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ADRENERGIC AND OPIOIDERGIC MODULATION OF A SPINAL REFLEX IN THE DECEREBRATED RABBIT

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SUMMARY

1. In the decerebrated and spinalized rabbit, electrical stimulation of the sural nerve evokes a short-latency reflex in the ipsilateral ankle extensor gastrocnemius medialis (GM) which is tonically suppressed by endogenous opioids. In the present study we have investigated the inhibitory influences affecting this reflex in non-spinalized, decerebrated rabbits.

2. In non-spinalized rabbits, the thresholds and latencies of the sural–GM reflex were significantly higher than in spinalized preparations. The opioid antagonist naloxone and the α-adrenoceptor antagonist idazoxan potentiated the reflex in both preparations. Naloxone was significantly more effective in spinalized rabbits whereas idazoxan had a much larger effect in non-spinalized animals.

3. When the spinal cord was sectioned in the presence of naloxone alone, the GM reflex always increased in size. An ipsilateral hemisection of the cord was as effective as total section in this respect. When the section was performed in the presence of idazoxan and naloxone, the response usually decreased in size.

4. The α_2-adrenoceptor agonist clonidine depressed the reflex in spinalized rabbits, an action that was reversed by idazoxan but not by naloxone.

5. These data show that in the decerebrated, non-spinalized rabbit, the sural–GM reflex is tonically suppressed by endogenous opioids, presumably acting at the segmental level, and by an ipsilateral descending pathway which involves an α-adrenoceptor-mediated synapse. Activity in this descending pathway masks the facilitatory effects of opioid antagonists on spinal reflexes in this preparation.

INTRODUCTION

The reflex evoked in the ankle extensor gastrocnemius medialis (GM) by stimulation of the ipsilateral sural nerve in the decerebrated, spinalized rabbit is potently and stereospecifically enhanced by opioid antagonists (Catley, Clarke & Pascoe, 1983; Clarke & Ford, 1987), and it is likely that some neurones in this reflex arc are subject to a tonic inhibition by endogenous opioids. In preliminary experiments it was found that naloxone had a relatively weak effect on the sural–GM reflex when the spinal cord was intact (Clarke & Ford, 1985). This paper gives a full

account of the differences between reflexes recorded from non-spinalized as compared to spinalized decerebrated rabbits, both in their physiological characteristics and in their sensitivity to opioid antagonists. The role of descending adrenergic systems in mediating the differences between these two preparations has also been investigated. Some of these data have been published in the form of abstracts (Clarke & Ford, 1985; Clarke, Ford & Taylor, 1987).

METHODS

Experiments were performed on fifty-six rabbits of either sex weighing from 2·2 to 3·5 kg. Anaesthesia was induced by intravenous injection of methohexitone sodium (Brietal, Eli Lilly, 20 mg initially and supplemented as necessary), and maintained after cannulation of the trachea by 2–4% halothane (Fluothane, ICI) in oxygen or oxygen–nitrous oxide (30:70). One carotid artery and a jugular vein were cannulated and the second carotid artery was ligated.

All rabbits were decerebrated by the following method. An extensive mid-line craniotomy was performed to reveal the cerebral hemispheres. The dura was peeled back and the contents of the cranium as far as the rostral edge of the superior colliculi were removed by gentle suction. Large blood vessels were closed with aluminium clips and small bleeds were controlled with activated cellulose (Surgicel, Johnson & Johnson). A laminectomy was performed at T11–L1 in all rabbits, and twenty-three animals were spinalized at this level prior to decerebration. All animals were paralysed with gallamine triethiodide (Flaxedil, May & Baker; 4 mg/kg initially and supplemented as necessary) and artificially ventilated to maintain end-tidal CO_2 below 5·5%. In some experiments the inspired gas was supplemented with 100% O_2. Arterial blood pressure was monitored through the carotid cannula and core temperature was maintained at $38 \pm 0{\cdot}5$ °C by a thermostatically controlled heating blanket.

The sciatic nerve and its branches were exposed in the left popliteal fossa. The sural nerve was cut and the central end stimulated electrically at 1 Hz with square-wave pulses of 0·1 ms duration applied through paired platinum electrodes; evoked afferent activity was recorded at a more central location. The sural nerve was stimulated at a strength sufficient to excite the Aβ and Aδ axons. Reflex responses to these stimuli were recorded from the ipsilateral GM muscle nerve using twin platinum electrodes. Responses to eight successive stimuli were averaged and quantified as the voltage–time integral (area). Anaesthesia was discontinued after the nerve dissection was completed, and a recovery period of at least 1 h was allowed before recording was commenced. The size and latency of the reflex was monitored every 4 min and the threshold for each response, which was expressed in multiples of the threshold for the most excitable axons in the sural nerve (T_{su}), was determined every 10 min.

Twelve non-spinalized and seven spinalized rabbits were given naloxone in increasing doses from 0·5 to 200 μg/kg I.V. (total dose 388·5 μg/kg, dose interval 24 min). Where the spinal cord was still intact, it was sectioned at T11–T12 after the last dose of naloxone. This was completed either at one stroke, or after hemisections of the cord.

Fifteen non-spinalized and nine spinalized rabbits were used to study the effects of the α_2-adrenoceptor antagonist idazoxan, and a group of seven spinalized animals was used to investigate the effects of the α_2-agonist clonidine. Where arterial blood pressure fell below 60 mmHg with drug treatment, it was restored with a slow intravenous infusion of adrenaline tartrate (Antigen, 20–40 μg/ml given to effect). Infusions of adrenaline alone did not affect reflex responses.

The drugs used in these experiments were naloxone hydrochloride (a gift of Sterling-Winthrop), dissolved in Ringer–Dale solution or 0·9% NaCl solution to 1 or 2 mg/ml; idazoxan hydrochloride (a gift of Dr S. L. Dickinson, Reckitt & Colman), dissolved in Ringer–Dale solution so that the total volume of each injection was 1 ml; and clonidine hydrochloride (Sigma) dissolved as described above for idazoxan.

The absolute sizes of responses in GM were widely variable between experiments, and the data were normalized for most analyses. Statistical comparisons were by Student's t test. The use of the word 'significant' implies a probability level of less than 5%.

TABLE 1. The thresholds, latencies and absolute magnitudes (areas) for the sural–GM reflex in spinalized and in non-spinalized rabbits

		Threshold T_{su}			Latency (ms)		
	n	Mean	S.D.	Range	Mean	S.D.	Range
Spinalized	23	2·3	1·1	1·0–5·1	5·6	0·3	4·8–6·3
Non-spinalized	33	18	27	1·3–93	6·6	0·8	5·1–8·5

	Area (μV ms)		
	Mean	S.D.	Range
Spinalized	144	116	33–441
Non-spinalized	93	138	5–542

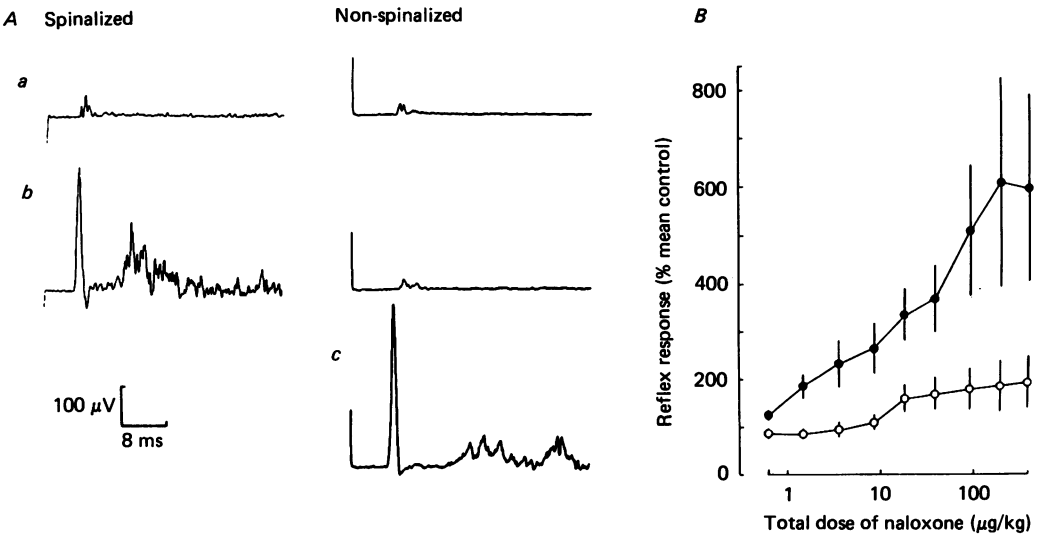

Fig. 1. *A*, GM reflex responses to stimulation of all myelinated sural nerve fibres in spinalized and in non-spinalized decerebrated rabbits: *a*, in the control period; *b*, after a cumulative dose of naloxone (388·5 μg/kg I.V.); *c*, after spinalization. Each trace is the average of eight sweeps, and the stimulus was applied at the beginning of each sweep. *B*, normalized dose–effect curves for the action of naloxone on the sural–GM reflexes in spinalized (●, $n = 7$) and non-spinalized (○, $n = 12$) rabbits. Each point is a mean ± S.E. of mean.

RESULTS

The mean thresholds, latencies and absolute sizes of GM responses to sural nerve stimulation in spinalized and in non-spinalized rabbits are shown in Table 1. The latencies and thresholds were significantly lower in the spinalized than in the non-spinalized rabbits ($P < 0·05$).

The effects of naloxone

In spinalized animals, naloxone caused a dose-dependent increase in the size of the reflex response to an average of 641 % of pre-treatment controls when the total dose given was 188·5 μg/kg (Fig. 1). In addition, the latency of the GM response fell by

an average of 0·7 ms and the threshold decreased by $1 \times T_{su}$. Naloxone was significantly less effective in enhancing the reflex in non-spinalized rabbits. At the saturating dose of 188·5 μg/kg, the GM response was 194 % of pre-treatement controls (Fig. 1). When naloxone was given to non-spinalized rabbits as a bolus dose of 250 μg/kg the response increased to 214 $\pm$ 68 % (mean $\pm$ S.E.M., $n = 10$) of controls. In non-spinalized animals naloxone caused a significant decrease in the latency of the

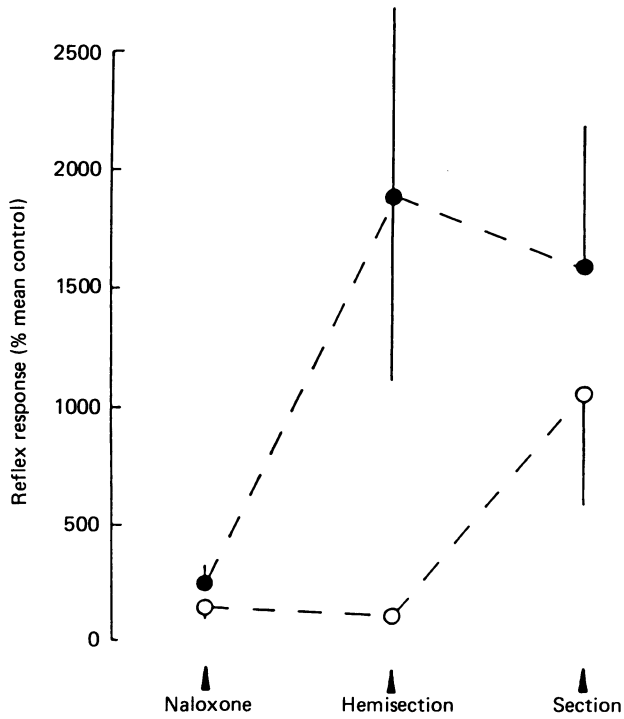

Fig. 2. The effects of spinal hemisections and subsequent total cord section on the sural–GM reflex in non-spinalized rabbits treated with naloxone (388·5 μg/kg I.V. total dose). ●, ipsilateral cord sectioned first ($n = 4$). ○, contralateral cord sectioned first ($n = 4$). Each point is a mean $\pm$ S.E. of mean.

reflex (mean change 0·3 ms, $P < 0{\cdot}025$), and there was also a decrease in threshold (by an average of $13 \times T_{su}$, $P < 0{\cdot}025$). In the spinalized rabbits naloxone always caused the GM reflex to increase in size, whereas only fourteen of the twenty-two non-spinalized animals showed increases, in one there was no change and in seven the response decreased to less than 90 % of pre-drug levels.

In the twelve animals which received the 388·5 μg/kg cumulative dose of naloxone, the spinal cord was sectioned without further treatment. After total section of the cord, the GM response increased to 1016 $\pm$ 246 % of pre-naloxone control levels (e.g. see Fig. 1). The latency and threshold of the reflex decreased so that they were statistically indistinguishable from those obtained in the spinalized rabbits in the presence of saturating doses of naloxone. The effects of ipsilateral and contralateral hemisections are shown in Fig. 2. It is clear that cutting the ipsilateral cord at the lower thoracic level completely released the reflex from descending

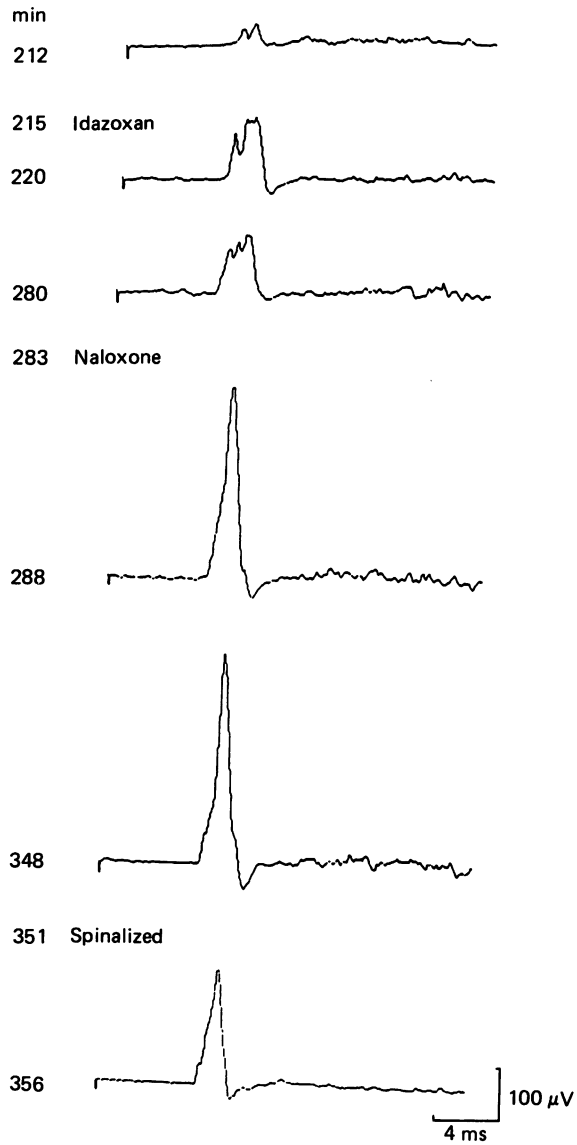

Fig. 3. Non-spinalized rabbit. Records of the sural–GM reflex before and after a single dose of idazoxan (2 mg/kg I.V.); a subsequent dose of naloxone (0·25 mg/kg I.V.); and after a spinal section. Each record is the average of eight sweeps, and the stimulus was given at the beginning of each sweep. The numbers to the left of each trace indicate the time into the experiment in minutes.

inhibition. Hemisections of the cord caused mean arterial blood pressure to decrease by an average of 14 mmHg regardless of the side sectioned.

The effects of idazoxan

Seven untreated, non-spinalized rabbits received the α_2-adrenoceptor antagonist idazoxan. When this drug was given at 2 mg/kg I.V. the reflex was potentiated

to a mean of 699 ± 116 % of pre-drug levels and latencies and thresholds decreased significantly ($n = 7$). Significant enhancement was seen at a dose of 0·02 mg/kg (Fig. 4). When naloxone (0·25 mg/kg) was administered in the presence of idazoxan (2 mg/kg) the size of the discharge in GM always increased, to an average of 943 ± 156 % of pre-drug controls, (see Fig. 3) and the latency decreased in six of the seven animals. Naloxone caused significantly larger increases in the absolute sizes of the response when given in the presence of idazoxan than when given alone ($P < 0·025$).

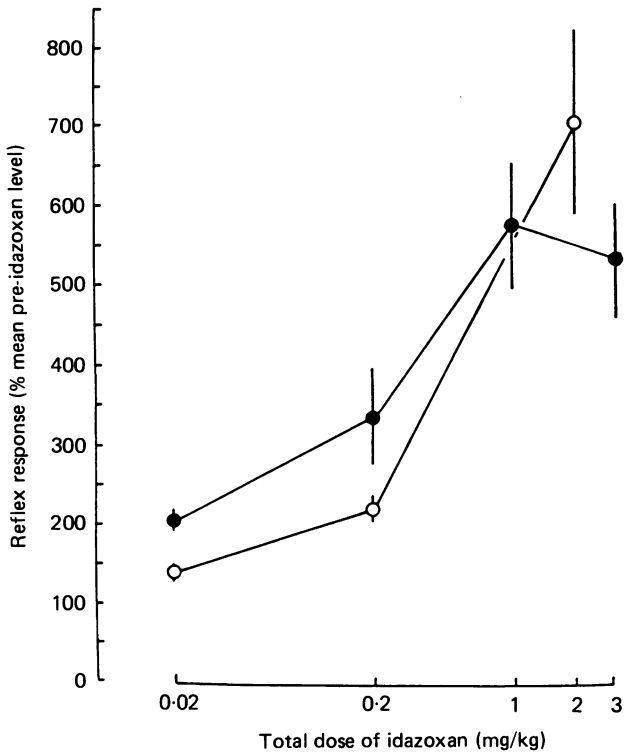

Fig. 4. Normalized dose–effect curves for the action of idazoxan on the sural–GM reflex in non-spinalized rabbits pre-treated with naloxone (●) or not pre-treated (○). Each point is a mean ± S.E. of mean. For doses < 1 mg/kg, $n = 5$; for doses > 1 mg/kg, $n = 8$ (naloxone pre-treated) or $n = 7$ (no pre-treatment).

Eight non-spinalized rabbits which received the bolus dose of naloxone were subsequently given idazoxan (0·02–3 mg/kg). After idazoxan (1 mg/kg) the sural–GM reflex increased to an average of 1142 ± 162 % of pre-drug controls, i.e. the size of the response increased to five times the post-naloxone level (Fig. 4). This was accompanied by significant decreases in latency compared to post-naloxone values (mean changes were 0·6 ms and $1·5 \times T_{su}$, $P < 0·01$ for both). In the presence of naloxone, the lower doses of idazoxan (0·02 and 0·2 mg/kg) caused significantly larger percentage increases in the GM response than when administered alone (Fig. 4).

Fourteen rabbits were spinalized in the presence of saturating doses of naloxone

and idazoxan. In nine animals the GM response decreased in size (Fig. 3), in three there was no change and two showed an increase. Overall there was a statistically significant decrease in the area of this response on spinalization ($P < 0{\cdot}05$). The threshold for the reflex also decreased but there were no significant changes in latency.

As a control, idazoxan was given in a dose of 2 mg/kg to nine untreated, spinalized rabbits. The GM response increased in size to $138 \pm 3\%$ of controls but no other parameters showed consistent changes.

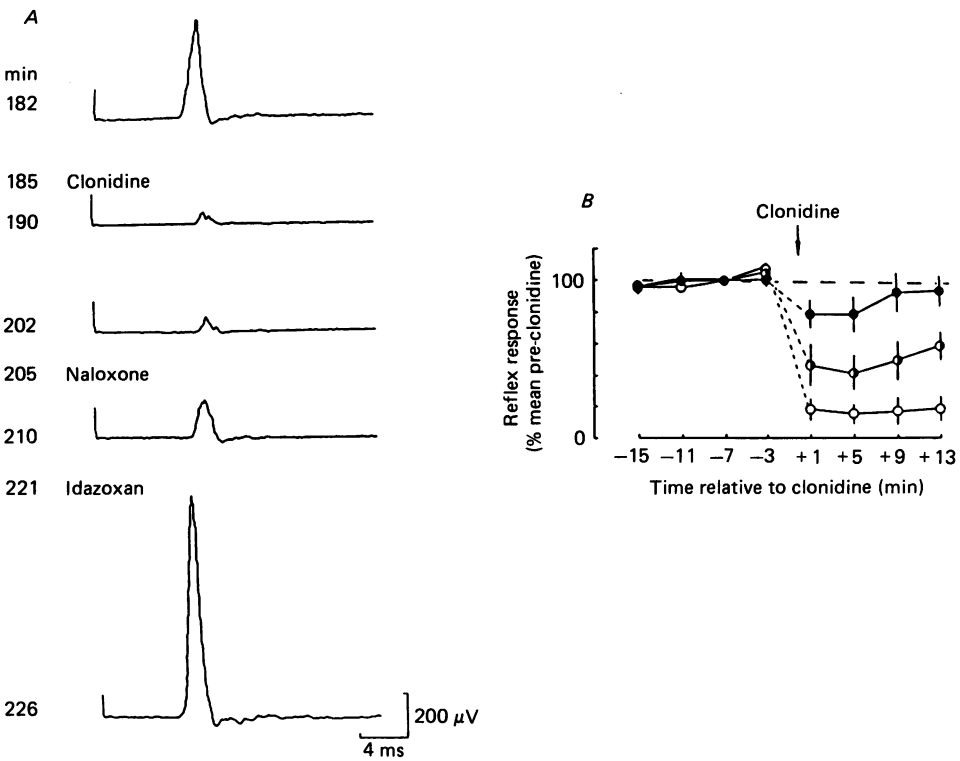

Fig. 5. *A*, spinalized rabbit. Records of the sural–GM reflex before and after clonidine (0·7 mg/kg), naloxone (0·25 mg/kg) and idazoxan (1 mg/kg). Each record is the average of eight sweeps and the stimulus was applied at the beginning of each sweep. The numbers on the left show the time in minutes into the experiment. *B*, the effects of clonidine at 0·01 (●), 0·1 (◑) and 0·7 (○) mg/kg on the sural–GM reflex in spinalized rabbits. Each point is the mean ± S.E. of mean; $n = 7$ for all points.

The effects of clonidine

The α_2-agonist clonidine was given to seven spinalized rabbits. This drug caused a dose-dependent suppression of the sural–GM reflex so that within 5 min of an injection of 0·7 mg/kg I.V. the response was $16{\cdot}6 \pm 6\%$ of pre-injection controls (Fig. 5). There were concomitant increases in the threshold and latency of the reflex. When naloxone (0·25 mg/kg) was given in the presence of the last dose of clonidine, the reflex increased to an average of $235 \pm 15\%$ of post-clonidine levels (i.e. 33% of pre-clonidine levels). Six animals were given idazoxan (1 mg/kg) after clonidine and

naloxone. In these preparations the area of the reflex increased and the latency and threshold decreased to levels which were not significantly different from those obtained when naloxone was given alone, i.e. idazoxan antagonized the effect of clonidine and released the action of naloxone (see Fig. 5*A*).

DISCUSSION

The effects of naloxone

In spinalized rabbits, naloxone had a powerful facilitatory effect on the GM response evoked by all myelinated sural afferents which was indistinguishable from that which it has on responses evoked by Aβ afferents alone (cf. Clarke & Ford, 1987). This effect is almost certainly the result of antagonism of endogenous opioids present in the spinal cord, which suppress non-selectively the reflex drive from all sural myelinated afferent fibres (Catley *et al.* 1983; Clarke & Ford, 1987).

The sural–GM reflex in the non-spinalized rabbit was significantly less sensitive to the action of naloxone than that recorded in the spinalized preparation. A similar phenomenon has been observed in the cat, in which species naloxone enhances the hindlimb C-fibre-evoked flexion reflex in the spinalized but not in the intact preparation (Bell & Martin, 1977; Bell, Sharpe & Pickworth, 1985). The recent finding that damage to the spinal cord causes a naloxone-reversible decrease in blood flow to the cord (Faden, Jacobs, Smith & Zivin, 1984) suggests that spinalization itself could be the stimulus to the release of endogenous opioids and that their action may be secondary to alterations in blood flow. However, the fact that spinal hemisections had differential effects on the sural–GM response renders this explanation unlikely.

The high thresholds and latencies of reflex responses recorded in non-spinalized animals suggest that there is tonic descending inhibition of the reflex, and this probably accounts for the relatively weak effect of naloxone in these rabbits. It appears that this naloxone-insensitive descending system is so powerful that removal of the segmental opioidergic inhibition by naloxone has only a small effect on transmission through the reflex pathway as a whole.

The role of noradrenaline

In the decerebrated cat, both excitatory and inhibitory reflex interneurones in the spinal cord are under a powerful, tonic descending inhibition which arises from the brain stem (see Lundberg, 1964; Engberg, Lundberg & Ryall, 1968). The possibility that noradrenaline is involved in brain stem control of spinal neurones has been under consideration for many years (Engberg *et al.* 1968; see also Duggan, 1985; Fitzgerald, 1986; for reviews), but studies on the tonic or evoked activity of adrenergic inhibitory systems have been hampered by the lack of selective antagonists (Duggan, 1985). Idazoxan (formerly RX781094) is an antagonist at adrenergic α-receptors with some selectivity for the α_2-subtype (Doxey, Roach, Strachan & Virdee, 1983; Berridge, Roach, Strachan & Virdee, 1984; Megens, Leysen, Awouters & Niemegeers, 1986). In the rabbit, doses up to 1 mg/kg I.V. failed to antagonize the increase in blood pressure caused by the selective α_1-agonist phenylephrine (Hannah, Hamilton & Reid, 1983). There is little evidence that idazoxan has actions at other types of receptor.

In the present experiments idazoxan caused a greater increase in the sural–GM reflex in the non-spinalized than in the spinalized preparations. The results obtained in non-spinalized rabbits could be due to blockade of descending inhibition, to enhancement of descending facilitation or to a combination of these actions. The results of spinalizing animals in the presence of naloxone alone and in the presence of naloxone with idazoxan show that descending systems of opposite action are active in the decerebrated rabbit. Recent work in this laboratory has shown that idazoxan applied directly to the spinal cord has a facilitatory effect on reflexes which is similar to that seen on intravenous administration (Clarke, Ford, Harris & Taylor, 1988). Further, the actions of clonidine in spinalized rabbits show that activation of α-receptors in the spinal cord causes a profound suppression of reflex responses. On the basis of these observations we believe that the major effect of idazoxan is mediated by an antagonist action at inhibitory adrenergic synapses in the spinal cord. The fact that clonidine is effective in spinalized rabbits indicates that the α-receptors responsible for causing depression of reflexes are probably postsynaptic to adrenergic terminals. It is interesting to note that clonidine restricted the potentiating action of naloxone in spinalized rabbits; this shows that activity in an adrenergic descending pathway could well explain the weak effects of naloxone in non-spinalized rabbits.

The finding that idazoxan caused a small potentiation of reflexes in spinalized animals was quite unexpected. Fleetwood-Walker, Mitchell, Hope & Molony (1987) have reported that responses to noxious stimuli of spinocervical tract neurones are inhibited by microelectrophoretic application of κ-opioids and that this effect can be blocked by idazoxan. Although endogenous opioids are tonically active in spinalized rabbits, their effects are mediated almost exclusively through μ-opioid receptors (Clarke & Ford, 1987), which do not appear to activate spinal adrenergic mechanisms (Fleetwood-Walker *et al.* 1987). The effects of idazoxan in spinalized rabbits may have been due to residual release of noradrenaline from the terminals of severed axons. The doses of idazoxan and clonidine used in the present studies were too high to be certain of the types of α-receptor involved, but other laboratories have shown that α_2-receptors are of major importance in descending inhibition (see Fitzgerald, 1986, for review), and in the mediation of the inhibitory effects of noradrenaline on spinal neurones (Fleetwood-Walker, Mitchell, Hope, Molony & Iggo, 1985).

The catecholamine-containing cell groups of the rabbit brain and their projections to the thoracic spinal cord have been carefully mapped by Blessing and co-workers (Blessing, Chalmers & Howe, 1978; Blessing, Goodchild, Dampney & Chalmers, 1981). In agreement with our findings, most catecholaminergic projections to the spinal cord are organized ipsilaterally.

Interactions between opioid and adrenergic systems

The opioid receptors involved in the tonic suppression of reflexes in the rabbit are predominantly of the μ-type (Clarke & Ford, 1987). In neurones from rat locus coeruleus, activation of opioid μ-receptors increases a potassium conductance which is also increased by adrenergic α_2-receptor agonists (North & Williams, 1985). This raises the possibility that adrenergic descending inhibition and segmental opioidergic effects on reflexes are mediated on the same population of neurones in the spinal

cord; it could also account for the synergistic effects of co-administration of idazoxan and naloxone in non-spinalized animals.

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