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Behavioural analysis of changes in nociceptive thresholds produced by remoxipride in sheep and rats

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Abstract

The antinociceptive potential of remoxipride was investigated in sheep and rats with concurrent motor function assessments. Previous studies of sheep given intravenous remoxipride have revealed increases in mechanical nociceptive thresholds. Here, further investigation in sheep demonstrated elevated thermal nociceptive thresholds with no effect on subjectively assessed sedation or motor impairment scores. However, in rats, the dose of remoxipride (100 mg/kg i.p.) required to produce nociceptive thresholds similar to those elicited by morphine (30 mg/kg i.p.), itself reduced rotarod performance. Medetomidine (200 μg/kg i.p.) evoked sedation without influencing rotarod performance or antinociception. The antinociceptive, motor deficit and cataleptogenic actions of remoxipride were similar to those induced by two other dopamine antagonists, haloperidol (5 mg/kg) and raclopride (16 mg/kg i.p). Tocainide (100 mg/kg i.p.) induced thermal antinociception with normal rotarod performance and no catalepsy suggesting that Na^+ channel blockade by remoxipride is not responsible for the changes in nociceptive thresholds. This study emphasizes the importance of motor function assessment during acute antinociceptive testing.

Keywords: Remoxipride; Antinociception; Catalepsy; Rotarod; (Sheep)

1. Introduction

Remoxipride is a substituted benzamide with potent, selective, dopamine D_2 receptor blocking actions (Ögren et al., 1984) and has been used for treatment of acute schizophrenia (Lewander et al., 1990). Remoxipride has other, dose-related actions which include the ability to elevate serum prolactin levels and to induce a 'weak, atypical form of catalepsy' (Ögren et al., 1990). It is also a potent sigma ligand and is capable of blocking voltage-activated Na^+ channels in central and peripheral neuronal membranes (Westlind et al., 1992).

A dopaminergic system has been implicated in the descending control of analgesia (see Fitzgerald, 1986; Jensen, 1986) and dopamine agonists have been shown to produce analgesia in various models. For example, selective dopaminergic agents were used by Barasi and Duggal (1985) and Barasi et al. (1987) to establish that the dopamine D_2 receptor was responsible for the analgesia produced in the rat by apomorphine, a mixed dopamine D_1 and D_2 agonist.

Somewhat paradoxically, there is evidence that dopamine receptor antagonists may also be analgesic. Ramaswamy and Bapna (1986) reported an analgesic effect in mice of metoclopramide, a non-selective dopamine antagonist. In addition, Rosenblatt et al. (1991) demonstrated a reduction of both pain and of morphine requirements in man after metoclopramide pre-medication whilst Parry (1980) reported a reduction in narcotic requirements in children after droperidol pre-medication. These findings are not universal, however, since Lisander (1993) and Judkins and Harmar (1982) failed to obtain a post-operative analgesic effect of either metoclopramide or haloperidol, respectively.

Classically, opioids have been the mainstay of analgesic practise but it has been suggested that an interaction between dopamine antagonists and opioids can be

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discerned. Thus, potentiation of fentanyl by droperidol has been demonstrated in mice using the hot plate test (Greene, 1972) and in sheep using mechanical blunt pin stimulation (Kyles et al., 1993). On the other hand, recent work by this group (Main et al., 1994) could not demonstrate a synergism between remoxipride and fentanyl in sheep. Nevertheless, it did reveal a remoxipride-induced elevation of mechanical nociceptive thresholds.

Methods for measurement of a drug's antinociceptive effects were reviewed by Hammond (1989) and Carstens (1992). Broadly, the methods can either assess a drug's ability to modulate hyperalgesia (by reducing induced nociceptive hypersensitivity) or, as we have employed in the present study, to produce acute antinociception (by diminishing normal levels of nociceptive sensitivity). These methods depend upon an animal's reflexive or organised behavioural response and yet these responses will almost certainly be influenced by drugs that produce aberrant motor function. It is therefore essential that careful analysis of the animal's ability to respond is made. Rotarod performance has often been used for assessing the extent of motor function impairment following administration of analgesics (Pearl et al., 1969; Sher et al., 1992; Cartmell and Mitchell, 1993). Concurrent assessment of catalepsy may also be useful as catalepsy tests can evaluate an animal's response to a non-noxious stimulus, e.g. an externally imposed abnormal posture (Sanberg et al., 1988).

The initial aim of this study was to analyse the behavioural changes associated with remoxipride-induced increases in nociceptive thresholds in sheep. Later, locomotor function and antinociceptive assessments were made concurrently to allow us to judge the antinociceptive potential of remoxipride in rats.

2. Materials and methods

2.1. Sheep studies

Three experimental protocols were carried out in sheep to assess the effect of systemically administered remoxipride on (a) mechanical and (b) thermal nociceptive thresholds. Sheep behaviour was assessed in a separate trial by using a subjective visual analogue scoring system.

A total of 23 adult Suffolk/mule cross sheep were used in this study (weight 81–100 kg) with six used in each trial (five for the romifidine mechanical threshold trial). The sheep were placed in crates (50 × 120 × 100 cm) for mechanical and thermal antinociceptive testing. Individual sheep were never isolated and prior to testing they were allowed at least 1 h to acclimatise to the laboratory environment.

Drugs

Remoxipride hydrochloride (Astra Arcus) and romifidine (Sedivet, Boehringer) were prepared using intravenous infusion-grade saline (150 mM/0.9% NaCl). The drugs were administered into the jugular vein via a 19 gauge 25 mm-long needle using a constant injection volume of 0.2 ml/kg (mechanical thresholds) or 0.1 ml/kg (thermal thresholds and behaviour trial). Sheep were always allowed at least a 7-day interval between drug treatments.

Mechanical nociceptive thresholds

Threshold response to blunt pin stimulation of the antebrachium was assessed using a device based on that described by Nolan et al. (1987). The blunt pin (2 mm diameter) was housed in a lightweight Perspex box attached to the sheep's foreleg so that the pin pressed into the cranial aspect of the antebrachium. An increasing force was applied to the pin via remote operation. The minimum force required to elicit a clear raising of the forelimb was defined as the threshold response. The force exerted was recorded via strain gauges to give an output in mV. The output was calibrated in Newtons prior to testing and the maximum force applied was limited to 20 N in order to prevent tissue damage. Control measurements were made at 5 min intervals until four baseline values were obtained. Readings were taken after drug injection at 3 min intervals for the first 15 min and then at 5 min intervals for a maximum of 1 h.

Saline (0.9%) and four doses of remoxipride hydrochloride (3.16, 5.6, 7.5 and 10 mg/kg) were given using a randomized protocol. The observer was unaware of the treatments the animal received.

In a separate trial, the anti-nociceptive effect of the α_2-adrenoceptor agonist, romifidine (5 μg/kg i.v.) was compared with saline also given intravenously to five sheep. The observer was not blind to treatment in this trial.

Thermal nociceptive thresholds

The thermal thresholds were assessed using a heat source mounted on a small clip which was attached to a sheep's ear as described by Nolan et al. (1987). A small resistor and thermocouple produced a constant rate of temperature increase. The threshold response was defined as the minimum temperature that produced a clear flicking motion of the head. The maximum temperature applied was 70°C in order to prevent tissue damage. Control measurements were made at 5 min intervals until four baseline values were obtained. Readings were taken at 5 min intervals after drug injection for the first 20 min and then at 10 min intervals for the next 40 min.

Saline (0.9%) and a single dose of remoxipride hydrochloride (10 mg/kg) were given to the sheep in a

crossover protocol. The observer was aware of the drug treatment in this trial.

Assessment of sheep behaviour

Sheep were placed in a small pen (three at a time) and allowed 30 min to become accustomed to the laboratory environment. Behaviour was assessed using a visual analogue score (VAS) for sedation or motor impairment. A single observer, blinded to the drug treatments, placed a mark on a 100 mm line using subjective assessment and with the maximum effect represented by a mark 100 mm from the left. Behaviours used for sedation scoring were drooping eyelids, head drooping and reduced arousal when stimulated by a human voice. Behaviours suggesting motor impairment were dragging of feet, 'knuckling' of fore limbs, ataxia or recumbency. The sheep were assessed prior to drug injection to ensure that they all scored zero on the VAS. VAS assessments were made every 5 min for the first 10 min and then every 10 min for the next 50 min.

Remoxipride (10 mg/kg), romifidine (an α_2-adrenoceptor agonist, 5 μg/kg) or saline (0.9%) were given to the sheep using a randomised protocol.

Statistical analysis

The results for the antinociceptive tests are presented as the mean ± S.E.M. of the change in threshold. The threshold change is defined as the difference between the force or temperature required for a response after drug treatment and the mean pre-drug control value. The behaviour scores are presented as the mean ± S.E.M. of the actual scores. The areas under the effect (threshold change and behaviour score) vs. time curve values were calculated and used for the statistical analysis. The nonparametric Kruskal-Wallis test was used for demonstrating differences between groups and Dunn's multiple comparison test was used for post-test comparisons.

2.2. Rat studies

A preliminary trial was performed to assess the effect of four lower doses (15.8, 25.1, 39.8 and 63.1 mg/kg) of i.p. remoxipride on hot plate response latencies. These doses failed to elevate nociceptive thresholds in this trial so a higher dose of 100 mg/kg was used in subsequent experiments. The doses of the other dopamine antagonists (haloperidol and raclopride) used in trial B were also relatively high so that they were comparable to the high remoxipride dose.

Two trials were then performed on rats in order to study the antinociceptive effects of remoxipride together with a concurrent assessment of motor function using established tests. All trials were conducted blind so that the observer was unaware of the treatments used.

In trial A, remoxipride was compared with morphine and medetomidine (positive analgesia and sedation controls) with reference to antinociceptive effects (paw pressure test and hot plate) and motor function performance (rotarod).

The second trial (trial B) compared the efficacy of remoxipride with other dopamine antagonists (raclopride and haloperidol) and a local anaesthetic agent (tocainide). In this trial, antinociception and motor function were assessed as before but catalepsy scores (vertical grid and platform test) were also assessed. In both trials, subjective assessments of behaviour were made and scored using a VAS system.

Male Sprague-Dawley rats (mean bodyweight 150 ± 15 g) were used in these studies. All rats were acclimatised to handling for at least 4 days prior to testing and acclimatised to the testing environment for 24 h. Rats were also familiarised to the test equipment by completion of two runs of the test protocol on each of the test days and the previous day. Rats were never isolated and the ambient temperature of the testing environment was maintained between 21 and 26°C.

Drugs

Remoxipride hydrochloride, raclopride tartrate (Astra Arcus), tocainide hydrochloride (Astra Hässle) and morphine sulphate (Sigma) were dissolved in intravenous infusion-grade saline (150 mM/0.9% NaCl). Haloperidol (Sigma) was dissolved in a vehicle containing glacial acetic acid (25 μl/mg haloperidol) and distilled water. Medetomidine (Domitor, SmithKline Beecham) was diluted in 0.9% saline. All drugs were injected intraperitoneally in a volume of 2 ml/kg.

Thermal nociceptive thresholds

An open-topped hot plate was used with a metal base of 18 × 24 cm and clear Perspex sides (35 cm high). The metal base was maintained at 55 ± 0.5°C by placement over a heated water bath. The latency to respond after placement on the hot plate was recorded and the rat was removed immediately after a positive response. If animals failed to respond, a maximum of 30 s contact with the hot plate was allowed in order to prevent tissue damage. The endpoint responses used were hind paw licking, jumping or vigorous hind limb kicking.

Mechanical nociceptive thresholds

A purpose-built paw pressure testing device (Ugo Basile, Biological Research Apparatus, Italy) was used for measurement of mechanical thresholds. The rat was held loosely in the hand and the right hind paw placed on a flat circular platform (1 cm diameter). A

blunt-ended Perspex pin (2 mm diameter) was then placed on the dorsum of the paw and the pressure exerted by the pin increased via a weight which moved along a scored rotating rod. The mechanical threshold was defined as the distance, in mm, that the weight had moved before a withdrawal of the limb or whole body movement occurred. To prevent tissue damage, the maximum force applied was limited to that produced by a 200 mm deflection of the weight. The device was calibrated prior to testing with a beam-type device so that the force produced by a 200 mm deflection of the weight was 2.03 N.

Rotarod

Rats were placed on a cylindrical bar (7 cm diameter) around which was wrapped a coarse, elasticated bandage. The rat was restricted to a 25 cm length of rod by a 25 cm-high partition which rotated with the rod at 6 r.p.m. The endpoint was defined as the time the rat could remain on the rotarod before falling on soft landing material. If the rat remained on the rotating bar for 3 min then it was assumed to have normal motor function.

Sedation and motor impairment assessment

The rats were assessed using a VAS index in a similar manner to that used for the sheep studies. This included subjective assessment of sedation or motor impairment during the handling period and an assessment of the ability to walk on the moving rotarod. Criteria used for sedation scores were drooping eyelids and ease of arousal. Criteria used for motor impairment were slower walking, falling to one side or ataxia while on the rotarod.

Catalepsy

Rats were scored for the presence or absence of catalepsy using two methods: the vertical grid and hindlimb platform tests. The apparatus was based on that described by Fuenmayor and Vogt (1979) and Fog et al. (1970), respectively. The vertical grid test was scored by recording the time taken for the rat to move one forelimb after placement on the grid (0.8×0.8 cm mesh of 1 mm diameter wire). The platform test involved recording the time taken for the rat to remove its hind paw after placement of its plantar surface on a small rubber stopper (1.6 cm high and 1 cm diameter). If the rat maintained its position in either test for at least 120 s then catalepsy was deemed to be maximal.

Protocol for drug administration and testing

(a) Trial A. Morphine (30 mg/kg), medetomidine (200 μg/kg), remoxipride (100 mg/kg) and saline (0.9%, 2 ml/kg) were each given intraperitoneally to nine rats in a blind, randomised manner. Each rat was assessed with the paw pressure, rotarod and hot plate tests together with behavioural VAS. Rats were assessed at 20, 40 and 60 min following drug injection. At each time point, the following tests were made: paw pressure at 0 min, rotarod at 1 min, hot plate at 5 min and behaviour scores during the handling period.

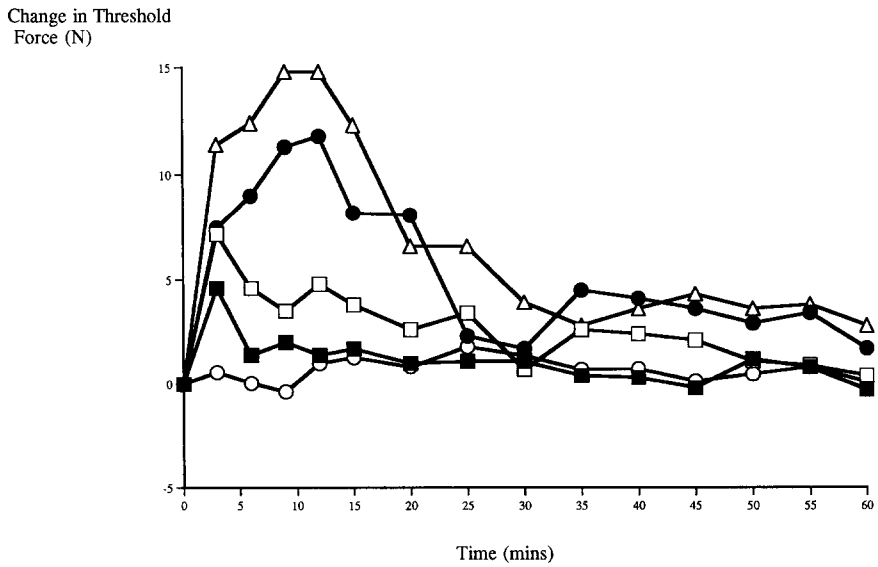

Fig. 1. The effect of four i.v. doses of remoxipride on the threshold force required to elicit the forelimb-raising response in sheep. Each point represents the mean of the change in threshold value from pre-drug control in six animals. ○ = saline, ■ = 3.16 mg/kg, □ = 5.6 mg/kg, ● = 7.5 mg/kg and △ = 10 mg/kg remoxipride hydochloride. The standard error values, which ranged from 0.15 to 2.54 N in this study, are excluded from the graph for the sake of clarity.

(b) Trial B. Tocainide (100 mg/kg), raclopride (16 mg/kg), haloperidol (5 mg/kg), remoxipride (100 mg/kg), haloperidol vehicle and saline (0.9%, 2 ml/kg) were each given intraperitoneally to nine rats in a blind, randomised manner. Each rat was tested as in trial A but in addition, these rats were also assessed for the presence of catalepsy. The rats were assessed over a 10 min handling period commencing 5 min before the 1 and 2 h post-drug injection time points. During each handling period, the rats were assessed in the following order: platform test, vertical grid, paw pressure, rotarod and hot plate at 0, 2, 4, 5 and 9 min respectively.

Statistical analysis

The results were analysed by the nonparametric Kruskal-Wallis test. This revealed no significant differences between the different time points within each group so the results for the post-drug time points were combined and analysed by the same Kruskal-Wallis test and post-hoc tests were performed using Dunn's multiple comparison test.

3. Results

3.1. Sheep studies

Mechanical nociceptive thresholds

Remoxipride produced a dose-dependent increase in mechanical nociceptive thresholds (Fig. 1). At a dose of 10 mg/kg, all six sheep tested reached the maximum threshold (20 N) for at least two of the five time points during the first 15 min. The area under the threshold change vs. time curve (AUC) for the first 60 min with 10 mg/kg of remoxipride was significantly ($P < 0.05$) greater than that obtained with saline (Table 1).

Romifidine also produced elevations in the mechanical nociceptive thresholds (Fig. 2) and the AUC values were significantly greater than the saline control ($P < 0.01$) (Table 1).

Thermal nociceptive thresholds

Remoxipride at 10 mg/kg produced an increase in thermal nociceptive thresholds (Fig. 3) which followed a similar time course as the mechanical threshold changes. The AUC for the first 60 min with 10 mg/kg of remoxipride was significantly ($P < 0.01$) greater than that obtained with saline (Table 1).

Behaviour testing

All sheep, either when given saline or at the pre-drug behavioural assessment, scored zero on both VAS indices. Romifidine, an α_2-adrenoceptor agonist, produced sedation and motor impairment as determined by the VAS (Fig. 4a and b) with significantly ($P < 0.01$ and $P < 0.05$, respectively) greater areas under the VAS vs. time curves compared with the saline control

Table 1
The pre-injection values and calculated values for the area under the effect vs. time curve for nociceptive thresholds (mechanical and thermal) and behaviour scores following either intravenous remoxipride or romifidine in sheep

Remoxipride (mg/kg)	Romifidine (μg/kg)	Pre-injection values	Area under effect vs. time curve (0–60 min)
Change in mechanical nociceptive thresholds		*(N)*	*(Ns)*
0 (saline)	–	4.5	35.1
3.16	–	5.27	27.3
5.6	–	5.9	174.7
7.5	–	5.43	258.0
10	–	4.76	358.0 *
	0 (saline)	4.1	30.1
	5	5.3	426.1 * *
Change in thermal nociceptive thresholds		*(°C)*	*(°Cs)*
0 (saline)	–	57.7	75.8
10	–	54.0	259.0 * *
Behaviour – visual analogue scores		*(mm)*	*(mms)*
(1) Sedation			
0 (saline)	–	0	0
10	–	0	207.5
–	5	0	1738.0 * *
(2) Motor impairment			
0 (saline)	–	0	0
10	–	0	0
–	5	0	350.0 *

The median values are presented ($n = 6$). * $P < 0.05$ and * * $P < 0.01$ when compared to saline values.

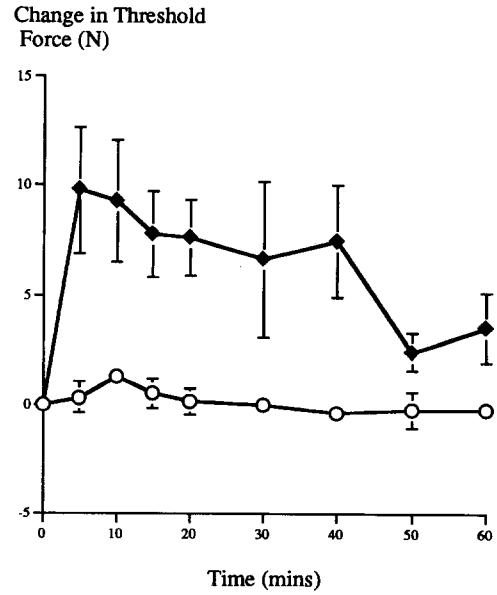

Fig. 2. The effect of i.v. romifidine on the threshold force required to elicit the forelimb-raising response in sheep. Each point represents the mean (± S.E.M.) of the change in threshold value from pre-drug control in five animals. ○ = saline, ♦ = 5 μg/kg romifidine.

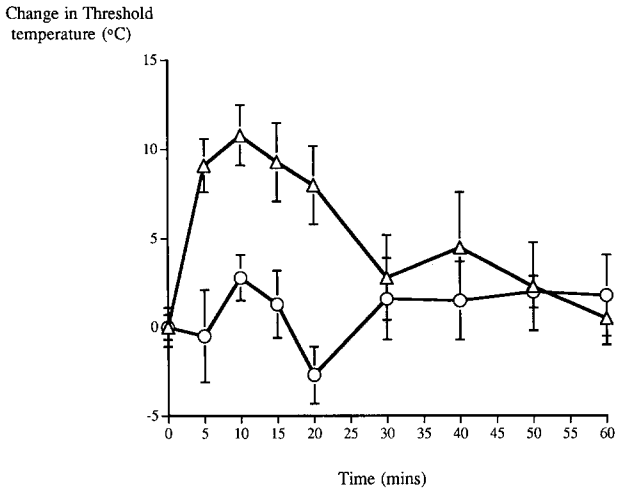

Fig. 3. The effect of i.v. remoxipride on the threshold temperature applied to the ear which produced the head-shaking response in sheep. Each point represents the mean (±S.E.M.) of the threshold change from pre-drug control value ($n = 6$). ○ = saline and △ = 10 mg/kg remoxipride hydrochloride.

group (Table 1). For remoxipride, the AUC for sedation and motor impairment was not significantly different from that obtained with saline (Table 1). When the sheep were given remoxipride, they appeared bright and alert and most responded to vocal stimulation. One sheep, however, was reluctant to move during the first 15 min but would walk when made to do so.

3.2. Rat studies

Preliminary work (data not shown) revealed that i.p. doses (15.8, 25.1, 39.8 and 63.1 mg/kg) of remoxipride did not elevate hot plate response latencies significantly. Subsequently a higher dose of 100 mg/kg i.p. remoxipride was used in trials A and B.

Trial A

Remoxipride and morphine significantly elevated thresholds in the hot plate and paw pressure tests ($P < 0.001$, Fig. 5a and b), while medetomidine did not alter the nociceptive thresholds. Behavioural assessment (Fig. 5c and d) revealed that all three drugs produced sedation and apparent motor impairment ($P < 0.001$). At these doses, medetomidine produced more sedation than morphine ($P < 0.01$) and remoxipride produced more motor impairment than morphine ($P < 0.05$). However, only remoxipride produced a significant reduction in rotarod performance ($P < 0.001$, Fig. 5e) with all nine rats unable to maintain position for the full 3 min. In contrast, only three of the rats given morphine and one rat given medetomidine were unable to complete the rotarod test. All pre-drug injection and post-saline injection assessments of rats revealed zero behaviour scores and all these rats were capable of completing 3 min on the rotarod.

Trial B

Remoxipride-, raclopride- and haloperidol-treated rats all had elevated thresholds in both the hot plate and paw pressure tests ($P < 0.05$, Fig. 6a and b) compared with the control saline or haloperidol vehicle groups. On the other hand, the group that received tocainide only displayed elevated thresholds during the hot plate test ($P < 0.001$). Remoxipride-, raclopride- and haloperidol-treated rats all had significantly greater sedation and motor impairment scores than the control groups ($P < 0.001$, Fig. 6c and d). Following tocainide, 5/9 rats produced positive scores for sedation and 4/9 for motor impairment scores but the combined scores were not significantly different from the saline-treated groups. In addition to the demonstrable sedation and

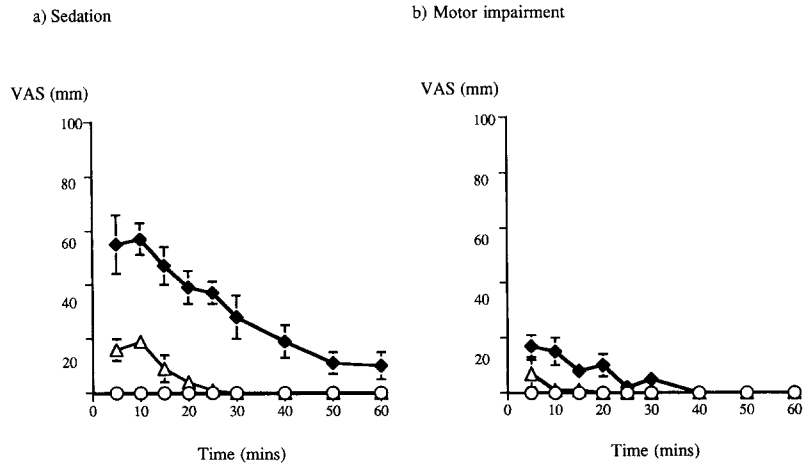

Fig. 4. The effects of i.v. remoxipride (△, 10 mg/kg), romifidine (◆, 5 μg/kg) and saline (○) on the visual analogue scores (VAS) for sedation and motor impairment in six sheep. Each point represents the mean (±S.E.M.).

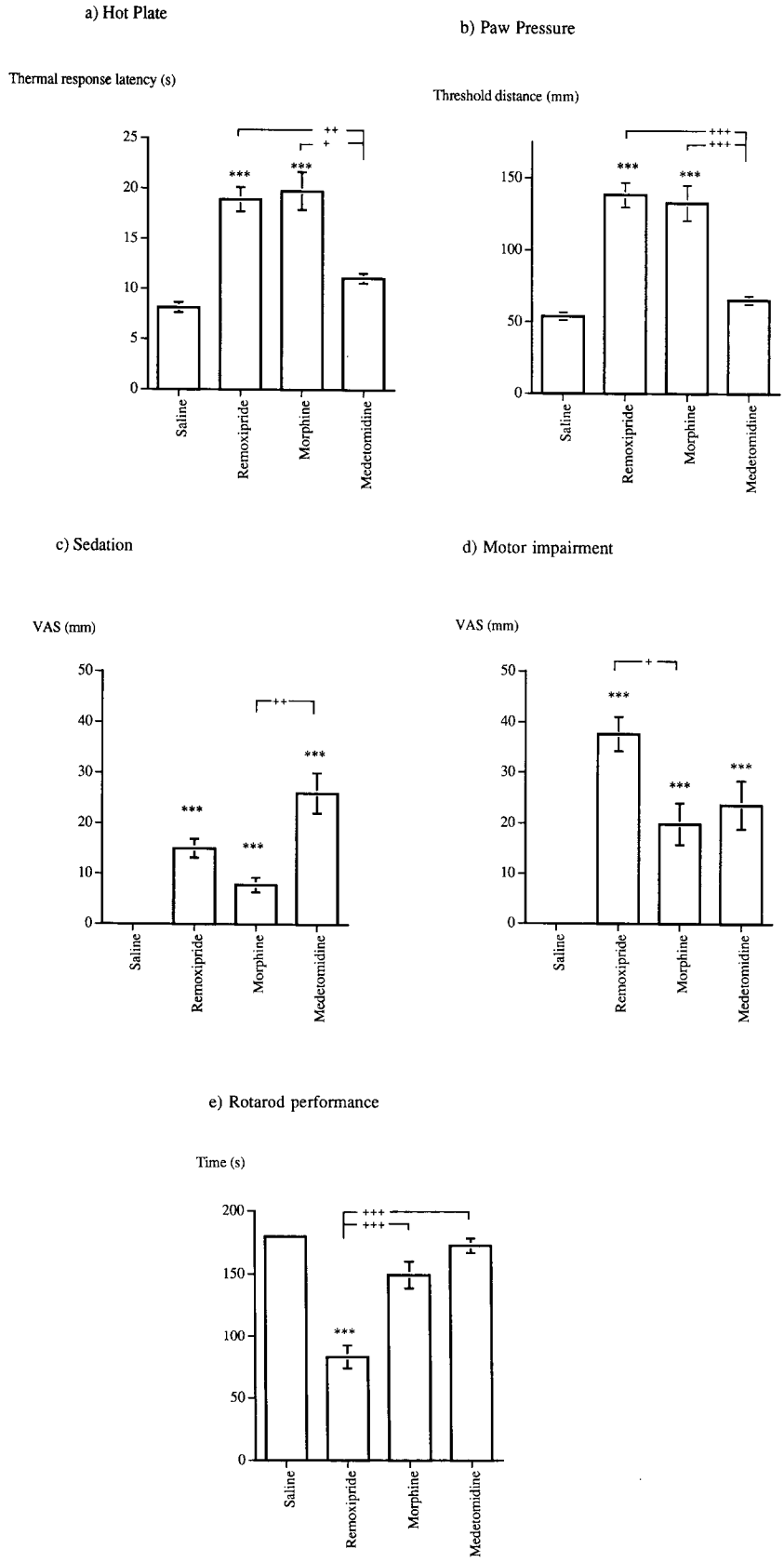

Fig. 5. Trial A. The effects of i.p. remoxipride (100 mg/kg), morphine (30 mg/kg) and medetomidine (200 μg/kg) on post-injection values (mean ± S.E.M.; $n = 9$) for (a) hot plate test, (b) paw pressure test, (c) sedation visual analogue scores (VAS), (d) motor impairment VAS, and (e) rotarod performance. The displayed probability values are derived from comparisons with saline (*) and between the drug groups (+). $^{*,+}$ $P < 0.05$, $^{**,++}$ $P < 0.01$ and $^{***,+++}$ $P < 0.001$.

motor impairment, catalepsy was also present in the remoxipride, raclopride and haloperidol groups, as judged by the vertical grid and platform tests ($P < 0.001$, Fig. 6e and f). These three drug-treated groups also yielded significantly poorer performances in the rotarod test ($P < 0.001$, Fig. 6g) compared with control

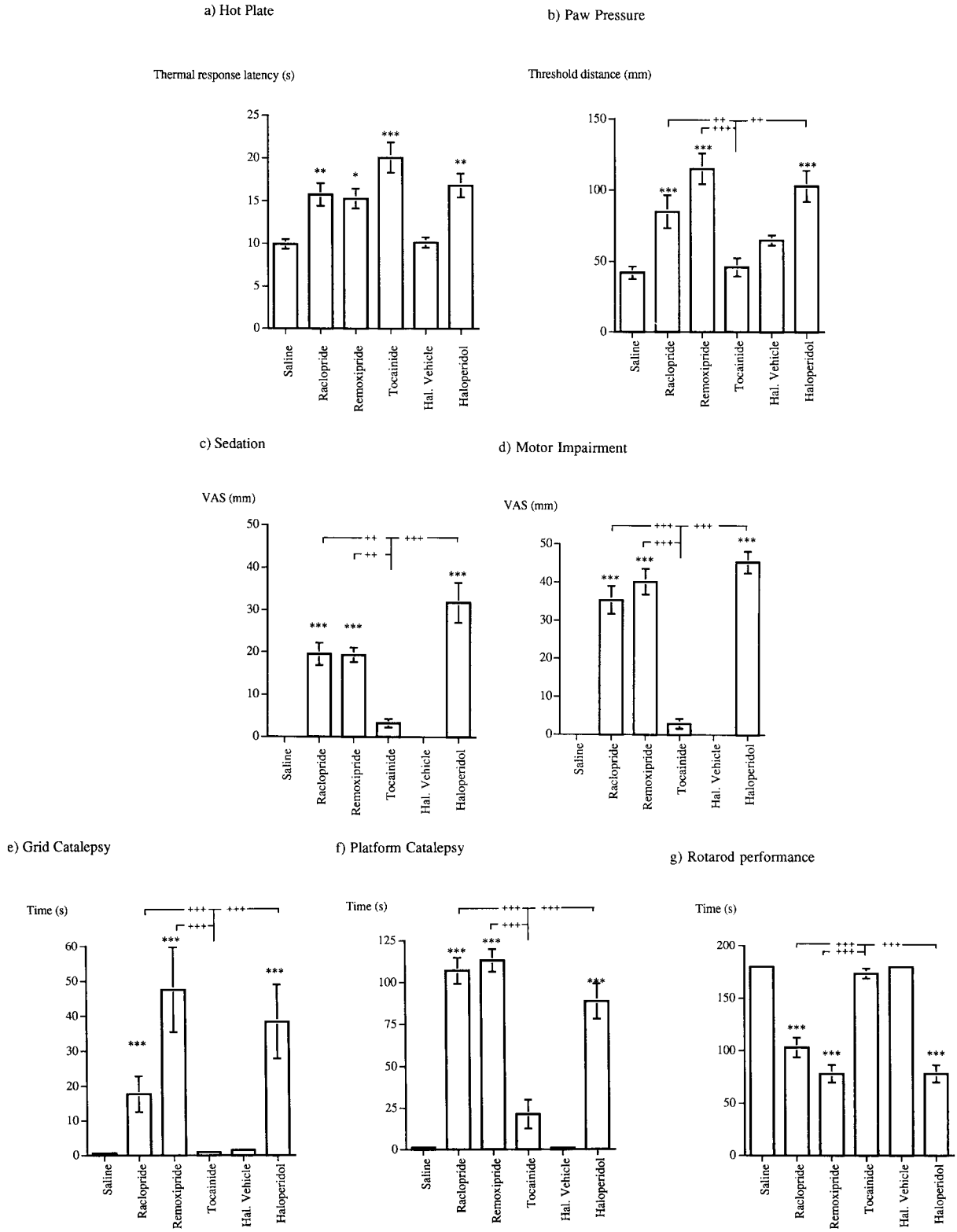

Fig. 6. Trial B. The effects of i.p. raclopride (16 mg/kg), remoxipride (100 mg/kg), tocainide (100 mg/kg) and haloperidol (5 mg/kg) on post-injection values (mean ± S.E.M.; $n = 9$) for (a) hot plate test, (b) paw pressure test, (c) sedation visual analogue scores (VAS), (d) motor impairment VAS, (e) grid catalepsy scores, (f) platform catalepsy scores, and (g) rotarod performance. The displayed probability values are derived from comparisons with saline (*) and between the drug groups (+). $^{*,+}P < 0.05$, $^{**,++}P < 0.01$, $^{***,+++}P < 0.001$.

groups. Again, all pre-drug-, saline- and haloperidol vehicle-treated rats had zero sedation VAS, zero motor impairment VAS and were capable of completing the 3-min rotarod test.

It is noteworthy that in the two separate groups of remoxipride-treated rats used in trial A and trial B, neither the VAS indices nor the rotarod abilities of either group were significantly different from each other.

4. Discussion and conclusions

This study has demonstrated that remoxipride produces an elevation in mechanical and thermal nociceptive thresholds in sheep with no change in the behavioural indices of sedation or motor impairment. However, the significance of these results with regard to the analgesic effect of remoxipride must be questioned in the light of further behavioural studies carried out in the rat which revealed diminished performance in the rotarod test.

4.1. Methodological considerations

The species difference seen in motor effects may be a consequence of differences in pharmacokinetics and metabolite production. After oral administration, rats, hamsters and mice but not dogs and humans, produce phenolic and catecholic metabolites which are known to be active dopamine D_2 receptor antagonists (Widman et al., 1993). It is not known whether sheep produce these active metabolites and it is possible that sheep may not demonstrate motor impairment and catalepsy because of a lack of production of these metabolites. It is known that i.p. administration of remoxipride produces these active metabolites in rats (Ögren et al., 1993). Other routes of administration (s.c. or i.v.) would produce less of these active metabolites and investigation of their antinociceptive potential would be interesting. Ögren et al. (1993) demonstrated that catalepsy after i.v. administration of remoxipride declined from 20 min after injection, which would make concurrent assessment of all of the behavioural parameters used in this study difficult. Behavioural analysis following subcutaneous dosing may also be of interest, since there is less metabolite production than with i.p. administration (Ögren et al., 1993). However, preliminary work revealed a marked localized hyperaemia accompanied by an apparently painful reaction after s.c. dosing, so use of this route of administration was difficult to justify ethically and may have also altered the animal's behavioural responses.

Whilst the data imply that remoxipride may raise nociceptive thresholds in sheep without producing any motor impairment, it is possible that the subjective assessment tests used were not subtle enough to reveal small reductions in motor performance. For example, the vocal stimulation used to arouse the sheep may be a more severe stimulus than the rotating rod used in the rat assessment so reducing sensitivity of the test. In addition, the sheep were not assessed for their ability to respond to placement of a limb or whole body in an abnormal position (as in the catalepsy tests used for the rats), since preliminary work failed to produce a reliable test of catalepsy in the sheep.

The mechanical nociceptive threshold increases seen with remoxipride in sheep were similar to those produced by romifidine (in this trial), medetomidine (Muge et al., 1994) and other α_2-adrenoceptor agonists and opioid analgesics in sheep in the same test system (Livingston et al., 1992). However, in these studies the sheep given opioids were noticeably agitated and those given α_2-adrenoceptor agonists were sedated compared to the minimal behavioural effects seen after remoxipride administration.

The changes in nociceptive threshold seen in the sheep were mirrored by increases in the paw pressure and hot plate thresholds in the rats. The endpoints used for analgesia tests are obviously important for the reproducibility and reliability of the tests (see Hammond, 1989) and the response endpoint used must, therefore, be appropriate for the stimulus used. It is also preferable to use one, clear, behavioural endpoint because a drug may affect different behaviours with differing potencies. We used more than one behaviour as an endpoint in each test, i.e. either withdrawal or struggle for the paw pressure and jump, hindpaw lick or vigorous kicking for the hot plate test. It was decided to forego some sensitivity in the tests for ethical reasons; it was considered unacceptable to leave the rat on the hot plate or on the paw pressure device beyond the first appearance of an aversive behavioural response. The behavioural endpoints used in the present study were each organised behaviours except for the spinal reflex involved in the withdrawal response for the paw pressure test. Carter (1991) demonstrated that including the jump as an endpoint in the hot plate test was useful in excluding drugs that altered the lick response but which did not have analgesic efficacy. Different endpoints have been used for the paw pressure test, with struggle being used by Randall and Selitto (1957) and withdrawal used by Hargreaves et al. (1988). Careful training and handling is an essential requirement for the paw pressure test as naive rats are obviously stressed but after acclimatization they appear calm and relaxed.

4.2. Motor impairment and apparent antinociception

The concurrent assessment of motor function in the rats revealed that remoxipride-treated animals displaying elevated nociceptive thresholds were less able to complete the rotarod test. Morphine produced similar

increases in thresholds to remoxipride but these rats were not significantly worse than the saline group at completing the rotarod test. This discrimination between impairment of motor function and analgesic potency has also been demonstrated in mice by Pearl et al. (1969) who compared morphine and a phenothiazine neuroleptic, chlorpromazine. Morphine was an effective analgesic in the writhing test at a lower dose than that needed to produce a reduction in rotarod performance. Chlorpromazine, however, could only produce apparent analgesia at doses that significantly reduced rotarod performance.

Interestingly in the present study, medetomidine, an α_2-adrenoceptor agonist, failed to raise thresholds in either the hot plate or paw pressure test. Pertovaara (1993) has reviewed the antinociceptive effects of medetomidine in various behavioural pain tests. Thus, an intraperitoneal dose of only 55 μg/kg was effective in the rat formalin test whereas 300 μg/kg, a highly sedative dose, was required to suppress the rat tail flick response. In the current trial, rats given medetomidine were clearly sedated and had apparent motor impairment. Despite this, they were still capable of performing well on the rotarod. This suggests that a degree of sedation and apparent motor impairment does not necessarily adversely affect either rotarod performance or the threshold responses to the hot plate and paw pressure tests.

In the second rat trial (B), the remoxipride-induced elevation of nociceptive thresholds in the paw pressure and hot plate tests was found not to be a unique property of this drug as it also occurred with other dopamine antagonists (raclopride and haloperidol). Moreover, the reduced performance in the rotarod test and the sedation and motor impairment scores that were seen with remoxipride in trial A were also seen with all three dopamine antagonists in trial B. Importantly, all three drugs produced catalepsy as assessed by the vertical grid and platform tests.

Since the three dopamine antagonists produce catalepsy as well as an increase in nociceptive thresholds, it could be argued that catalepsy permits or even generates an increase in nociceptive thresholds. Catalepsy can be defined as a reduced response to an abnormal posture or proprioceptive stimulus. If a drug promotes a reduced response to an abnormal proprioceptive stimulus by a reduction in the animal's *ability to respond*, then it might also reduce their ability to respond to a noxious stimulus and the drug cannot, therefore, be assumed to be analgesic. Furthermore, if the drug reduces the animal's *ability to detect* an abnormal posture then it is unlikely to be a clinically useful analgesic if locomotor side-effects are generated concurrently. Although catalepsy was not assessed in trial A, De-Ryck et al. (1980) demonstrated that morphine can also produce elevations in catalepsy scores at the dose employed here (30 mg/kg i.p.). As shown both in the present study and by Pearl et al. (1969), the rotarod test can be used to separate the doses of morphine that cause motor impairment or analgesia. This suggests that the rotarod test should be used in addition to catalepsy tests to assess motor function.

4.3. Contribution of non-dopaminergic mechanisms to remoxipride action

In addition to their dopamine antagonist properties, remoxipride and other neuroleptics can cause selective Na^+ channel blockade in peripheral and central nerve membranes (IC_{50} ~ 300 μM and IC_{50} ~ 20 μM, respectively; Westlind et al., 1992). In this regard, these compounds may be viewed to act in a similar manner to local anaesthetics. Systemic administration of local anaesthetics can (a) induce changes in acute nociceptive thresholds, as demonstrated with tocainide both in this trial and by Wiesenfeld-Hallin and Lindblom (1985), (b) induce a selective depression of afferent C-fibre-evoked activity in the spinal cord (Woolf and Wiesenfeld-Hallin, 1985), and (c) block nerve injury-induced hyperalgesia (Abram and Yaksh, 1994). However, the antinociceptive and behavioural profile of tocainide in this study was different from that of the dopamine antagonists, i.e. tocainide promoted only a thermal antinociception but without an accompanying reduction in rotarod performance or the induction of catalepsy.

The behavioural side effects seen with these high doses of neuroleptics do not preclude their use as potential analgesics at lower doses. Clinically, low doses of local anaesthetics have been used for treatment of diabetic neuropathy (Kastrup and Peterson, 1987). Neuropathic pain is a considerable clinical problem (Hamman, 1993), justifying attempts at finding suitable drug treatments and so the selective Na^+ channel blockade by remoxipride or other neuroleptics could potentially be utilized to treat neuropathic pain. However, this trial clearly demonstrates that if these drugs are to be assessed in neuropathic pain models then motor function tests must also be performed concurrently.

In conclusion, these results indicate that remoxipride can induce apparent antinociception in sheep with minimal behavioural side effects. However, the measure of antinociception which also occurred with this compound in rats was accompanied by marked motor impairment in this species.

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