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ANTINOCICEPTIVE ACTIONS OF DESCENDING DOPAMINERGIC TRACTS ON CAT AND RAT DORSAL HORN SOMATOSENSORY NEURONES

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SUMMARY

1. The actions of dopamine (DA) and DA receptor specific agonists and antagonist ionophoretically applied in the spinal dorsal horn, and of focal electrical stimulation in the region of the supraspinal DA cell groups (A9 and A11) were assessed on the somatosensory responses of dorsal horn neurones, in both the rat and cat. The neurones tested were multireceptive, giving reproducible responses to both noxious (using a mechanical pinch or radiant heat) and innocuous (using a motorized brush) cutaneous stimuli, as well as to ionophoretically applied DL-homocysteic acid (DLH, a direct excitant). In the cat, all neurones tested were identified as belonging to the spinocervical tract (SCT) and were located in the dorsal horn laminae III–V, whilst in the rat, spinothalamic tract (STT) and spinomesencephalic (SMT) neurones located in the region of lamina I and laminae III–V were tested.

2. Ionophoretically applied DA and RU24213, a D_2 DA receptor agonist, caused a selective inhibition of the responses to noxious stimuli of SCT, STT and SMT neurones, whilst the responses to non-nociceptive stimuli, spontaneous activity and DLH-evoked activity were unaffected. This action was reversed in the presence of sulpiride, the highly selective D_2 DA receptor antagonist. Neither sulpiride alone nor SKF38393, a D_1 DA receptor agonist, altered evoked or spontaneous activity when ionophoretically applied.

3. Focal electrical stimulation in the region of the A11, but not the A9, DA cell group selectively suppressed nociceptive responses of spinal, multireceptive neurones in the rat. This stimulus-evoked effect was consistently and rapidly reversed by ionophoresis of sulpiride, in the vicinity of the dorsal horn neurone being tested. In contrast, naloxone and idazoxan (RX781094), an α_2-antagonist, were not effective.

4. This study presents data supporting a selective antinociceptive role for DA at the spinal level, where it has a widespread antinociceptive influence, on cells in both the superficial and deeper dorsal horn. The A11 DA cell group was shown to be a supraspinal site from which a selective antinociceptive action could be electrically evoked and which was mediated by DA at the level of the dorsal horn.

INTRODUCTION

Several studies have indicated the presence of a population of nerve terminals that contain dopamine (DA), but not other catecholamines, in the dorsal horn of the spinal cord of the rat and cat (Fleetwood-Walker & Coote, 1981; Skagerberg, Bjorklund, Lindvall & Schmidt, 1982). In 1964, Dahlstrom & Fuxe described the location of a number of supraspinal catecholaminergic cell groups, designated A1 to A14. Of particular interest to this study are the A9 and A11 dopaminergic cell groups. The DA-containing cells of the diencephalic A11 group have been shown by histofluorescence techniques to provide a prominent projection to the spinal cord of the rat (Hökfelt, Phillipson & Goldstein, 1979; Skagerberg *et al.* 1982; Skagerberg & Lindvall, 1985). A partial contribution to the spinal dopaminergic innervation from the nigral A9 DA cell group, has also been suggested by Commissiong, Gentleman & Neff (1979) on the basis of a partial decrease in spinal DA levels following lesions of the nigral region. However, it is possible that these lesions may have interrupted the descending projection from the A11 DA cell group, and other anatomical studies of the A9 region do not support a direct dopaminergic nigrospinal (A9) projection (Hökfelt *et al.* 1979; Skagerberg *et al.* 1982). Radioligand binding studies have indicated the presence of specific DA receptor sites localized in the dorsal horn (Demenge, Feuerstein, Mouchet & Geurin, 1981; Scatton, Dubois & Cudennec, 1984). These receptors may be involved in a behavioural analgesic action of DA at the spinal level, since intrathecal administration of apomorphine, a DA receptor agonist, increased the latency of aversion response in the hot-plate test (Jensen & Yaksh, 1984). This antinociceptive action of apomorphine and also of DA can be attenuated by the stereospecific antagonists, (+)-butaclamol and *cis*-flupenthixol, but not by their enantiomers, indicating a specific receptor-mediated effect (Jensen & Smith, 1983*a*).

The present experiments examine the effects of DA on the responses of dorsal horn neurones to different modalities of cutaneous input. We have also investigated any effects that may be exerted on somatosensory transmission by the supraspinal dopaminergic innervation of the spinal cord by using focal electrical stimulation in the regions of the candidate DA cell groups (A9 and A11) while recording from spinal neurones.

METHODS

Surgical procedures

Experiments were performed on Wistar rats (260–320 g) and cats (2·5–3·5 kg). After induction with halothane, rats were anaesthetized with a mixture of α-chloralose (35 mg/kg I.V.) and urethane (700 mg/kg I.V.) and cats were anaesthetized with α-chloralose (60–70 mg/kg I.V.). In both species, supplementary doses of anaesthetic were given as required, core temperature was maintained at 37–38 °C and carotid blood pressure monitored. Animals were placed in a suitable stereotaxic head holder and spinal frame and dorsal laminectomies were carried out to expose spinal segments, thoracic 13 to lumbar 3 in rats and lumbar 5 to sacral 1 in cats. The dura and pia were retracted and the pool, formed by skin flaps, was filled with mineral oil to prevent dehydration.

In rats, respiration was spontaneous throughout the experiment. Humidified O_2 (0·1–0·2 l/min) was passed through one arm of a Y-shaped tracheal cannula to enrich the inspired air. A craniotomy was performed to allow stereotaxic placement of electrodes at supraspinal sites in rats. Cats were paralysed with gallamine triethiodide (Flaxedil, 15 mg/kg) and artificially respired with room air following bilateral pneumothorax. The effects of gallamine were allowed to wear off

periodically to allow the state of anaesthesia to be assessed. End-tidal CO_2 was maintained between 3·5 and 4·0%. In addition to the lumbar laminectomy, a cervical laminectomy (cervical 1–5) was performed in all cats to allow antidromic identification of spinocervical tract (SCT) neurones, as previously described (Brown, House, Rose & Snow, 1976; Fleetwood-Walker, Mitchell, Hope, Molony & Iggo, 1985).

Electrophysiological methods

Extracellular recordings were made via the central barrel (4 M-NaCl, pH 4·0–4·5) of a seven-barrelled glass microelectrode. Electrode tip sizes were 4·0–4·5 μM and DC resistances were 5–8 MΩ. The bandwidth of the recording amplifier was 1 Hz–7 kHz. One side barrel contained 1 M-NaCl (pH 4·0–4·5) for automatic current balancing and current controls (Neurophore BH2 Ionophoresis System, Medical Systems Corporation). Other barrels contained: 0·1 M-dopamine hydrochloride, 0·01 M-(±)-sulpiride hydrochloride, 0·3 M-SKF38393 hydrochloride, 0·4 M-RU24213 hydrochloride, 0·5 M-naloxone hydrochloride, 0·1 M-idazoxan (RX781094) and 0·1 M-clonidine hydrochloride (all in aqueous solution at pH 4·0–4·5), DL-homocysteic acid (DLH) (in aqueous solution at pH 8·0–8·5) and Pontamine Sky Blue dye (2% in 0·5 M-sodium acetate for marking recording sites at 50 μA minutes). The drug RU24213 hydrochloride was donated by Roussel Laboratories, Swindon; idazoxan by Reckitt & Colman, Hull; clonidine hydrochloride by Boehringer-Ingelheim, Bracklesham; SKF 38393 hydrochloride from Research Biochemical Inc., Waylands, U.S.A.; sulpiride hydrochloride by Ravizza Laboratories, Italy; and naloxone hydrochloride, Endo Laboratories, New York, U.S.A. All drugs were applied with a cathodal current, except DLH (anodal). Retaining currents of 10–15 nA were applied to minimize drug leakage between tests.

All dorsal horn neurones tested were multireceptive, having excitatory responses to both noxious and innocuous cutaneous stimuli. In the rat, some neurones were specifically identified as belonging to the spinothalamic tract. Concentric bipolar electrodes (Rhodes Medical SNE-100) were placed in the region of the contralateral medial lemniscus (anterior (A) 2·5 mm, ventral 5·0 mm, lateral 2·0 mm, with respect to bregma), to provide a search stimulus. The placement of these electrodes was confirmed by recording compound action potentials at the level of the medial lemniscus, evoked from the thoracic ventrolateral quadrant of the spinal cord. Neurones activated from the region of the medial lemniscus were also identified from the ventrolateral posterior thalamus (anterior 3·0–3·5 mm, ventral 4·0 mm, lateral 2·5 mm, with respect to bregma), using standard antidromic criteria (Lipski, 1981) and were defined as spinothalamic (Fig. 1*A*). The stimulation parameters used were: 0·4 ms monophasic square-wave pulses, 50–150 μA, at 1 Hz for the routine search stimulus and 100–330 Hz for tests of following frequency. Using the same stimulation parameters, neurones were also antidromically identified as belonging to the spinomesencephalic tract, in the rat, using bipolar electrodes stereotaxically placed posterior 9·5–10·0 mm, ventral 6·5–7·0 mm and lateral 0·5–1·0 mm, with respect to bregma.

In other rats, bipolar or monopolar electrodes were stereotaxically placed, either in the region of the A11 DA cell group (anterior 3·0–4·0 mm, ventral 4·5–5·0 mm, lateral 0·5 mm, with respect to bregma) or the A9 DA cell group (anterior 2·0–2·5 mm, ventral 2·0 mm, lateral 7·5–8·0 mm, with respect to bregma). The stimulation parameters used were 0·4 ms monophasic square-wave pulses, 10–175 μA at 10–100 Hz. The positions of all stimulating electrodes, were marked by iron deposition from the electrode (30–60 s, 30 μA DC) and verified histologically, following ferrocyanide staining.

In cats, all neurones were identified as belonging to the spinocervical tract (SCT) by their antidromic activation from silver ball electrodes placed on the dorsal cervical cord, according to the criteria of Brown *et al.* (1981).

Test protocols

Action potentials of the recorded neurones could be clearly discriminated from other field potentials and could be evoked by controlled noxious pinch (10 s duration) or radiant heat (ramp 30–48 °C, 5–10 s duration) and innocuous motorized brush (10 s duration). These stimuli were applied to adjacent sites of the ipsilateral receptive field. Responses to noxious and innocuous cutaneous stimuli and DLH-evoked activity (ionophoresed at 5–60 nA for 10–15 s) were regularly repeated over 3 or 4 min cycles. These responses were approximately matched in terms of neuronal firing rate and were always submaximal. Duplicate or triplicate controls varied by less than 15%. Neuronal firing was recorded on FM tape and firing rates (400 ms bins) were plotted on-line,

together with analog signals from the stimulators and the Neurophore, by computer (Cromemco System III). Responses to pinch, heat, brush, DLH and epochs of spontaneous activity were integrated over times appropriate for the particular stimulus and normalized against control responses. These normalized values were expressed graphically to facilitate comparisons of drug effects on different classes of evoked activity. All drugs, except RU24213, were continuously ionophoresed for 1 min before and for the duration of the test cycle. Ionophoretic currents were increased stepwise between each cycle to allow the construction of cumulative-current effect curves for each response. The D_2 DA receptor agonist RU24213 was ionophoresed 30 s before and for the duration of each stimulus-evoked response.

A similar protocol was used to investigate the spinal effects of electrical stimulation in the region of the A9 or the A11 DA cell groups. Stimulation commenced 15–20 s before and continued for the duration of each stimulus-evoked response. To determine whether an effect evoked from either DA cell group was mediated by spinal DA, the selective DA receptor antagonist, sulpiride (Seeman, 1981) was ionophoresed in the vicinity of the dorsal horn neurone being tested.

RESULTS

Characteristics and receptive field properties of the neurones

Spinocervical tract neurones could be driven at frequencies of up to 300 Hz. Latencies were constant within $\pm 0{\cdot}1$ ms and conduction velocities were $53{\cdot}5 \pm 0{\cdot}7$ m/s ($n = 20$, mean $\pm$ S.D.). In rats, spinothalamic tract (STT) and spinomesencephalic (SMT) neurones were antidromically driven from the contralateral ventrolateral thalamus at frequencies up to 300 Hz (Fig. 1*B*), latencies being constant within $\pm 0{\cdot}1$ ms. The conduction velocities of STT cells were $16 \pm 2{\cdot}4$ m/s ($n = 19$, mean $\pm$ S.D.), and of SMT cells were $18 \pm 3{\cdot}5$ ($n = 5$, mean $\pm$ S.D.).

The receptive field characteristics and location of these dorsal horn neurones were similar to those previously described, in both the rat (Menetrey, Giesler & Besson, 1977) and cat (Cervero, Iggo & Molony, 1977; Brown, 1981). Pontamine Sky Blue marks, corresponding to the recording sites of SMT neurones in the rat established that the majority were located in the region of lamina I, whilst STT neurones in the rat and SCT neurones in the cat were located in the deeper dorsal horn laminae III–V. All neurones had cutaneous receptive fields on the ipsilateral hindlimb which remained topographically constant throughout the recording. Prominent excitatory responses could be evoked by both noxious and innocuous cutaneous stimuli. Inhibitory components were rarely observed and were not analysed in detail.

Effects of ionophoretically applied dopamine, dopamine receptor agonists and an antagonist

Dopamine consistently and selectively inhibited neuronal responses to noxious cutaneous stimulation, whilst the responses to innocuous stimulation or DLH-evoked activity were unaffected (Fig. 2). This selective antinociceptive effect was exerted on neurones in both rats (thirty-two out of thirty-four) and cats (fifteen out of seventeen). Cumulative-current effect curves were constructed and enabled direct comparisons of the relative sensitivity to drugs of different neurones and different responses. A summary graph, constructed from seventeen neurones in the rat, tested with the same DA ejection currents over the same time course, is shown in Fig. 3. The nociceptive responses of neurones in both the region of lamina I and laminae III–V of the rat were selectively inhibited by ionophoretically applied DA (Fig. 3). The

selective antinociceptive action of DA was observed irrespective of the order in which the stimuli were tested. Ejection currents of DA (0–49 nA) required to give a 50% reduction in the nociceptive response did not cause any significant change from control levels of either innocuous, DLH-evoked or spontaneous activity (Table 1), thus indicating that the inhibitory action of DA was very highly selective for

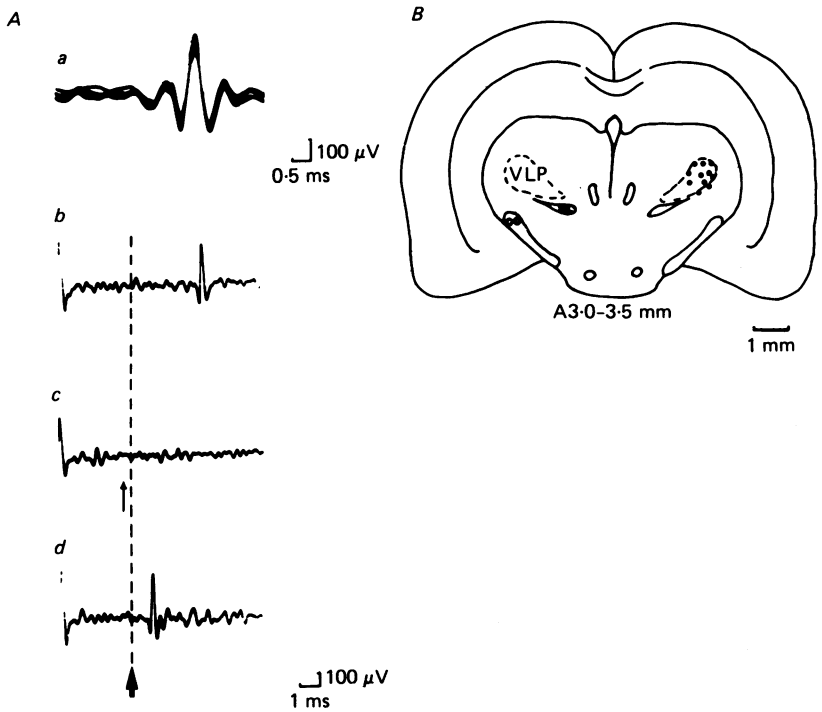

Fig. 1. Antidromic identification of STT or SCT neurones. *A*, collision testing. The example shown was recorded in the rat, from a neurone activated from the contralateral ventrolateral thalamus. Spontaneous action potentials were used to trigger the antidromic stimulus after a delay, here, of 3·5 ms, shown by the dashed line and large arrow. *a*, five superimposed sweeps of an antidromic action potential recorded at the cell body. *b*, the antidromic potential was triggered 3·5 ms after spontaneous action potential. *c*, the trigger delay is reduced below the critical period (3·4 ms), shown by a small arrow, and collision has occurred. *d*, cancellation with a spontaneous action potential which occurred within the critical period. *B*, the positions of stimulating electrodes used to activate STT neurones are shown on a representative transverse section (A 3·0–3·5 mm). VLP, ventrolateral posterior thalmic nucleus.

nociceptive responses. At higher ejection currents (80–120 nA) DA caused some depression of all types of activity monitored. Sodium chloride current controls (0–150 nA) did not affect the levels of any of the evoked responses tested. Dopamine had no effect on two out of thirty-four neurones in the rat and two out of seventeen neurones in the cat, with up to 80 nA ejection current.

The selective antinociceptive action of DA was found to be consistently reversed in the presence of sulpiride, a highly-selective D_2 DA receptor antagonist, ionophoresed in the vicinity of the dorsal horn neurone being tested (Figs 2 and 3).

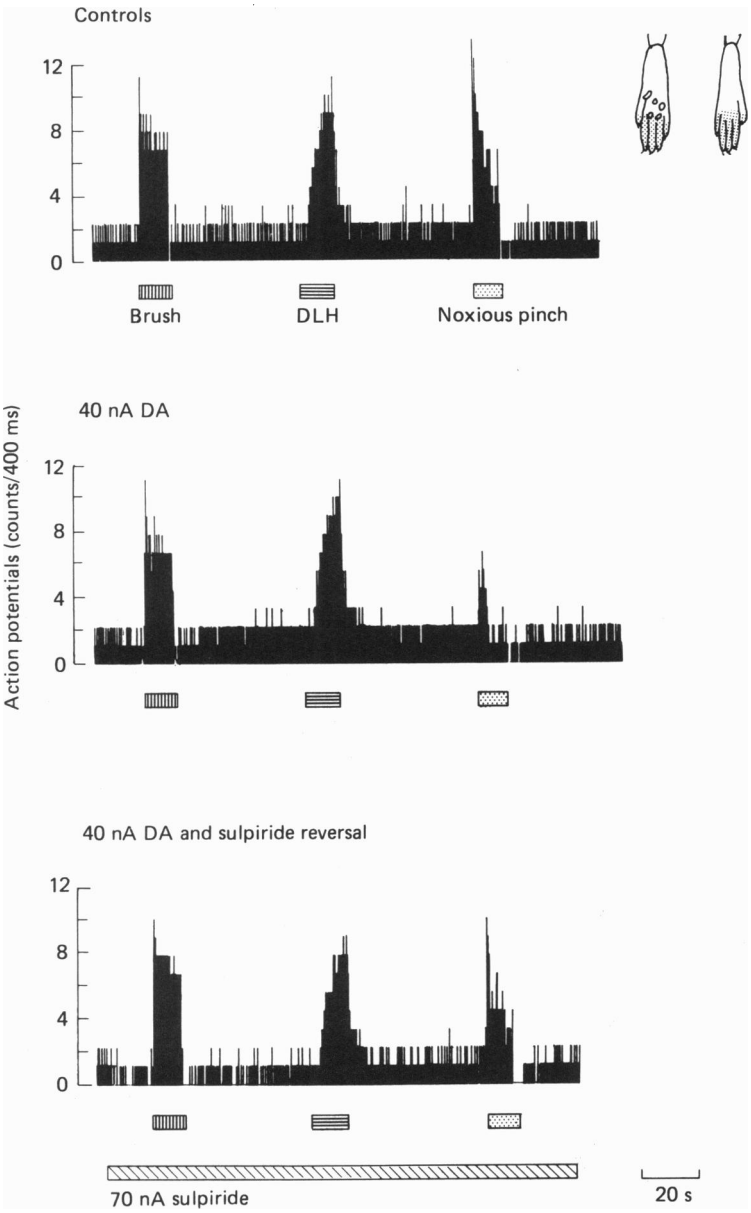

Fig. 2. Effects on ionophoretically applied dopamine on sensory responses of dorsal horn neurones. Continuous records of firing rates of a multireceptive STT neurone, as number of action potentials (counts/400 ms) against time. Prominent excitatory responses to brush, DLH and noxious pinch can be seen on the top row (controls). Typical effects of DA applied ionophoretically, 1 min prior to the test, are shown (middle row), where DA caused a selective inhibition of the response to noxious pinch, with no prominent change in the other responses. Reversal of the action of DA by sulpiride (70 nA) is shown on the bottom row.

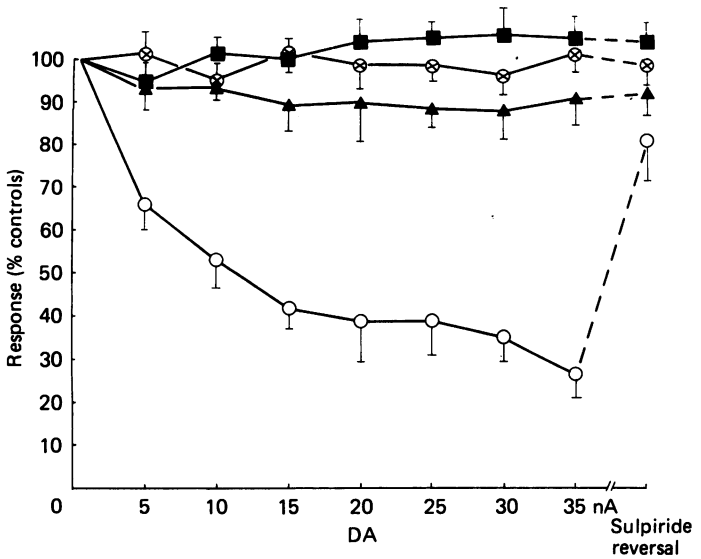

Fig. 3. Selective antinociceptive effect of dopamine on dorsal horn neurones. The graph shows the mean values (±S.E.M.) of the responses to brush (▲), DLH (■), noxious pinch (○), and spontaneous activity (⊗) of seventeen multireceptive dorsal horn neurones. Each neurone was tested using the same ejection currents and time course for the ionophoretic application of DA. The consistent reversal of the action of DA by sulpiride (70–85 nA) is shown. Neurones were located in the region of lamina I and in laminae III–V (twelve out of seventeen).

TABLE 1. Selectivity of the action of ionophoretically applied DA on different populations of multireceptive dorsal horn neurones

Types of dorsal horn neurones tested	Mean ionophoretic currents of DA which caused 50% inhibition of nociceptive responses (nA)	Effects of corresponding ionophoretic currents of DA on other activity (% of initial control values)		
		Brush	DLH	Spontaneous activity
SCT (cat), $n = 15$	22·8±3·5	88·8±2·3	101·0±2·3	96·5±4·5
STT (rat), $n = 8$	24·4±4·1	93·0±1·6	98·1±3·4	104·6±5·2
SMT (rat), $n = 5$	20·3±3·5	94·5±2·6	99·2±4·1	98·2±6·2
Unidentified (rat), $n = 19$	21·2±4·9	92·7±2·7	95·8±3·0	98·2±2·2

Mean and standard error values are shown. Statistical analysis by Student's matched pair t test on raw data, or sign test R statistic on normalized data indicated no significant changes in non-nociceptive activity under these conditions.

Sulpiride alone, ionophoresed continuously for 5–10 min at 50–70 nA, had no effect on any responses monitored and failed to modify the antinociceptive effect of clonidine, an α_2-adrenoreceptor agonist (Fleetwood-Walker *et al.* 1985) in five out of five neurones, even when ionophoresed at 70 nA for 10 min.

The high degree of antagonism of the action of DA by sulpiride (> 80%) suggested

that DA acts primarily via a D_2 DA receptor (Fig. 3). This was investigated further by the use of selective D_1 and D_2 DA receptor agonists. A highly selective D_2 DA receptor agonist, RU24213, (Euvrard, Ferland, DiPaolo, Beaulieu, Labrie, Oberlander, Raynaud & Boissier, 1980) mimicked the action of the DA (Fig. 4*A*). Continuous ionophoresis of this drug was not required to elicit selective antinociception. Intermittent ionophoresis (5–15 nA) of RU24213, as described in the Methods, powerfully inhibited nociceptive responses, whilst other activity was unaffected in all five neurones tested. In contrast, continuous ionophoresis of SKF38393, the highly selective D_1 DA receptor agonist (Setler, Sarau, Zirkle & Saunders, 1978), at 20–100 nA for 5–10 min had no effect (Fig. 4*B*).

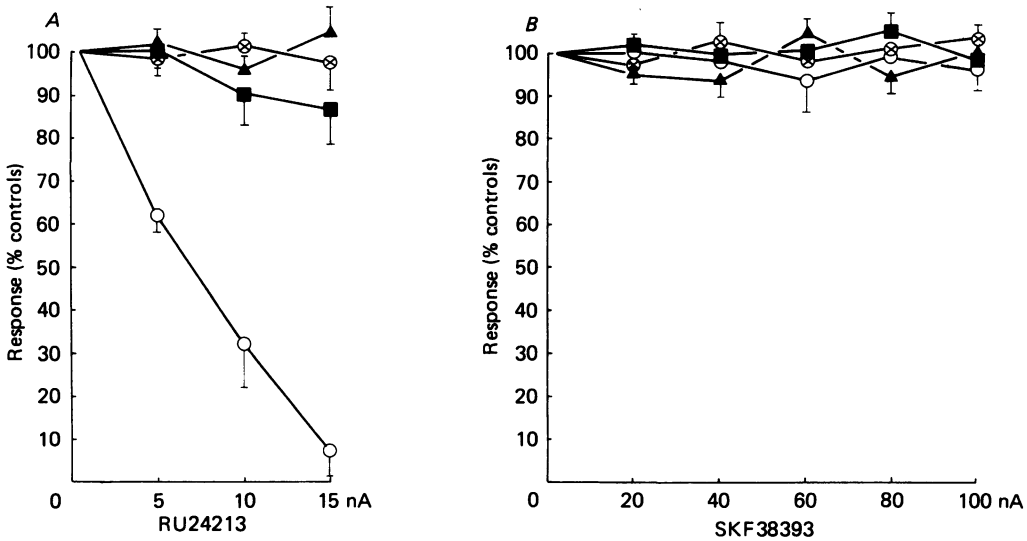

Fig. 4. Effects of agonists selective for dopamine receptor subtypes. Responses to brush (▲), DLH (■), noxious pinch (○) and spontaneous activity (⊗). *A*, the selective D_2 DA receptor agonist, RU24213, potently mimicked the selective antinociceptive action of DA. The graph shows the mean values (±S.E.M.) from five neurones tested. *B*, the selective D_1 DA agonist, SKF 38393, did not affect the evoked responses tested, using up to 100 nA. The graph shows the mean values (±S.E.M.) from three neurones tested.

Effect of focal electrical stimulation in the region of dopamine cell groups, A9 and A11

Focal electrical stimulation of the region of the ipsilateral A11 DA cell group of the rat caused a selective inhibition of the nociceptive responses of nineteen out of twenty-one multireceptive dorsal horn neurones, located in both lamina I and laminae III–V. Three to six minutes after electrical stimulation was discontinued, nociceptive responses returned to within 80% of control levels (Figs 5 and 6). In contrast, innocuous, DLH-evoked and spontaneous activity were unaffected throughout these tests (Figs 5 and 6). The selective stimulus-evoked effect could only be elicited from a restricted area that contained the dopaminergic cells of the A11 group, as described by Bjorklund & Nobin (1973) and Jacobowitz & Palkowitz (1974) and observed in histofluorescent studies undertaken in this laboratory (P. J. Hope &

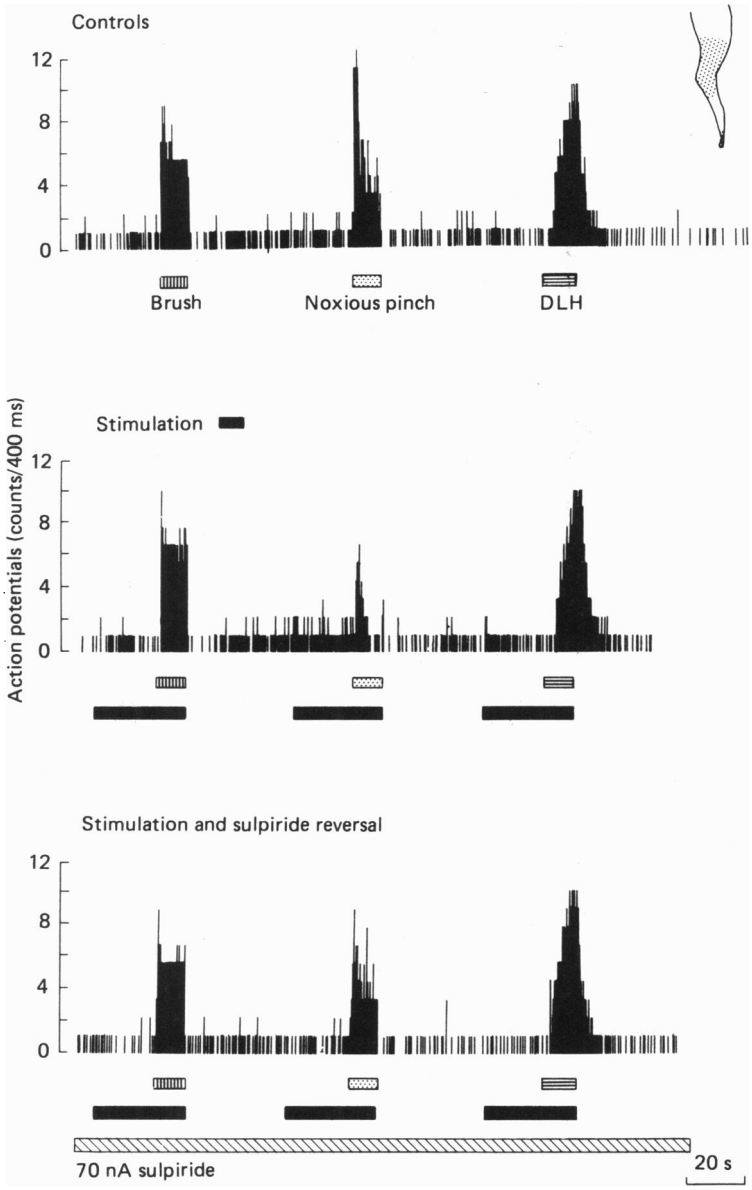

Fig. 5. Effects of electrical stimulation in the region of the A11 cell group. Continuous firing rates of a multireceptive dorsal horn neurone as number of action potentials (counts/400 ms) against time. Prominent excitatory responses to brush, DLH and noxious pinch can be seen on the top row. Typical effects of focal electrical stimulation in the region of the A11 group (40 μA, 100 Hz) are shown (middle row), where stimulation caused a selective inhibition of the response to noxious pinch, with no prominent change in the other responses. This effect was reversed when sulpiride was ionophoresed in the vicinity of the dorsal horn neurone being tested (bottom row).

S. M. Fleetwood-Walker, unpublished observations). Similar effects could not be evoked 0·5–1·0 mm outside the region of the A11 DA cell group (Fig. 7) or from the contralateral region. The onset of the antinociceptive effect was optimally observed 15–20 s after electrical stimuli had commenced, using a range of stimulation frequencies (10–100 Hz). At lower frequencies (10–33 Hz) it was found that generally higher stimulation currents (mean = 120 μA) were required whilst at higher frequencies (50–100 Hz) lower current levels (mean = 30 μA) were sufficient to evoke similar effects on the same neurone.

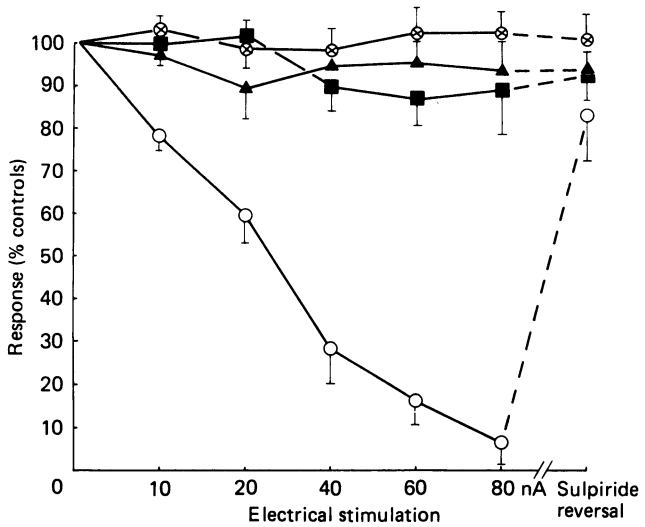

Fig. 6. Selective antinociception elicited by electrical stimulation in the region of the A11 cell group. The graph shows the mean values (± S.E.M.) of the responses to brush (▲), DLH (■), noxious pinch (○) and spontaneous activity (⊗), of seventeen multireceptive dorsal horn neurones, tested using the same time courses and levels of stimulation current (100 Hz) in the region of the A11 DA cell group. The consistent reversal of this stimuli-evoked antinociceptive effect by ionophoretically applied sulpiride (70–85 nA) is shown.

The selective inhibition of nociceptive responses by focal electrical stimulation in the region of the A11 DA cell group was reversed by ionophoresis of sulpiride in the vicinity of dorsal horn neurones being tested (Figs 5 and 6). This reversal of the stimulus-evoked effect was consistently observed in all seventeen neurones so tested (Fig. 6). Using the same protocol, ionophoretic application of naloxone, the opioid receptor antagonist, or idazoxan, an α_2-adrenoreceptor antagonist (70–100 nA, 5–10 min), failed to mimic the action of sulpiride in all four neurones tested. Subsequent testing confirmed that ionophoretically applied DA had a consistently selective antinociceptive action on these neurones.

Using the same stimulation protocol, no significant effect was evoked from either the contralateral or ipsilateral region of the A9 cell group, on five multireceptive neurones tested, although ionophoretically applied DA was still effective (Fig. 7).

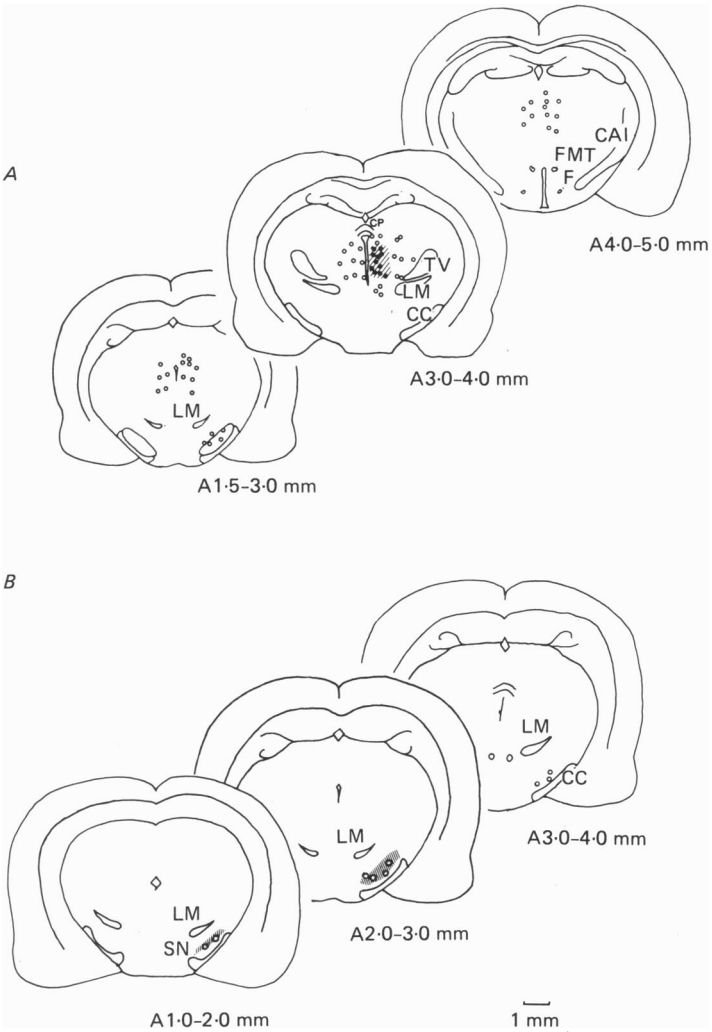

Fig. 7. Location of diencephalic stimulation sites eliciting antinociceptive effects. Shows the stereotaxic placement of stimulating electrodes on representative transverse sections of the rat brain (A2·0–5·0 mm, and according to Jacobowitz & Palkowitz, 1974). ●, sites from which a selective antinociceptive effect on multireceptive dorsal horn neurones was electrically evoked. These sites were all ipsilateral to the neurone being tested and were located in the region of the A11 DA cell group (*A*) (as described by Bjorklund & Nobin, 1973; Jacobowitz & Palkowitz, 1974; and as seen in this laboratory), shown by the hatching. Stimulation outside this region or contralateral to the neurone being tested or in the region of A9 DA cell group (*B*) had no apparent effect (○). FMT, fasciculus mamillothalamicus; LM, lemniscus medialis; TV, nucleus ventralis thalamis; CC, crus cerebri; CAI, capsule interna; F, fornix; SN, substantia nigra.

DISCUSSION

The use of compounds highly selective for D_1 and D_2 DA receptor subtypes provides direct evidence that ionophoretically applied DA exerts its antinociceptive actions via D_2 DA receptors, at the level of the dorsal horn.

Spinal D_2 DA receptors are also implicated in the mediation of the selective antinociceptive effect elicited by discrete electrical stimulation in the region of the A11 DA cell group. However, the precise location in the spinal cord of these receptor sites is not clear. A direct action on the recorded multireceptive neurone might be expected to result in non-selective inhibition of all types of neuronal activity, and indeed electron microscopic investigation of the dendritic trees of SCT cells has so far failed to reveal synaptic profiles with the dense core vesicles that are characteristic of monoamines (D. J. Maxwell, unpublished observations). These findings indicate a presynaptic action or an action on an interneurone, rather than postsynaptic dopaminergic action, although final confirmation that DA does not act directly requires intracellular recordings.

The most likely location of these DA receptors may be interneurones, perhaps closely antecedent to the neurones tested in this study, considering the profound action of low ionophoretic currents (5–20 nA) used. In view of the action of DA on neurones in both lamina I and laminae III–V, such interneurones may be located throughout the dorsal horn.

An antinociceptive action of DA, mediated via the ventral horn, is unlikely. Dopamine has been reported to increase the amplitude of ventral root field potentials (Barasi & Roberts, 1977). If this was the primary action, DA may be expected to enhance spinal reflexes; however, DA has been shown to inhibit the spinal tail-flick response in behavioural studies, when administered intrathecally in spinal rats (Jensen & Smith, 1983*a*; Barasi & Duggal, 1985*a*).

Behavioural studies also support the proposal that the selective antinociceptive actions of DA, described in this study, are mediated via D_2 DA receptors. Barasi & Duggal (1985*a*) reported that intrathecal administration of LY171555, a D_2 DA receptor agonist, significantly increased the tail-flick latency of lightly anaesthetized rats, whilst SKF39383, a D_1 DA receptor agonist, had no effect. In accordance with these observations, autoradiographic studies using ligands predominantly selective for D_2 DA receptors, have reported binding sites concentrated in the dorsal horn of the rat (Demenge *et al.* 1981; Scatton *et al.* 1984).

The involvement of the D_2 DA receptors in the spinal antinociceptive effects resulting from electrical stimulation of the region of the A11 DA cell group is also indicated by its attenuation due to sulpiride released in the immediate vicinity of the neurone tested (Fig. 6). The failure of both ionophoretically applied naloxone and idazoxan to reverse the antinociceptive action of focal electrical stimulation in the region of the A11 DA cell group is evidence against the involvement of opioid or α_2-receptors in this study, and supports the specificity of the effect. Furthermore, the antinociceptive action of clonidine (Fleetwood-Walker *et al.* 1985) was not attenuated by the presence of ionophoretically applied sulpiride. The focal electrical stimulation protocol used in this study most probably did not activate only the dopaminergic cells of this region. However, the potent antagonism of this stimulus-evoked effect by sulpiride implies that the antinociceptive effect was primarily mediated by DA, at

least at the spinal level, and therefore originated from the cells of the A11 DA group. These findings also support the view that DA is the major catecholamine present in cells of A11 group cells, as indicated from immunohistofluorescence studies (Hökfelt *et al.* 1979).

The finding that discrete electrical stimulation of the region of the A11 DA cell group was effective on dorsal horn neurones ipsilateral, but not contralateral, to the stimulation site is in agreement with histofluorescence studies which have reported that the spinal catecholaminergic projection from this group is predominantly uncrossed (Hökfelt *et al.* 1979; Skagerberg *et al.* 1982). The spinal D_2 DA receptors that mediated this stimulus-evoked effect are presumably the same population as those mediating the antinociceptive action of ionophoretically applied DA on the dorsal horn neurones.

In agreement with our earlier observations (Fleetwood-Walker & Hope, 1985) and those of Barasi & Duggal (1985*b*), the present study failed to demonstrate any stimulus-evoked action, from the region of the A9 DA cell group, on somatosensory transmission through the dorsal horn. Therefore, the A11 DA cell group may provide the major (and perhaps only) source of dopaminergic innervation of the spinal cord that affects somatosensory processing.

Previous studies have implicated direct diencephalo-spinal projections in the modulation of spinal neurones (Hancock, 1976; Hosoya, 1980; Skagerberg & Lindvall, 1985). The present study provides strong evidence that diencephalic A11 cells provide a direct projection that has a selective antinociceptive role, and is mediated by DA at the spinal level. The selective inhibition by DA of nociceptive inputs to neurones with long ascending axons in both the superficial and the deeper dorsal horn (SCT, SMT and STT neurones) indicates that the dopaminergic system can regulate not only spinal reflex processing but also the transfer of information to supraspinal levels. In this preparation, the dopaminergic system is not tonically active and therefore the question as to how such antinociceptive systems are normally activated remains to be answered. Furthermore, evidence for interactions between spinal dopamine and other antinociceptive systems, such as serotonin and noradrenaline (Jensen, Schroder & Smith, 1984; Jensen & Smith, 1983*b*), suggests that the integrated influence of multiple antinociceptive systems should be considered.

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