previously described L4 neurones which relay cortico- and rubrospinal actions (see e.g. Vasilenko, Kostyukov & Pilyavskij, 1972; for other references see Edgley & Jankowska, 1987*b*).

In eleven interneurones (out of forty-nine tested) stimuli applied in the contralateral pyramidal tract produced EPSPs which were classified as having been evoked monosynaptically, because they were evoked by single stimuli and faithfully followed each stimulus of a train. Furthermore, they appeared with fixed latencies, which were between 4·5 and 7·2 ms from the stimuli and corresponded to segmental latencies of 0·7–2·8 ms from the earliest components of the descending pyramidal volleys; at least the earliest of these latencies did not allow any additional interposed interneurones. All neurones with such responses were located at depths between 1·60 and 2·75 mm from the cord surface (predominantly in laminae V–VII), they

projected to motor nuclei and/or were excited by both group I and II afferents. Longer-latency EPSPs which appeared only in response to a train of stimuli, and were therefore classified as having been evoked polysynaptically, were seen in fourteen other interneurones. The latter neurones were located within a wider area of the grey matter. Distinct IPSPs, exemplified in Fig. 4*I*, were seen in only three of these neurones.

Stimuli applied in the red nucleus had similar effects. In seventeen interneurones (of seventy tested) they evoked EPSPs with latencies between 3·8 and 4·7 ms from the stimulus. The segmental latencies of these EPSPs were 0·2–0·7 ms; the EPSPs were therefore classified as having been evoked monosynaptically. They were found in interneurones located at depths between 1·6 and 3·15 mm from the cord surface, in an area overlapping with, but extending further ventrally than, the area where monosynaptic actions from the pyramid were found. All but two of these interneurones were found to project to motor nuclei and/or to have input from both group I and II afferents. Longer-latency EPSPs which only appeared in response to a train of stimuli to the red nucleus (Fig. 4*H*) were found in twenty-nine other neurones. The segmental latencies of these EPSPs were more than 1·2 ms and for both these reasons the EPSPs were classified as having been evoked di- or polysynaptically. Like the interneurones with polysynaptic input from the pyramidal tract, these interneurones were found throughout the grey matter. IPSPs were found in sixteen of the seventy interneurones. The segmental latencies of only six of them were less than 1·5 ms, indicating a disynaptic coupling, the remaining ones, illustrated in Fig. 3*L*, being late.

General features of the descending actions on L4 interneurones with group II input

In almost all of the interneurones we have investigated the PSPs evoked from the tested descending pathways were smaller than the PSPs evoked from group II afferents. This was particularly the case for EPSPs evoked from the rubrospinal and pyramidal tracts. Selective excitation from only one of the descending systems (when the other ones were adequately tested; in thirty-two neurones) was seen in four cells from the pyramidal tract, in six cells from the red nucleus and in four cells following stimulation in the cuneiform nucleus. Co-excitation from two or all three pathways was found in more than half of these thirty-two neurones, the cuneiform nucleus stimulation acting in concert with either red nucleus (see Fig. 4*G–H*) or pyramidal tract stimulation, or both. The three descending pathways could thus reinforce each other's actions and thereby increase the probability of discharging the interneurones. However, opposing actions from these descending pathways were also found. For instance, in some neurones EPSPs were evoked from the cuneiform nucleus while IPSPs were evoked from the red nucleus (Fig. 3*L*) or from the pyramidal tract (Fig. 4*I*).

The effects of DOPA on activity of L4 interneurones

Investigation of the actions of DOPA on individual, functionally identified interneurones of reflex pathways is greatly hampered by the fact that its effects are short lasting and cannot be repeated in the same experiment (see Anden *et al.* 1966*a*). The effects of DOPA were therefore investigated on only a small number of neurones

($n = 6$) the responses of which were monitored continuously, both before and after its application. The neurones selected for testing were all antidromically activated by stimuli applied within either the triceps surae or hamstring motor nuclei and all were discharged by stimulation of the quadriceps or sartorius nerves at strengths suprathreshold for group II afferents (five times the threshold (T) of the most excitable fibres). The latencies at which the interneurones were activated by such

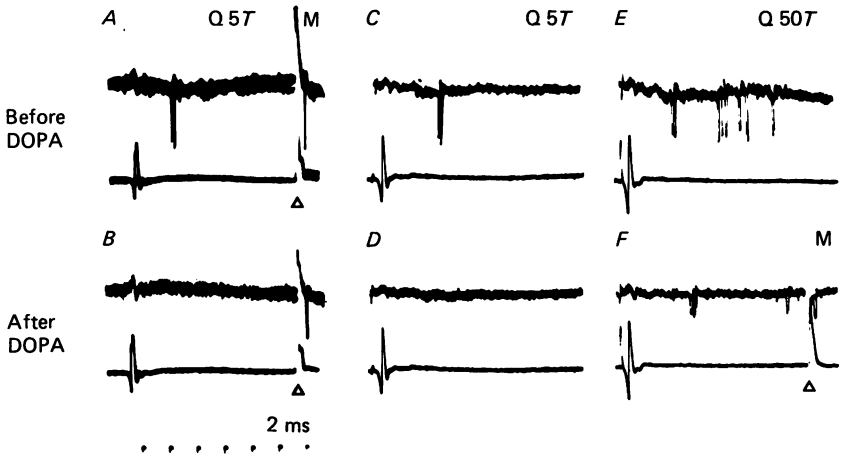

Fig. 5. Effects of DOPA on two L4 interneurones (A and B; C–F) which had group II input and projected to L7 motor nuclei. Note in B and D that in both neurones DOPA abolished responses evoked by single stimuli sufficient to activate group II afferents ($5T$) of the quadriceps (Q) nerve. Stronger stimuli were more effective in activating the 2nd interneurone (E and F; note that the early response was evoked at a shorter latency); such stimuli were also effective in activating this neurone after administration of DOPA, although at a longer latency (F). A, B and F also show antidromic activation of the neurone by stimuli applied in motor nuclei (M; arrow-heads) and confirm that the neurone was not lost. The variation in amplitude of the spike potentials in C–F was due to slight adjustments of the position of the electrode.

single stimuli (2·4–3·0 ms from the group I volleys recorded at the L5 segment) were fully compatible with monosynaptic excitation by group II afferents (see Edgley & Jankowska, 1987*b*). The neurones were located at the same depths (2·6–3·1 mm from the cord surface) as the majority of the interneurones activated from the cuneiform nucleus. They are therefore likely to have belonged to the same functional population. Descending actions onto these neurones could not be tested since the classical effects of DOPA appear only in decerebrate, spinalized, non-anaesthetized preparations.

In addition to monosynaptic activation by group II afferents, the effects of stimuli sufficient to activate group III muscle afferents and high (electrical) threshold afferents of cutaneous and joint nerves were investigated by increasing the stimulus intensity to 20–50 times threshold. Prior to the application of DOPA, single stimuli in this range evoked a short-latency burst of discharges which lasted 20–50 ms (Fig. 5E). These were prolonged to about 100 ms (Fig. 6C and E) when short trains of stimuli were used.

The effects of DOPA on the responses of interneurones were tested in parallel with its effects on discharges of motoneurones recorded in peripheral nerves (a branch of

the semitendinosus nerve and the medial gastrocnemius nerve). The latter were recorded to ensure that DOPA modified the discharges of motoneurones in the previously described fashion (i.e. that it depressed the short-latency flexor reflex responses and that it released long-latency, long-lasting responses, with alternating activity in nerves to flexors and extensors, Jankowska *et al.* 1967*a*). The depression of the short-latency responses was seen in all experiments, bursts of activity alternating between flexor and extensor nerves being present during at least part of the recording period (see Fig. 6*H*). The alternation was most strongly developed and continued over longer periods in the cats which were pre-treated with nialamide.

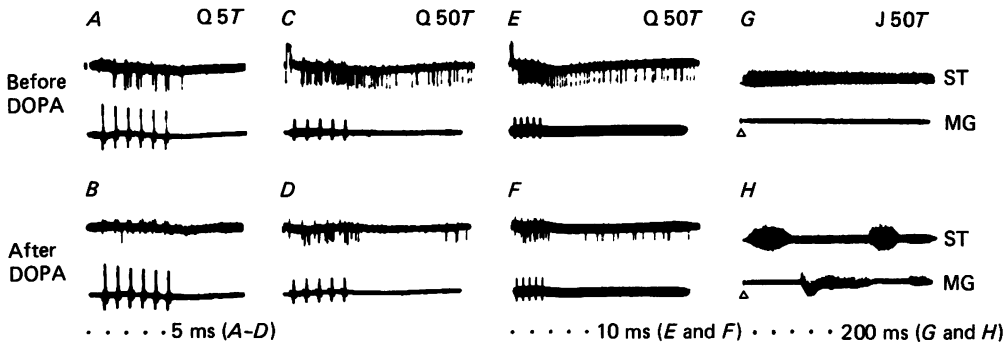

Fig. 6. Effects of DOPA on responses evoked by trains of stimuli sufficient to activate group II and group III afferents. In *A* and *B* and *C–F* are records from the two interneurones illustrated in Fig. 5. *B* illustrates that DOPA strongly depressed their responses to trains of stimuli but did not abolish all of them. *D* and *F* show that early excitation of neurones by stronger stimuli remained after the administration of DOPA (probably due to both spatial and temporal facilitation of monosynaptic group II actions) while the early polysynaptic burst of spikes was strongly depressed. *G* and *H* show responses from branches of the nerves to semitendinosus (ST) and medial gastrocnemius (MG) muscles upon stimulation of the posterior knee joint (J; arrow-heads) nerve before (*G*), and after (*H*) DOPA.

Soon after the application of DOPA all six tested neurones either ceased responding to single stimuli in the group II range, or showed a considerable depression of such responses. The responses were abolished in the four preparations pre-treated with nialamide (e.g. Fig. 5*B* and *D*) whereas they were only depressed in the two cats which were given DOPA alone. The depression involved a reduction in the proportion of trials in which the cell responded to stimulation of group II afferents (from 100% down to 21 and 3% of trials) and an increase in the latency and threshold of the responses (cf. Fig. 5*C* and *F* and Fig. 6*A* and *D*). The responses evoked by stronger stimuli, which were applied to muscle, skin and joint nerves, were also depressed after application of DOPA (cf. Fig. 5*E* and *F* and Fig. 6*C*, *E* and *D*, *F*), while the antidromic responses of the neurones to stimuli delivered in the motor nuclei were unaffected (Fig. 5*D* and *F*), confirming that the recording conditions were unchanged.

None of the selected neurones showed any late, long-lasting discharges in phase with the discharges of flexor or extensor motoneurones as has been found in interneurones studied previously (Jankowska *et al.* 1967*b*; Fu, Lundberg &

Jankowska, 1975). On the other hand, such discharges were seen in four L4 neurones of an apparently different subpopulation which were not antidromically activated from the motor nuclei or excited by group II afferents at the time of testing.

One to two hours after the application of DOPA, the late long-lasting discharges of motoneurones in response to strong stimuli began to wane. At the same time the responses of the tested neurones to group II volleys reappeared or became more secure (i.e. the latencies decreased and the proportion of trials in which the responses were seen increased).

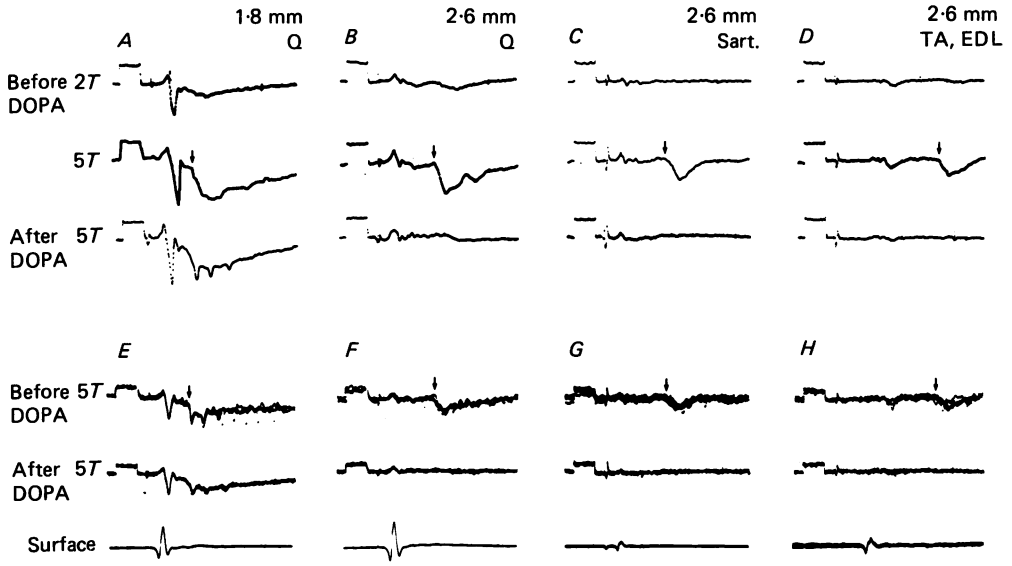

Fig. 7. Effect of DOPA on extracellular field potentials evoked from quadriceps (Q), sartorius (Sart.) and anterior tibial and extensor digitorum longus (TA, EDL) nerves. Upper panel: averaged records of field potentials evoked by stimuli at $2T$ and $5T$ before the administration of DOPA and at $5T$ after DOPA. Lower panel: single-sweep records of field potentials and of the corresponding afferent volleys recorded from the surface of the spinal cord evoked by stimulation at $5T$. Comparison of control responses evoked by $2T$ and $5T$ stimuli allows the field potentials indicated by arrows to be attributed to group II muscle afferents. Note that those recorded at a depth of 2·6 mm (*B–H*) were abolished by DOPA while those at 1·8 mm depth (*A* and *B*) were practically unchanged. Shorter-latency field potentials attributable to group I afferent were less affected. The calibration pulses are 1 ms, 200 μV.

Effects of DOPA on field potentials evoked by group II muscle afferents

Since the effects of DOPA could be examined on only a small number of single interneurones, the experiments described above were supplemented with observations on changes in extracellular field potentials evoked on stimulation of group II and group I muscle afferents which reflect monosynaptic EPSPs evoked by these afferents in their target neurones (Edgley & Jankowska, 1987*a*). Field potentials were recorded at different depths to compare the effects of DOPA on neuronal populations in the dorsal, intermediate and ventral regions of the spinal cord. We found that after the application of DOPA the group II field potentials recorded in the

intermediate zone or ventral horn were very strongly depressed or were abolished. This is illustrated in Fig. 7*B*, *C* and *D* with both averaged (upper panel) and single-sweep records (lower panel) of the same potentials. In contrast, the group II field potentials in the dorsal horn remained practically unchanged (Fig. 7*A*). The comparison of effects of DOPA on the early field potentials (of group I origin, see Edgley & Jankowska, 1987*a*) and on the late field potentials (of group II origin) in the intermediate zone (Fig. 7*B–D* and Fig. 8*A*) revealed that the depressant effect of DOPA was considerably stronger on the group II than the group I field potentials.

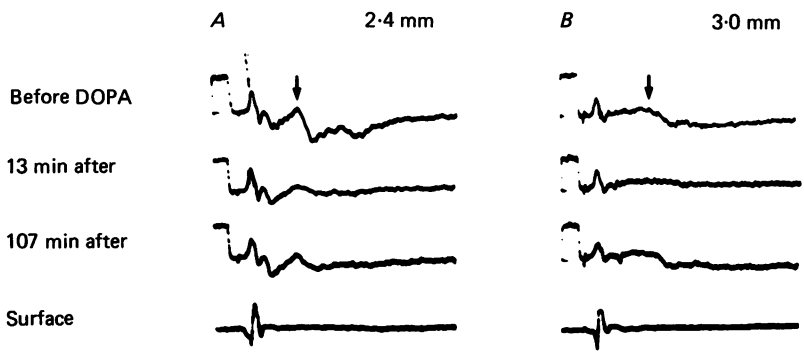

Fig. 8. Reversible depressive effects of DOPA on group II field potentials evoked at two locations. The records are averaged field potentials and incoming volleys as in Fig. 7. Note in *A* the potent depression of group II field potentials (indicated by arrows) and lack of effect upon the earlier group I field potential at 2·4 mm depth 13 min after administration of DOPA. Note also some recovery of group II field potentials 107 min after DOPA. The calibration pulses are 1 ms, 200 μV.

The depression of group II field potentials followed a similar time course to the depression of neuronal responses and also began to recover 1–2 h after the application of DOPA (cf. records in Fig. 8 before, just after and over 100 min after the application of DOPA).

DISCUSSION

The results of this study show that electrical stimuli which can induce locomotion in decerebrate cats (as described by Shik *et al.* 1967; see Grillner, 1981; Armstrong, 1986; Jordan, 1986) excite a large proportion of the L4 interneurones which are interposed in reflex pathways from group II afferents to motoneurones. They also demonstrate that DOPA has a depressant effect on these neurones. These are properties expected of neurones involved in locomotion. The results of this study thus support the hypothesis that the activity of L4 interneurones with group II input is closely related to locomotion (Edgley & Jankowska, 1987*b*). However, our evidence for this is still preliminary and far from being conclusive. For instance, we cannot be sure that the activation of L4 interneurones by stimuli applied in the cuneiform nucleus was evoked via the same neuronal system, or systems, which initiate locomotion. Stimulation in the cuneiform nucleus can excite a variety of cells

and fibres, both within and outside this nucleus (see e.g. Armstrong, 1986). It may also give rise to a number of parallel effects (Shefchyk & Jordan, 1985; Noga, Kriellaars, Brownstone, Mallory & Jordan, 1986). Of these we could only eliminate the effects mediated by the rubrospinal tract (by verifying that our stimuli did not activate red nucleus neurones, either antidromically or synaptically). The differences between the effects evoked by stimuli applied in the cuneiform nucleus and in the red nucleus (Fig. 3*K* and *L*; Fig. 4*G* and *H*) support this conclusion. Furthermore, the brief latency of the observed actions allows one to attribute them to the most direct cuneiform–reticulospinal pathways (see Orlovsky, 1970; Shefchyk, Jell & Jordan, 1984; see also Lloyd, 1941) and to eliminate effects mediated by less direct pathways (Mori, Shik & Yagodnitsyn, 1977).

Similarly, the depressant actions of DOPA on L4 interneurones do not implicate these neurones as directly subserving locomotion. According to the original hypothesis (cf. Lundberg, 1969, 1979), activation of the neuronal networks involved in locomotion by DOPA is secondary to disinhibition, i.e. to depression of transmission via neurones which normally prevent the activation of these networks. The L4 interneurones examined in this study might well be used for this purpose, both directly (the inhibitory interneurones) and indirectly. However, the actions of DOPA are unlikely to be limited to only one category of neurone. Consequently, the depression of L4 neurones by DOPA may, but does not have to be, causally related to the release of activity of the intrinsic networks of locomotion.

Of the total population of L4 interneurones with group II input, only those located in the ventral horn and in the intermediate zone have been found to be disynaptically excited from the cuneiform nucleus and depressed by DOPA. However, we do not know whether they are the only interneurones to be affected in this way, nor whether they are the only interneurones which mediate the short-latency excitation of motoneurones from the cuneiform nucleus (Shefchyk & Jordan, 1985). They are the first excitatory premotor interneurones to have been functionally identified in the lumbar spinal cord to date, and the first functionally defined spinal neurones in which input from the mesencephalic locomotor region has been investigated. They are also the first ones in which DOPA has been demonstrated to depress monosynaptically evoked responses, an indication that they might be subject to its direct actions. Future studies may thus reveal other neurones with similar properties in the more caudal or rostral segments. However, only a very small proportion of the more dorsally located L4 interneurones with group II input were excited by stimuli applied in the cuneiform nucleus. Furthermore, if responses of any of these interneurones were depressed by DOPA, the effect must have been rather weak in view of hardly any depression of the focal field potentials in the dorsal horn. Their function is unknown but our observations do not indicate any essential role of these interneurones in locomotion.

The opposing effects of stimuli applied in the cuneiform nucleus (excitatory) and of DOPA (depressant) on L4 interneurones are not easy to interpret as a part of the same pattern of motor behaviour. A different type of experiment will have to be undertaken to determine if, and when, these neurones are active during locomotion. However, there are already some experimental data relevant to this question. For instance, we may expect their input via the cuneiform–reticulospinal pathway to be

phasic, because many cells in the mesencephalic locomotor region and many reticulospinal neurones are phasically active during locomotion (see Armstrong, 1986; Jordan, 1986). We may also expect that such a phasic descending input coinciding with strong peripheral input during specific phases of locomotion would be able to overpower inhibitory actions exerted by monoaminergic pathways.

Generally, our observations provide another example of neurones which might be used for several different purposes. One of the main tasks of the L4 neurones must be to adjust the degree of muscle contractions according to the length of the muscles, both in reflexly and centrally evoked movements (see Lundberg, Malmgren & Schomburg, 1987*b*). Powerful excitation of them from the cuneiform nucleus indicates that they may be involved in locomotion. Also the cortico- and rubrospinal actions on the L4 interneurones might be considered in terms of central initiation of locomotion, or as a mechanism for adjustment of leg position during stepping, since these actions would be influenced by changes in muscle length. However, even the closest linkage of L4 interneurones with the neuronal networks used during locomotion would not preclude their contribution to other movements.

We wish to thank Professor A. Lundberg for his comments on an earlier version of this paper and R. Källström for his assistance during all the stages of this study. The study was supported by the Swedish Medical Research Council (project 5648). S. A. E. received a Royal Society European Exchange Programme Fellowship and a Swedish Medical Research Council Visiting Scientist Fellowship.

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