An assessment of the peripheral antinociceptive potential of remoxipride, clonidine and fentanyl in sheep using the forelimb tourniquet

D.C.J. MAIN *
A.E. WATERMAN *
I.C. KILPATRICK † &
A. JONES *

**Department of Clinical Veterinary Science, University of Bristol, Langford House, Langford, Bristol, BS18 7DU, UK*
†Department of Pharmacology, School of Medical Sciences, University Walk, Bristol, BS8 1TD, UK

Main, D.C.J., Waterman, A.E., Kilpatrick, I.C., Jones, A. An assessment of the peripheral antinociceptive potential of remoxipride, clonidine and fentanyl in sheep using the forelimb tourniquet. *J. vet. Pharmacol. Therap.* **20**, 220–228.

A modification of the intravenous regional anaesthesia technique was used to assess the peripheral antinociceptive effect of remoxipride, clonidine and fentanyl. Drugs administered intravenously via peripheral catheters were restricted to the distal limb and nociceptive threshold test site by prior inflation of a tourniquet proximal to both the catheter and a threshold-testing device. Lignocaine (1 mg/kg) induced peripheral antinociception during tourniquet inflation. Clonidine (6 μg/kg) only induced significant elevations in thresholds after tourniquet deflation. A low dose of remoxipride (2 mg/kg), which had no systemic antinociceptive effect, produced antinociception after its restriction to the periphery. Peripheral administration of saline and tourniquet-induced restriction of blood flow to the distal limb did not alter threshold values. Peripheral administration of fentanyl was used to test a further modification of the injection protocol designed to reduce the incidence of leakage into the systemic circulation. Fentanyl administration (11.2 μg/kg) failed to elicit an increase in thresholds when it was restricted to the distal limb test site. The contribution of a peripheral mechanism to the antinociception induced by systemic administration of a higher remoxipride dose (7.5 mg/kg) was investigated using an inflated tourniquet to exclude remoxipride from the periphery. Exclusion of remoxipride from the periphery reduced its antinociceptive effect, i.e. threshold values were lower than if remoxipride was allowed free access to the limb prior to tourniquet inflation.

The technique described here was effective in demonstrating that the increase in noninflammatory nociceptive thresholds seen with clonidine and fentanyl is not peripherally mediated whilst that seen with remoxipride has a peripheral component.

(Paper received 29 July 1996; accepted for publication 2 December 1996)

D.C.J. Main, Department of Clinical Veterinary Science, University of Bristol, Langford House, Langford, Bristol, BS18 7DU, UK.

INTRODUCTION

Intravenous regional anaesthesia (IVRA) is a routine clinical procedure in humans (see Fagg, 1987) and animals (Prentice *et al.*, 1974). The technique depends upon the peripheral administration of local anaesthetic agents and the restriction of these agents to the distal limb by a tourniquet. Concurrent administration of other analgesic agents during IVRA in man has been attempted with variable results in order to improve postoperative analgesia following surgery. For example, Reuben *et al.* (1995) demonstrated that ketorolac, a nonsteroidal anti-inflammatory drug, was more effective at reducing tourniquet-related and postoperative pain in humans when given peripherally with the local anaesthetic for IVRA than if given systemically at the same dose. Other authors have failed to demonstrate a benefit of additional fentanyl during IVRA (Armstrong *et al.*, 1991; Abdulla & Fadhil, 1992; Arthur *et al.*, 1992). Pethidine, however, given via an IVRA protocol has been shown to produce a local anaesthetic-like motor and sensory blockade in humans (Armstrong *et al.*, 1993; Acalovschi & Cristea, 1995).

This paper demonstrates the use of this simple technique to define the potential of other drugs to have a peripheral antinociceptive effect when administered alone. In addition, a method is described which attempts to define the importance of a peripheral site of action after systemic administration of drugs by restricting the flow of blood and therefore, drug access, to the distal limb test site.

The agents investigated in this study were remoxipride, clonidine and fentanyl. Remoxipride is a substituted benzamide with potent, selective, dopamine D_2 receptor blocking actions

(Ögren *et al.*, 1984) and has been used for the treatment of acute schizophrenia (Lewander *et al.*, 1990). Previous studies in sheep have demonstrated elevated mechanical nociceptive thresholds after remoxipride administration (Main *et al.*, 1995a). In that same study, remoxipride also raised nociceptive thresholds in rats but this was accompanied by marked motor impairments and catalepsy. In sheep, however, there was no behavioural motor impairment at antinociceptive doses. The mechanism and site of action for this apparent antinociceptive effect are not known. In the present study, restriction of remoxipride to the distal limb was used to define its potential for generating peripheral antinociception whilst its exclusion from the periphery aimed to evaluate the importance of any peripheral mechanism in mediating the systemic effect.

Clonidine is an α_2- adrenoceptor agonist which induces elevations in mechanical and thermal nociceptive thresholds in sheep when given systemically (6 μg/kg intravenously (i.v.), Livingston *et al.*, 1992). Gaumann *et al.* (1992) and Chambers (1993) have demonstrated that clonidine and lignocaine have comparable inhibitory effects on C-fibre action potentials *in vitro*. The concentration of clonidine that exhibits these local anaesthetic properties is unlikely to be achieved *in vivo* after its systemic administration without producing severe concurrent cardiovascular side-effects. However, the *in vivo* clonidine concentration in the distal limb can be much higher if a dose of clonidine (6 μg/kg), which has antinociceptive properties after systemic administration, is given in an intravenous regional anaesthesia protocol. In the present study, the first trial compared the peripheral antinociceptive potential of this higher clonidine concentration with that of remoxipride and lignocaine.

Fentanyl is a μ-opioid agonist and a clinically useful parenteral and epidural analgesic. Opioids appear to have a more profound peripheral antinociceptive effect on inflammatory pain compared with noninflammatory pain as reviewed by Junien and Wettstein (1992) and Stein (1993). However, high concentrations of fentanyl have been shown to produce a weak, local anaesthetic-type effect on peripheral nerves (Gissen *et al.*, 1987; Power *et al.*, 1988). Thus, in a separate trial we aimed to evaluate the antinociceptive capability of a high peripheral concentration of fentanyl. Again, the dose chosen (11.2 μg/kg) was that which has been shown to increase nociceptive thresholds in sheep following its systemic administration (Waterman *et al.*, 1990). Also, concurrent hind limb threshold testing allowed us to assess whether a further modification of the injection protocol could prevent leakage of administered drug past the tourniquet into the systemic circulation.

A preliminary report of the use of this technique has been published (Main *et al.*, 1995b).

MATERIALS AND METHODS

Sheep

Adult female mule/Suffolk cross sheep (67–103 kg body wt) were used in this study. The sheep were placed in crates (50 × 100 × 120 cm) for 60 min prior to testing. Individual sheep were never isolated and were familiar both with the laboratory environment and experimental procedures.

Drugs

Remoxipride hydrochloride (Astra, Arcus AB, Södertälje, Sweden), clonidine hydrochloride (Sigma) and fentanyl citrate (Sigma, Poole, UK) were all dissolved in 0.9% saline. Lignocaine hydrochloride (2% Xylocaine, Astra) was used undiluted in the peripheral administration protocol. The drugs were injected in a volume of 0.05 mL/kg for peripheral administration and 0.1 mL/kg for systemic administration. The order of treatments in each study was randomized and the observer was unaware of the treatments given.

Mechanical nociceptive thresholds

Drug-induced elevations in nociceptive thresholds were assessed by blunt pin stimulation to the sheep foreleg (cranial aspect of metacarpus) using the device described by Chambers *et al.* (1994). This device used air pressure to apply a force to the pin at a constant rate of increase of 0.33 N/s. The threshold force was defined as the minimum force required to elicit a clear raising of the forelimb. A maximum applied force of 20 N ensured that there was no tissue damage. In all experiments, four baseline control measurements were taken prior to drug treatment.

Intravenous catheter placement

Forelimb peripheral vein. Either the lateral (IV) or medial (II) palmar digital vein, located proximal to the accessory digits at the level of the fetlock joint, was catheterized. The sheep was restrained in lateral recumbency and warm wet towels were wrapped around the distal forelimb to encourage temporary vasodilation and easier visualization of the superficial veins. The fetlock region was clipped and surgically scrubbed. A small skin incision (3 mm) was made overlying the vein to allow easier catheter placement. The peripheral veins were raised to allow a 24G 19 mm intravenous catheter (Jelco, Critikon, Bracknell, UK) to be inserted into the vein. This was maintained in position using cyanoacrylate glue and elasticated adhesive bandage. The catheter was connected to extension tubing attached to the fleece at the shoulder to allow both flushing and drug administration without further handling of the limb. The tubing and catheter was flushed with heparinised saline (10 international units heparin/mL 0.9% saline) immediately after placement within the vein. The injection tubing had a dead space of 2.5 mL. Prior to drug administration, the intravenous position of the catheter was confirmed by drawing back blood, minimal resistance during flush injection and no subcutaneous lump after flush injection.

Jugular vein. For systemic administration of drugs, the jugular vein was catheterized at least 60 min prior to testing with 18 G 44 mm catheters (Jelco, Critikon). Catheters with three way taps attached were maintained in position with cyanoacrylate glue and flushed with heparinised saline.

Peripheral drug administration protocol

Trial A. The peripheral antinociceptive potential of remoxipride (2 mg/kg), clonidine (6 μg/kg), lignocaine (1 mg/kg) and 0.9% saline were investigated. Each treatment was administered to six sheep (from a total of eight sheep, 76–102 kg body wt) via peripheral venous catheters placed on the previous day. Successive drug treatments on individual animals were separated by at least seven days.

To restrict the administered drug to the peripheral limb, a tourniquet sited proximal to the carpus was inflated immediately prior to and for 20 min after drug administration. Mechanical nociceptive thresholds distal to the tourniquet were then recorded at 5 min intervals before and during tourniquet inflation and for 20 min after tourniquet deflation.

The tourniquet used had a bladder size of 4.5 × 25 cm (Biomet Ltd, Mid Glamorgan, UK) and was placed around the limb above the carpus. The tourniquet was inflated to 700 mm Hg and the drug treatment (injection volume 0.05 mL/kg) followed by 5 mL heparinised saline was injected slowly over 1 min via the extension tubing. The tourniquet was then deflated to 300 mm Hg at 2.5 min and maintained at this pressure until 20 min after drug injection when it was completely deflated allowing normal blood flow to the distal limb.

The inflation pressures used were those preliminary trials had suggested would prevent the systemic spread of clonidine (6 μg/kg), which causes obvious behavioural signs of sedation if leakage occurrs. The initial high pressure was only maintained for a short period (2.5 min) as there is marked aversion and hyperalgesia after about ten minutes. However, the lower pressure of 300 mm Hg was well-tolerated.

Trial B. A modified tourniquet inflation and drug injection protocol were used in order to prevent systemic leakage during cuff inflation. First, a syringe pump (Ohmeda 9000, Steeton, UK) was used to administer a slow, constant injection rate (150 mL/hour). Secondly, excess intravenous pressures were prevented by monitoring the pressure within the injection tubing (via a pressure transducer (Druck, Groby, UK) & Multitrace 2 chart recorder (Lectromed, Letchworth, UK). Grice *et al.* (1986) demonstrated that intravenous pressure in the distal limb during tourniquet inflation in humans, could not exceed a defined maximum pressure whatever the injection rate, and that at this maximum pressure systemic leakage occurred. In that study the maximum pressure was 80–100 mm Hg less than the tourniquet pressure.

Accurate intravenous pressure recordings would require direct recordings via a second catheter. However, the intravenous pressure in this study could be estimated from the injection tubing pressure. This tubing pressure was assumed to be the sum of the intravenous pressure plus that induced by the resistance of catheters and tubing during injection. This resistance pressure was estimated prior to drug injection, using test saline injections at the same injection rate, with and without the tourniquet inflated. The increase in tubing pressure with the tourniquet was assumed to be the elevation in intravenous pressure, and this was restricted to 150 mm Hg during drug adminstration, i.e. 150 mm Hg less than the tourniquet. An example of a pressure recording is shown in Fig. 1. If this estimated venous pressure reached 150 mm Hg then the injection was temporarily halted to allow the pressure to fall. Therefore, leakage was extremely unlikely as this is a much lower increase in venous pressure than that demonstrated by Grice *et al.* (1986), to promote leakage past a tourniquet into the systemic circulation.

In order to evaluate the modified injection protocol, fentanyl (11.2 μg/kg) and saline were given to six sheep (54.5–76 kg body wt) via peripheral catheters as before, in a randomized order. The catheters were maintained in the sheep for 2 days. The sheep were randomly assigned so that three sheep received saline on Day 1 and fentanyl the next day, whilst the other three sheep were given fentanyl before saline. The observer, however, was unaware of the treatments given. Mechanical nociceptive thresholds were assessed on the catheterized foreleg and also on the hind leg of the same side to evaluate systemic spread during tourniquet inflation. Four control threshold readings were recorded, as before, from both legs. Following tourniquet inflation and drug administration, thresholds were recorded at 5, 11, 17, 23, 29, and 35 min on the forelimb and 8, 14, 20, 26 and 32 min on the hind limb.

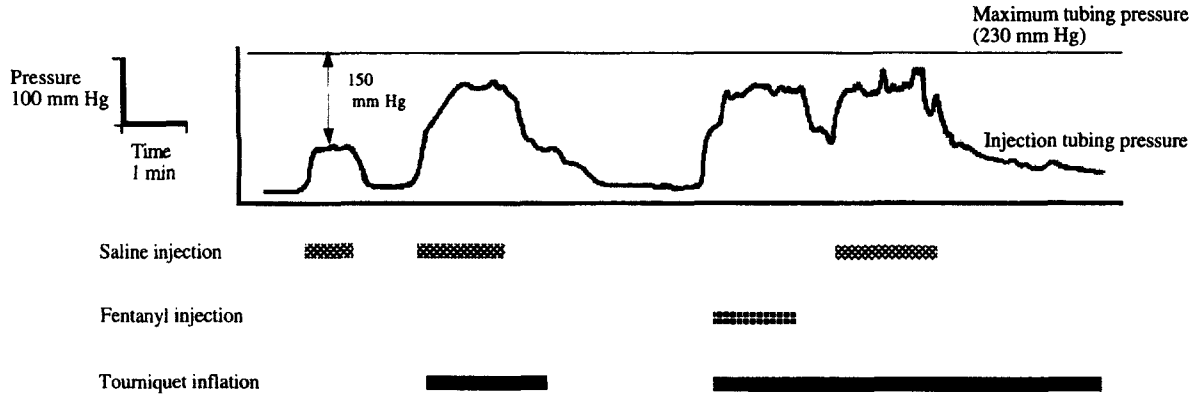

Fig. 1. A typical recording of the tubing pressure during test saline injection with and without tourniquet inflation and during drug administration. In order to prevent leakage into the systemic circulation, the intravenous pressure within the distal limb was limited by restricting the tubing pressure to less than 150 mm Hg greater than the pressure induced by the test saline injection without tourniquet inflation. Thus in this case the tubing pressure during drug administration was not allowed to exceed 230 mm Hg (80 + 150).

Systemic drug administration protocol

Trial C. A higher remoxipride dose (7.5 mg/kg), known to elevate nociceptive thresholds (Main *et al.*, 1995a), was given via the jugular vein catheter, either 90 s prior to, or immediately after tourniquet inflation. Therefore, in the former group, remoxipride was free to invade all tissues including the distal limb test site, whereas in the latter it was intended that remoxipride was excluded from the distal limb.

Eight sheep (73–109 kg body wt) all received two injections of either 0.9% saline or remoxipride (7.5 mg/kg) on two occasions, 7 days apart. On one occasion the remoxipride was given 90 s prior to tourniquet inflation, and the saline given after inflation; on the other, the order of administration of the two infusions relative to the inflation was reversed. Both saline and remoxipride were given in an injection volume of 0.1 mL/kg and the observer was unaware of the order of drug treatments given.

Mechanical nociceptive thresholds were recorded prior to inflation for control values and every 5 min until 40 min after the first injection. The tourniquet used in this trial had a cuff size of 2.5 × 20 cm and was inflated 90 s after the first injection to a pressure of 300 mm Hg. The pressure was maintained for 20 min and then the tourniquet was completely deflated. This inflation pressure in preliminary trials was capable of completely blocking the arterial blood flow in the axial palmar digital (III) artery, as detected by Doppler transcutaneous ultrasound (Parks Electronics Lab, model 801-B, OR, USA).

Trial D. The systemic effect of the lower remoxipride dose used in the peripheral administration protocol, was assessed without peripheral injection or tourniquet restriction. The treatments were given via jugular catheters with no restriction of blood flow to the distal limb. Six sheep were given either saline or remoxipride (2 mg/kg) on two separate occasions and the observer was unaware of the treatment given. Mechanical nociceptive thresholds were recorded before injection and every 5 min after injection for 40 min.

Statistical analysis

For all experiments, the data are presented graphically as the mean ± SEM of the nociceptive threshold force (in N) and as the median (± interquartile range) of area under the threshold vs. time curve expressed as the percentage maximum possible effect (%MPE). The median values were presented since statistical analysis of the percentage MPE values was performed using nonparametric tests. The percentage MPE value was used as a measure of the effect of the treatment over a specified time period and was derived using the following equation:

Maximum Possible Effect (%MPE) = (Observed *AUC* – Baseline *AUC*) × 100 / (Maximum *AUC* – Baseline *AUC*)

i.e.

Observed *AUC* = Area under the observed threshold force vs. time curve;

Baseline *AUC* = Area under the curve if the baseline threshold force had been maintained throughout the experiment;

Maximum *AUC* = Area under the curve if the threshold force had increased to the maximum possible value (20 N) at all postdrug administration time points.

The percentage MPE values were used for the statistical analysis of differences between treatment groups. An assessment of differences between treatment groups in trial A was made using the Kruskal–Wallis nonparametric test, and a Dunn's multiple comparison test was used to make comparisons between groups at each time period. A paired, nonparametric, Wilcoxon signed rank test was used to assess differences between the treatment groups used in trials B, C & D.

RESULTS

Peripheral drug administration

Trial A. Tourniquet inflation and injection of saline had minimal effect on the threshold forces required to induce a limb response in this protocol (Fig. 2A). The peripheral administration of lignocaine produced elevations in thresholds whilst the tourniquet was inflated (Fig. 2B), with a significant ($P < 0.05$) increase in the percentage MPE during the tourniquet inflation time period compared with saline. Five out of the six sheep failed to respond to the maximum pin force (20 N) 5 min after injection. However, only two sheep failed to respond at all the time points during inflation, i.e. there was at least some sensation remaining in the distal limbs of the other sheep.

One sheep which had received clonidine appeared to be sedated and failed to respond to pin stimulation below 20 N before tourniquet deflation. This indicated that some leakage of clonidine past the tourniquet must have occurred. The results from this sheep were therefore not included in the overall results but an extra sheep was included in the trial as a replacement. The observer was still unaware of the treatment given.

In the remaining sheep that received clonidine, there was no significant increase in nociceptive thresholds during tourniquet inflation (Fig. 2B). During the inflation period, one sheep did have maximum values at two time points but all other sheep had thresholds below 10 N. Whilst this sheep did not appear sedated, a small amount of leakage may have occurred. After tourniquet deflation, the sheep which had received clonidine had significantly greater nociceptive thresholds than the saline group (Fig. 2B). All sheep appeared very mildly sedated after tourniquet deflation.

The sheep which had received remoxipride yielded significantly ($P < 0.05$) elevated percentage MPE values both during and after tourniquet inflation (Fig. 2B). There were no sedative or other behavioural effects after remoxipride administration.

Trial B. An example of the tubing pressure recording before and during drug administration is shown in Fig. 1. The tubing pressure reached the maximum pressure allowed in two out of the twelve drug administrations resulting in temporary cessation

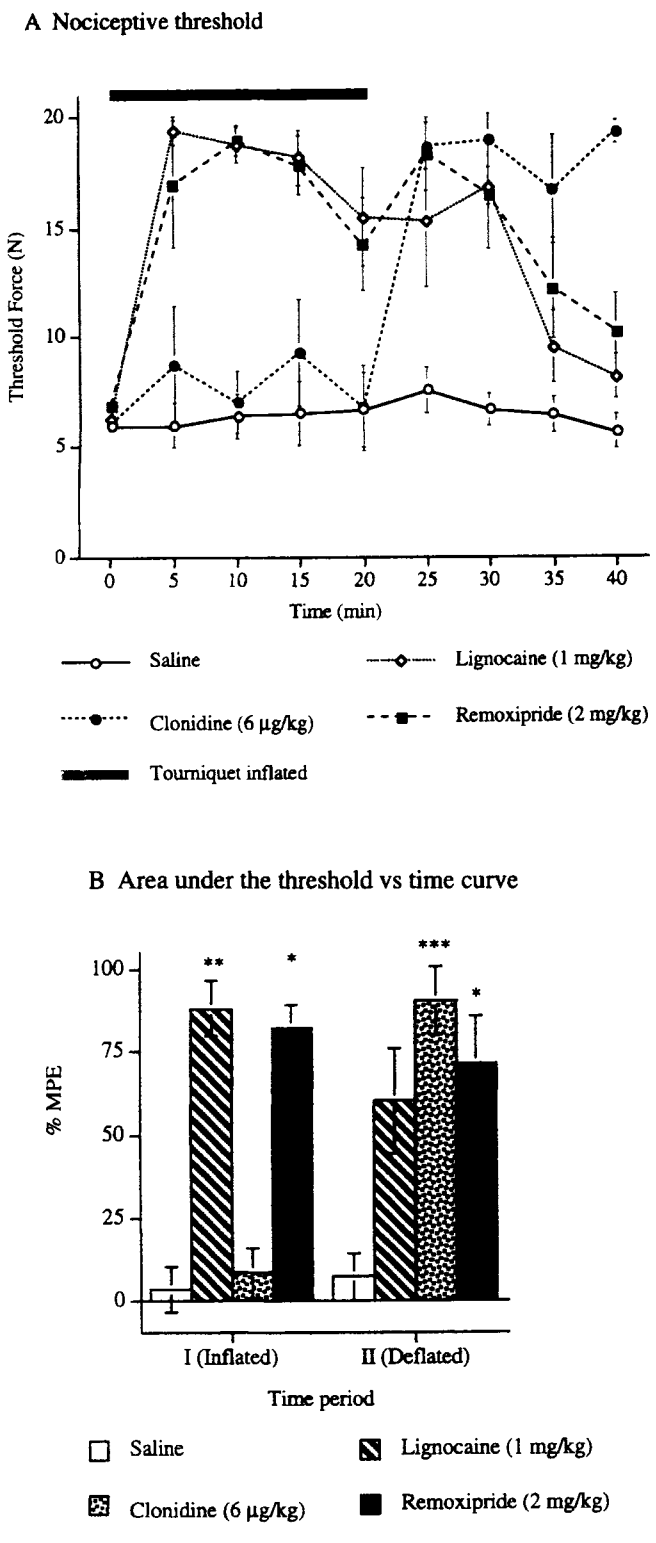

Fig. 2. Trial A. The effect of peripheral administration of saline, lignocaine (1 mg/kg), clonidine (6 μg/kg) and remoxipride (2 mg/kg) on (A) mean ± SEM threshold values (N) and (B) median (± interquartile range) area under the threshold vs. time curve values, expressed as percentage maximum possible effect (% MPE), for the tourniquet inflation time period (I, 0–20 min) and deflation period (II, 20–40 min) ($n = 6$). ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$: significantly different from the saline treated group (Kruskal–Wallis & Dunn's nonparametric tests).

of injection. The total time taken to administer the drug and saline flush ranged from 3 to 4.8 min.

Fentanyl administration resulted in higher thresholds in the forelimb only after tourniquet deflation ($P < 0.05$, Fig. 3B). The threshold changes seen in the hind limb were not significant, although there was a trend in those sheep which had received fentanyl towards an increase in their nociceptive thresholds when compared with the saline controls, but only after tourniquet deflation. There was also a trend for both saline- and fentanyl-treated groups to exhibit increased threshold values for the hind limb during tourniquet inflation. Since the fentanyl group did not appear to have higher threshold levels during this inflation period, it is likely that the modified injection protocol was able to prevent any significant systemic spread past the tourniquet. Behaviourally, those sheep with elevated thresholds following tourniquet deflation appeared both mildly agitated and restless.

Systemic administration

Trial C. Systemic administration of the high dose of remoxipride produced elevated thresholds in both groups (Fig. 4A). However, there was significantly ($P < 0.05$) greater antinociception in the group that had received remoxipride before rather than after tourniquet inflation (median values 67.8 vs. 53.4%; Fig. 4B), i.e. exclusion of remoxipride from the peripheral test site reduced its antinociceptive efficacy.

Trial D. The lower remoxipride dose (2 mg/kg), which had produced elevated nociceptive thresholds in the peripheral administration protocol (trial A), did not produce any change in nociceptive thresholds after its systemic jugular vein administration. The percentage MPE median value for saline and remoxipride was 8.22% (± 1.89 interquartile range) and −1.13% (± 1.42 interquartile range) respectively.

DISCUSSION

Two potential applications of this tourniquet technique have been explored in the present study. First, by restricting drugs to a local site, their potential for a peripheral antinociceptive site of action can be assessed. Second, the importance of such a peripheral site of action to the antinociception seen following its systemic administration can also be assessed.

Methodological considerations

The threshold changes and behavioural effects following clonidine administration give an interesting insight into the validity of the tourniquet protocol used in trial A. As previously mentioned, α_2- adrenergic agonists cause profound sedation in sheep when given intravenously (Livingston *et al.*, 1992). Sedation therefore indicates systemic spread of the drug. In the present study, sedation occurred in one sheep and that animal's data were consequently excluded from the results. Of course, it is

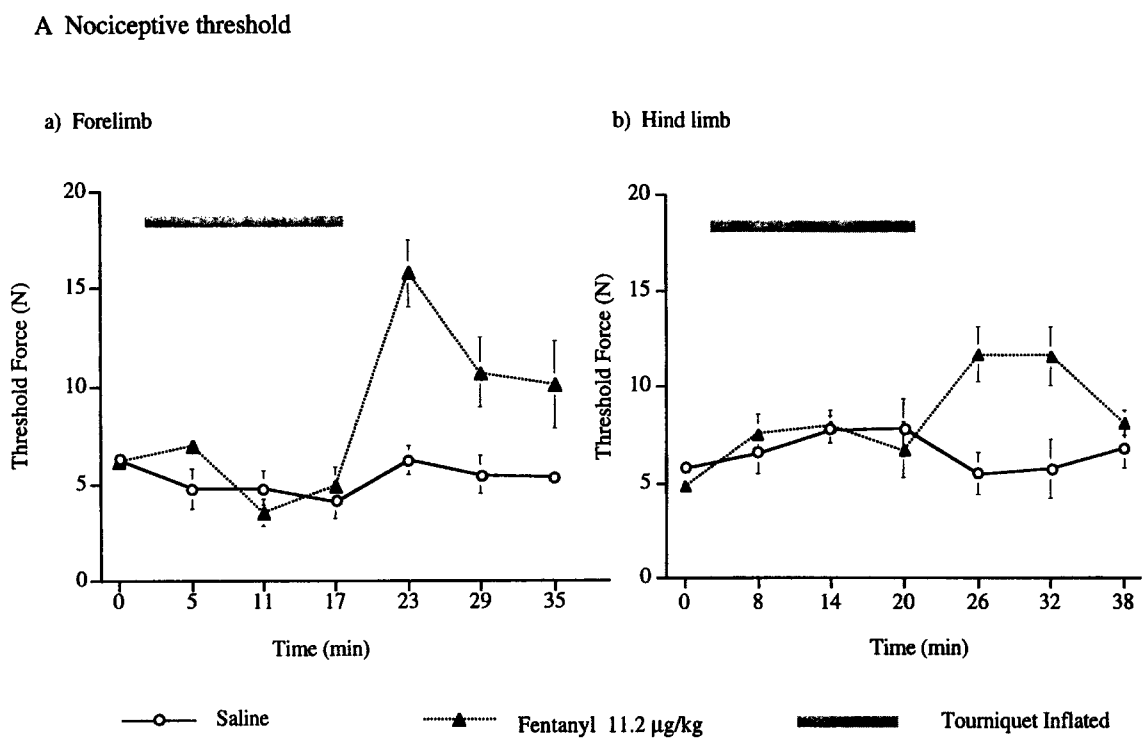

Fig. 3. Trial B. The effect of peripheral administration of saline and fentanyl (11.2 μg/kg) during and after tourniquet inflation on (A) mean ± SEM threshold values (N) and (B) median (± interquartile range) area under the threshold vs. time curve values, expressed as percentage maximum possible effect (% MPE), for the tourniquet inflation time period (I, 0–20 min) and deflation period (II, 20–40 min) and for both fore and hind limbs (n = 6). *P < 0.05; significantly different from the saline treated group (Wilcoxon signed rank nonparametric tests).

not possible to entirely rule out leakage of injected agents past the inflated tourniquet into the systemic circulation in any of the animals using the inflation protocol used in trial A, especially when treatments do not generate systemic behavioural actions. However, the threshold elevations seen in the clonidine group (excluding the prematurely sedated sheep) after tourniquet deflation, infer that release of the restriction was required in order to allow complete systemic spread of clonidine and its subsequent marked antinociceptive and mild sedative effects.

The peripheral administration of lignocaine did induce marked elevations in threshold force during the tourniquet inflation period, indicating that there was restriction to the distal limb. Lignocaine did not, however, completely abolish responses in four of the six sheep, suggesting that local anaesthetic blockade was not always complete. This may have been the result of: (1) an inadequate dose; (2) some leakage past the tourniquet into the systemic circulation; or (3) poor perfusion to the sensory tissues and/or relaying nerve fibres.

Remoxipride also induced marked elevations in nociceptive thresholds when administered via an IVRA protocol. Interestingly, the antinociception lasted longer than that seen with lignocaine and persisted after tourniquet release. This could be a result of a longer duration of action or a slower release from the limb tissues back into the circulation. The antinociception seen during the tourniquet inflation period was dependent upon peripheral administration and not systemic leakage, as trial D demonstrated that systemic administration of the same low dose (2 mg/kg) did not elevate mechanical nociceptive thresholds, i.e. even partial leakage could not explain the dramatic elevation in nociceptive thresholds seen after peripheral administration. Even though partial leakage during the IVRA protocol used in trial A does not invalidate the conclusions with regard to the peripheral antinociception of remoxipride, it is important for future studies to improve the technique by reducing leakage.

Leakage of local anaesthetics past an inflated tourniquet has been reported during intravenous regional anaesthesia

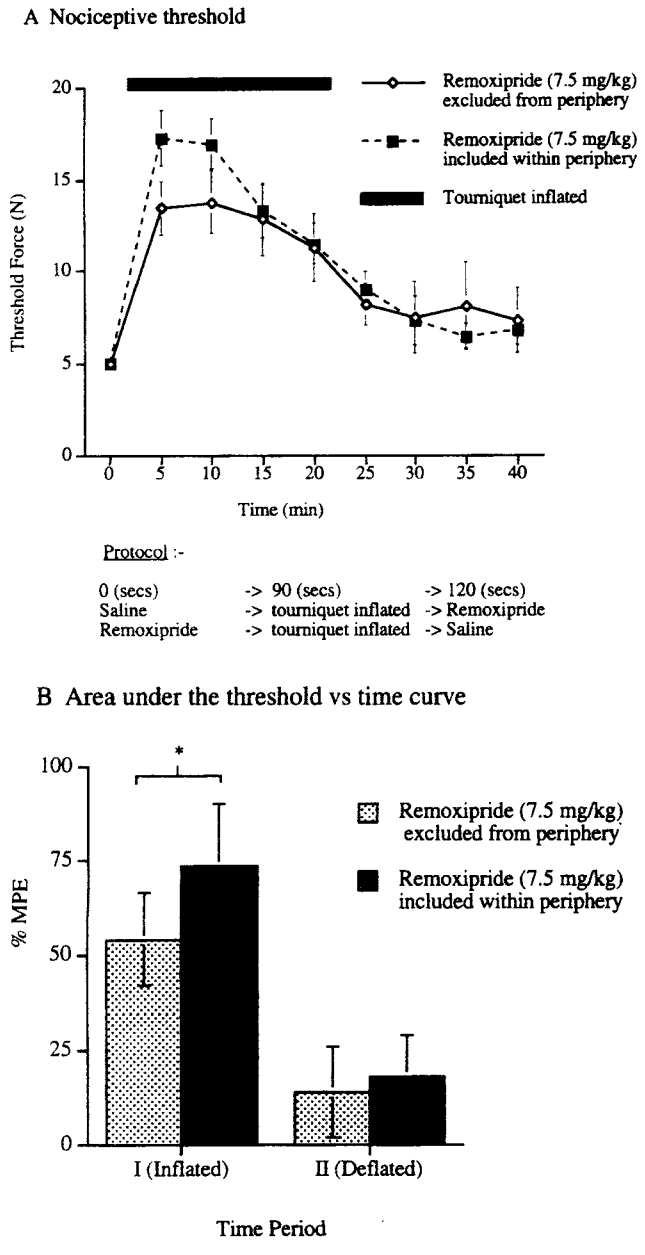

Fig. 4. Trial C. The effect of systemic remoxipride (7.5 mg/kg) given either immediately after or 90 s prior to tourniquet inflation on (A) mean ± SEM threshold values (N) and (B) median (± interquartile range) area under the threshold vs. time curve values, expressed as percentage maximum possible effect (% MPE), for the tourniquet inflation time period (I, 0–20 min) and deflation period (II, 20–40 min) (n = 8). *P < 0.05; significantly different between the two groups (Wilcoxon signed rank nonparametric tests).

(Davies *et al.*, 1983; Rosenberg *et al.*, 1983). Various studies have indicated that exsanguination, a wide tourniquet, high inflation pressures and slow injection rates are all important factors that can reduce leakage (Davies *et al.*, 1983; Grice *et al.*, 1986; Davies, 1989). The nociceptive threshold device was placed around the distal limb in this study which precluded exsanguination. The tourniquet width was also limited as the threshold response depended on free movement of the limb. Grice *et al.* (1986) demonstrated that leakage occurred when a predefined maximum intravenous pressure was reached. The injection protocol in trial B was modified by monitoring and subsequently limiting the venous pressure increase during injection. Presence of systemic leakage could be demonstrated by measuring fentanyl levels within the blood. However, concurrent hind limb threshold testing in this study allowed an immediate pharmacodynamic measure of systemic leakage. There was no elevation in the hind limb nociceptive thresholds during the tourniquet inflation period suggesting that there was no significant leakage of fentanyl into the systemic circulation.

The failure of clonidine and fentanyl to induce a peripheral effect could be the result of poor distribution of these agents to peripheral nerve fibres or nerve endings. Direct administration into peripheral limb tissues or use of a higher dose in this IVRA protocol may have demonstrated a peripheral effect. However, as clonidine and fentanyl did not produce a peripheral effect in this study with doses that could induce antinociception following their systemic administration, it is very unlikely that a peripheral mechanism contributes to the antinociception seen after systemic administration of these agents.

Welsh and Nolan (1994) reported significant hyperalgesia from 15 min onwards after application of a tourniquet to the sheep's forelimb. This hyperalgesia was reduced by prior systemic administration of two nonsteroidal anti-inflammatory drugs (flunixin and caprofen) and systemic fentanyl (5 μg/kg). The reason for the failure to observe hyperalgesia in our study is unclear, although it could be the result of different tourniquet designs. The inflation time in this study was deliberately restricted to 20 min to reduce the potential for hyperalgesia.

Peripheral mechanisms of action

The IVRA technique used in this study restricted agents to the limb periphery. An antinociceptive effect in the periphery is likely to be the result of a modulation of the excitability of the nerve endings or nerve fibres. Endogenous agents (e.g. prostaglandins and bradykinins) or exogenous agents such as local anaesthetics can influence the excitability of peripheral nociceptive neurons, via activation of specific receptors (often G protein-linked) or modulation of ion channel function. Lignocaine is an established local anaesthetic agent which has a well-known mechanism of action via inhibition of sodium channel permeability in nerve cell membranes. This is likely to be responsible for the elevations of nociceptive thresholds seen after peripheral administration of lignocaine in this study.

Clonidine has sedative, anaesthetic and analgesic properties which have been investigated for human clinical use (Maze & Tranquilli, 1991). Clonidine has been reported to exert a peripheral analgesic effect on sympathetically maintained pain after its topical application (Davis *et al.*, 1991). In noninflammatory nociceptive tests, however, clonidine and other α_2-adrenergic agonists are thought to induce antinociception by spinal and possibly supraspinal mechanisms (Pertovaara, 1993). The results of this study also support a nonperipheral site of action for clonidine-induced antinociception. Although one

sheep displayed nociceptive thresholds above 10 N during the inflation period, this may well have been because of partial leakage of clonidine into the systemic circulation.

Clonidine has been shown *in vitro* to inhibit C-fibre action potentials via a local anaesthetic-like mechanism at a concentration (500 μM; Gaumann *et al.*, 1992) which is similar to that administered in this study (450 μM). Using the IVRA protocol, the administered solution would have been diluted within the limb tissues so that clonidine concentrations at the sensory tissues were certainly lower. Drug dilution is likely to occur in the peripheral limb as the administered lignocaine concentration required for peripheral antinociception in this study was 73.9 mM, which is almost 150 times the effective *in vitro* concentration required for sodium channel blockade (Gaumann *et al.*, 1992).

Interestingly, Gaumann *et al.* (1992) were able to demonstrate an enhancing effect of a much lower clonidine concentration (500 nM) on lignocaine-induced inhibition of C-fibre action potentials. This is important as low doses of clonidine can prolong clinical peripheral nerve block duration and post-operative analgesia (Maze & Tranquilli, 1991). The model described here would be ideal to investigate such an interaction at a peripheral level.

The mechanism of the peripheral antinociceptive action of remoxipride is not known. However, remoxipride is able to block sodium current flow in rat CNS tissue *in vitro* (IC 50 $\approx$20 μM) and frog peripheral nerve membranes (IC 50 $\approx$ 300 μM; Westlind *et al.*, 1992). The remoxipride concentration in the peripherally administered solution (94 mM) was much greater than these values so that even with dilution after administration, the tissue concentration could well be adequate for sodium channel blockade. Remoxipride is also a dopamine D_2 receptor antagonist. However, we have been unable to find reports of peripheral dopamine receptors in peripheral limb nerves or vessels and so, whilst an action via blockade of these receptors cannot be excluded, it is unlikely.

The failure of fentanyl to induce peripheral antinociception in this study is not unexpected from previous studies in humans. Armstrong *et al.* (1991), Abdulla and Fadhil (1992) and Arthur *et al.* (1992), showed there was no benefit derived from adding fentanyl (1.25–2.5 μg/mL, total dose 50–100 μg) to the local anaesthetic solution used for intravenous regional anaesthesia. Our study involved administration of a much higher concentration of fentanyl (224 μg/mL, total dose 610–850 μg) but still we could not demonstrate peripheral antinociception. It is interesting to compare the *in vitro* capacity of fentanyl to block nerve conduction and the lack of an *in vivo* peripheral effect seen with our model. Gissen *et al.* (1987) demonstrated that fentanyl at 100 μg/mL induced a poor reduction in action potentials in intact C fibres, whereas Power *et al.* (1988)produced a reversible block on C-fibre conduction by fentanyl (ED50 170 μg/mL). However, in the present *in vivo* study, the administered fentanyl concentration (224 μg/mL) was inadequate to induce peripheral antinociception. As with clonidine, the most likely explanation here is the dilution of fentanyl in the tissue fluids that is inevitable *in vivo*. In some instances, opioids have a potential peripheral effect in the presence of inflammation (Stein, 1993). The methodology described in this study would be useful to investigate the influence of peripheral/systemic opioids on tourniquet-induced hyperalgesia following an extended inflation period (Welsh & Nolan, 1994), and hyperalgesia induced by chronic lameness in sheep (Ley *et al.*, 1995).

Contribution of peripheral mechanisms to a systemic effect

The peripheral administration of remoxipride indicated the potential for a peripheral site of antinociception. Interestingly, trial C demonstrated that when blood containing remoxipride was excluded from the distal limb and hence, the nociceptive test site, the elevations in thresholds were less than if remoxipride was allowed to enter the limb freely. This would suggest that at least a component of the antinociceptive effect seen following high systemic doses is the result of a peripheral site of action. The inability of tourniquet inflation, prior to administration, to suppress the systemic remoxipride-induced threshold elevation completely may have been because of leakage past the tourniquet into the distal limb, as well as an action at other nonperipheral sites.

The mechanism of this peripheral effect of remoxipride is unknown but it is possible that sodium channel blockade may be involved. The estimated remoxipride plasma concentration 90 s after a 7.5 mg/kg dose is 19 μM, using decay-derived estimates of plasma remoxipride concentrations following its systemic administration at a dose of 10 mg/kg (Main *et al.*, 1996). This value is of a similar order of magnitude to the effective *in vitro* concentrations of remoxipride required for voltage-dependent sodium channel blockade (Westlind *et al.*, 1992). Previous work (Main *et al.*, 1995a) has demonstrated thermal and mechanical antinociception in rats but this was accompanied by a significant degree of motor function impairment. This remoxipride-induced behavioural profile in this study was different from that produced by systemic administration of a local anaesthetic agent, tocainide (100 mg/kg), which produced clear antinociception to thermal but not mechanical stimuli with no reduction in rotarod performance (motor function). This would suggest that remoxipride-induced sodium channel blockade could not be solely responsible for the threshold changes seen in rats. In sheep, however, the apparent lack of motor impairment warranted further investigation into the site/mechanism of action and this study suggests a peripheral contribution to remoxipride-induced antinociception in sheep.

In summary, the technique described here proved effective for defining the lack of a peripheral site of antinociceptive action for clonidine and fentanyl, whilst demonstrating a peripheral site of antinociceptive action for remoxipride in sheep.

ACKNOWLEDGMENTS

D.C.J.M. is a Wellcome Trust Veterinary Clinical Research Training Scholar. Remoxipride was supplied by Astra Arcus AB (Södertälje, Sweden). We would also like to thank Dr B.D.X. Lascelles and Miss H.R. Whay for their help and support.

REFERENCES

Abdulla, W.Y. & Fadhil, N.M. (1992) A new approach to intravenous regional anesthesia. *Anesthesia Analgesia*, **75**, 597–601.

Acalovschi, I. & Cristea, T. (1995) Intravenous regional anesthesia with meperidine. *Anesthesia Analgesia*, **81**, 539–543.

Armstrong, P., Power, I. & Wildsmith, J.A. (1991) Addition of fentanyl to prilocaine for intravenous regional anaesthesia. *Anaesthesia*, **46**, 278–280.

Armstrong, P.J., Morton, C.P.J. & Nimmo, A.F. (1993) Pethidine has a local anaesthetic action on peripheral nerves *in vivo*. Addition to prilocaine 0.25% for intravenous regional anaesthesia in volunteers. *Anaesthesia*, **46**, 382–386.

Arthur, J.M., Heavner, J.E., Mian, T. & Rosenberg, P.H. (1992) Fentanyl and lidocaine vs. lidocaine for Bier block. *Regional Anesthesia*, **17**, 223–227.

Chambers, J.P. (1993) A comparison of lignocaine and alpha-2 adrenergic ligands as local anaesthetics. *Journal of Veterinary Anaesthesia*, **20**, 108–109.

Chambers, J.P., Waterman, A.E. & Livingston, A. (1994) Further development of equipment to measure nociceptive thresholds in large animals. *Journal of Veterinary Anaesthesia*, **21**, 66–72.

Davies, J.A.H. (1989) Intravenous regional analgesia with prilocaine for foot surgery. *Anaesthesia*, **44**, 902–906.

Davies, J.A.H., Hall, I.D., Wilkey, A.D., Smith, J.E., Walford, A.J. & Kale, V.R. (1983) Intravenous regional analgesia: The danger of the congested arm and the value of occlusion pressure. *Anaesthesia*, **39**, 416–421.

Davis, K.D., Treede, R.D., Raja, S.N., Meyer, R.A. & Campbell, J.N. (1991) Topical application of clonidine relieves hyperalgesia in patients with sympathetically maintained pain. *Pain*, **47**, 309–317.

Fagg, P. (1987) Intravenous regional anaesthesia for lower limb orthopaedic surgery. *Annals Royal College of Surgeons of England*, **69**, 274–275.

Gaumann, D.M., Brunet, P.C. & Jirounek, P. (1992) Clonidine enhances the effects of lidocaine on C-fiber action potential. *Anesthesia Analgesia*, **74**, 719–725.

Gissen, A.J., Gugino, L.D., Datta, S., Miller, J. & Covino, B.G. (1987) Effects of fentanyl and sufentanil on peripheral mammalian nerves. *Anesthesia Analgesia*, **66**, 1272–1276.

Grice, S.C., Morell, R.C., Balestrieri, F.J., Stump, D.A. & Howard, G. (1986) Intravenous regional anaesthesia: Evaluation and prevention of leakage under the tourniquet. *Anesthesiology*, **65**, 316–320.

Junien, J.L. & Wettstein, J.G. (1992) Role of opioids in peripheral analgesia. [Review]. *Life Sciences*, **51**, 2009–2018.

Lewander, T., Westerbergh, S.E. & Morrison, D. (1990) Clinical profile of remoxipride – a combined analysis of a comparative double-blind multicentre trial programme. *Acta Psychiatrica Scandinavia*, **358**, (suppl), 92–98.

Ley, S.J., Waterman, A.E. & Livingston, A. (1995) A field study of the effect of lameness on mechanical nociceptive thresholds in sheep. *Veterinary Record*, **137**, 85–87.

Livingston, A., Waterman, A.E., Nolan, A. & Amin, A. (1992) Comparison of the thermal and mechanical antinociceptive actions of opioids and alpha2-adrenoceptor agonists in sheep. In *Animal pain*. Eds Short, C.E. & Poznak A.V, pp. 372–377. Churchill Livingston, New York.

Main, D.C.J., Waterman, A.E. & Kilpatrick, I.C. (1995a) Behavioural analysis of changes in nociceptive thresholds produced by remoxipride in sheep and rats. *European Journal of Pharmacology*, **287**, 221–231.

Main, D.C.J., Waterman, A.E. & Kilpatrick, I.C. (1995b) The use of a forelimb tourniquet for evaluating the role of a peripheral site of action for analgesic drugs in sheep. *Journal of Veterinary Anaesthesia*, **22**, 38–39.

Main, D.C.J., Waterman, A.E. & Kilpatrick, I.C. (1996) Disposition of remoxipride in plasma and cerebrospinal fluid in sheep. *Journal of Veterinary Pharmacology and Therapeutics*, **19**, 402–404.

Maze, M. & Tranquilli, W. (1991) Alpha-2 adrenoceptor agonists: Defining the role in clinical anesthesia, *Anesthesiology*, **74**, 581–605.

Ögren, S.O., Hall, H., Köhler, C., Magnusson, O., Lindbom, L.O., Ängeby, K. & Florvall, L. (1984) Remoxipride, a new potential antipsychotic compound with selective antidopaminergic actions in the rat brain. *European Journal of Pharmacology*, **102**, 459–474.

Pertovaara, A. (1993) Antinociception induced by alpha-2-adrenoceptor agonists with special emphasis on medetomidine studies. *Progress in Neurobiology*, **40**, 691–709.

Power, I., Brown, D.T. & Wildsmith, J.A.W. (1988) Effect of fentanyl on nerve conduction. *British Journal of Anaesthesia*, **61**, 507–508P.

Prentice, D.E., Wyn-Jones, G., Jones, R.S. & Jagger, D.W. (1974) Intravenous regional anaesthesia of the bovine foot. *Veterinary Record*, **94**, 293–295.

Reuben, S.S., Steinberg, R.B., Kreitzer, J.M. & Duprat, K.M. (1995) Intravenous regional anesthesia using lidocaine and ketorolac. *Anesthesia Analgesia*, **81**, 110–113.

Rosenberg, P.H., Kalso, E.A., Tuominen, M.K. & Linden, H.B. (1983) Acute bupivacaine toxicity as a result of venous leakage under the tourniquet cuff during a Bier block. *Anesthesiology*, **58**, 95–98.

Stein, C. (1993) Peripheral mechanisms of opioid analgesia. [Review]. *Anesthesia Analgesia*, **76**, 182–191.

Waterman, A.E., Livingston, A. & Amin, A. (1990) The antinociceptive activity and respiratory effects of fentanyl in sheep. *Journal of Association of Veterinary Anaesthetists*, **17**, 20–23.

Welsh, E. & Nolan, A.M. (1994) Effects of non-steroidal anti-inflammatory drugs on the hyperalgesia to noxious mechanical stimulation induced by the application of a tourniquet to a forelimb of sheep. *Research Veterinary Science*, **57**, 285–291.

Westlind, D.A., Ericsson, G., Sandell, L., Elinder, F. & Arhem, P. (1992) High concentrations of the neuroleptic remoxipride block voltage-activated Na + channels in central and peripheral nerve membranes. *European Journal of Pharmacology*, **224**, 57–62.