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# Investigation of the antinociceptive activity of buprenorphine in sheep

A. Nolan,[1] A. Livingston & *A.E. Waterman

Departments of Pharmacology and *Veterinary Surgery, University of Bristol, Bristol BS8 1TD

**1** Buprenorphine given intravenously (6 $\mu g\,kg^{-1}$) was examined for its antinociceptive activity in unrestrained sheep using devices to measure thermal and mechanical thresholds

**2** The plasma levels of buprenorphine following intravenous injection over the time period of the antinociceptive testing were measured using a radioimmunoassay.

**3** Buprenorphine produced a clear antinociceptive effect lasting for up to three and a half hours when measured by the thermal threshold test, but no detectable antinociception in the mechanical test.

**4** The plasma levels of buprenorphine indicated that the drug was rapidly distributed in a manner not dissimilar to that reported in man, although individual animals showed a wide variation in some parameters.

**5** When plasma levels of the drug were high ($<700\,pg\,ml^{-1}$) during the first sixty minutes, no antinociceptive activity in the thermal test could be detected, which may be due to the slow receptor kinetics shown by this drug.

## Introduction

Most studies on the antinociceptive actions of opioids have been restricted to laboratory animals and man, although Pippi & Lumb (1979) and Kammerling *et al.* (1985, 1986) have examined their actions in horses.

In this study we used the sheep as an experimental animal and have compared the effects of buprenorphine on a thermal threshold stimulus and a mechanical pressure threshold stimulus.

The threshold stimuli were measured using a device which applied a ramped thermal stimulus to the pinna of the ear and one which produced a ramped increase in pressure via a mechanically driven pin which pressed against the anterior aspect of the radius. Both devices have been shown to produce a reliable series of values for nociceptive thresholds that do not vary with repeated stimuli, even if these are near maximal, and are not affected by sedation (Nolan *et al.*, 1987).

In addition the time course of the antinociceptive actions of buprenorphine was assessed in conjunction with the plasma levels of the drug using a sensitive radioimmunoassay. The techniques of assaying plasma buprenorphine using gas liquid chromatography (Blom & Bondesson, 1985; Cone *et al.*, 1985) were not sufficiently sensitive for the measurements in sheep and so a radioimmunoassay based on that described by Bartlett *et al.* (1980) was used to estimate the plasma levels. The nature of the spectrum of receptor activity of buprenorphine has been rather contentious, and whilst it has been generally agreed that the drug may affect both $\mu$ and $\kappa$ sites, the nature of the effects in terms of agonism and antagonism is less clear (Richards & Sadée, 1985).

[1]Author for correspondence.

## Methods

### *Animals*

Twelve adult female clun-cross ewes were used in the experiments. They were kept either at grass or housed and fed hay *ad libitum* depending on seasonal conditions. The animals weighed between 50 and 70 kg and were accustomed to handling. The techniques for measuring thermal and mechanical nociceptive thresholds have been described elsewhere (Nolan *et al.*, 1987).

### *Drug protocols*

A series of control readings were taken 15 to 30 min apart before the injection of any drugs.

*Thermal testing* Buprenorphine (6 $\mu g\,kg^{-1}$ i.v.) was given alone to seven sheep and tests carried out for

between 120 and 300 min. In two animals naloxone (0.2 mg $kg^{-1}$ i.v.) was injected 5 min before the buprenorphine (6 $\mu g\ kg^{-1}$ i.v.) and testing carried out for 120 min.

*Mechanical testing* Buprenorphine (6 $\mu g\ kg^{-1}$ i.v.) was given to five sheep and tests were carried out for 150 min, in addition one animal was dosed with 3 $\mu g\ kg^{-1}$ i.v. In two animals naloxone (0.2 mg $kg^{-1}$ i.v.) was given 5 min before buprenorphine (6 $\mu g\ kg^{-1}$ i.v.) and readings taken for 120 min, the injection of naloxone was then repeated and xylazine (50 $\mu g\ kg^{-1}$ i.v.) injected 5 min later and readings taken at 5, 15 and 30 min.

All drugs were washed in to a total volume of 10 ml with sterile 0.9% saline.

*Assay of buprenorphine*

Blood samples were taken under sterile conditions from the jugular vein via a 14 gauge cannula. Five ml samples were collected into heparinized syringes in the control period before injection of buprenorphine (6 $\mu g\ kg^{-1}$ i.v.) and at intervals up to 360 min after injection. Each sample was mixed and transferred to glass tubes which were refrigerated until centrifugation at 1000 *g* for 20 min. The resulting plasma was frozen and stored at −20°C until assayed.

*Antiserum*

The antibody used in the present studies was a generous gift from Reckitt and Colman, Hull. Details of characterization and preparation of the antiserum are given in Bartlett *et al.* (1980) along with information concerning cross-reactivity of the antiserum. The antibody used in the studies described here (L162) corresponded to L31 described by the above workers. The antibody was raised by immunization of rabbits with N-hemisuccinylbuprenorphine conjugated to bovine serum albumin. The antiserum was highly specific for buprenorphine and its N-dealkylated metabolite, but showed little affinity for the glucuronide metabolite.

*Assay procedure*

The assay method used was a modification of the method published by Bartlett *et al.* (1980). For every assay, a standard curve was constructed using amounts of buprenorphine varying from 1.95 to 8,000.0 pg per assay tube.

Each point on the standard curve was assayed in duplicate and samples were assayed in triplicate. For each set of samples, two were randomly chosen and 3 different dilutions, in a final volume of 200 $\mu l$, were assayed, each one a single determination, to check for parallelism with the standard curve.

Five hundred $\mu l$ of diluted [$^3$H]-buprenorphine (400 $\mu l$ stock made up to 40 ml with phosphate buffer) were pipetted into each assay tube followed by: 30% methanol (100 $\mu l$), plasma (200 $\mu l$) and 100 $\mu l$ antiserum (1:1600), to give a final dilution of 1:14,400 in a total assay volume of 900 $\mu l$; this final antiserum dilution had been previously estimated to yield 50% binding of ligand to antibody. For preparation of the standard curve, 80 $\mu l$ methanol (30%) and varying concentrations of buprenorphine (each contained in 20 $\mu l$) were added to each tube followed by 200 $\mu l$ of control plasma and finally 100 $\mu l$ antiserum (1:1600). Non-specific binding was determined by omitting antiserum and 'cold' buprenorphine from the tubes and replacing these with a saline/chlorhexidine solution. Maximum binding was determined in the absence of any displacing 'cold' buprenorphine. (In these tubes methanol (30%) was substituted). All assay tubes were vortexed and then left at room temperature for 2 h. Separation of bound and free [$^3$H]-buprenorphine was carried out using a dextran/charcoal suspension. The supernatants were then transferred to a liquid scintillation counter.

*Pharmacokinetic analyses*

Plasma concentrations of buprenorphine following intravenous injection were analysed using computer programmes for bi- and tri-exponential decay processes (Barlow, 1983). These programmes are based on the equations describing double and triexponential decays respectively.

$$C_p = Ae^{-\alpha t} + Be^{-\beta t}$$
$$C_t = Pe^{-\pi t} + Ae^{-\alpha t} + Be^{-\beta t}$$

where $C_p$, $C_t$ are concentrations of the drug in plasma at time t, $\alpha$, $\beta$, $\pi$ = rate constants for a given exponential process and A, B, P = concentration of the drug at time 0, i.e. maximum concentration for each exponential. The half-time ($t_{\frac{1}{2}}$) for each exponential decay describes the time taken for the concentration to fall by a half; it is constant at any part of the curve and can be expressed by $t_{\frac{1}{2}} = 0.693/k$. The computer programme estimates the half-lives of each exponential process mathematically from the rate constants (k = rate constant). Other terms used in the description of pharmacokinetic analysis are defined as follows: $t_{\frac{1}{2}\alpha}$ = distribution half-time (min). $t_{\frac{1}{2}\beta}$ = elimination half-time, also known as the half-life of the drug (min). $Vd_{(area)}$ = specific apparent volume of drug distribution based on the total area under the concentration vs time curve = Dose/A/$\alpha$ + B.$\beta$/$\beta$ (l $kg^{-1}$). Vc = specific volume of the central compartment (l $kg^{-1}$). $Cl_\beta$ = body clearance of a drug = $\beta$.$Vd_{(area)}$ i.e. the volume of blood cleared of a drug per unit time measured in ml $kg^{-1}$ $min^{-1}$.

*Drugs used*

Buprenorphine HCl (Temgesic; Reckitt & Colman), xylazine HCl (Rompun; Bayer), and naloxone HCl (Endo Laboratories).

*Statistical analysis*

The nociception data were examined using the Chi squared test, since the imposition of the maximum value rendered the data non-parametric. An effect was valued as different when it was two standard deviations from the mean pre-test values, and results were judged to be significantly different when a value of $P < 0.05$ was obtained.

## Results

*Thermal nociceptive testing*

Buprenorphine (6 $\mu g\,kg^{-1}$) gradually raised the temperature at which a response was evoked from 55.6 ± 1.2°C to the maximum (70°C) after 45 min. The temperature at which the sheep responded fell slowly from this time onwards but remained elevated for 210 min before returning to control values after 240 min (Figure 1). This prolonged elevation in nociceptive threshold was abolished for at least 120 min by pretreatment with naloxone (0.2 $mg\,kg^{-1}$)

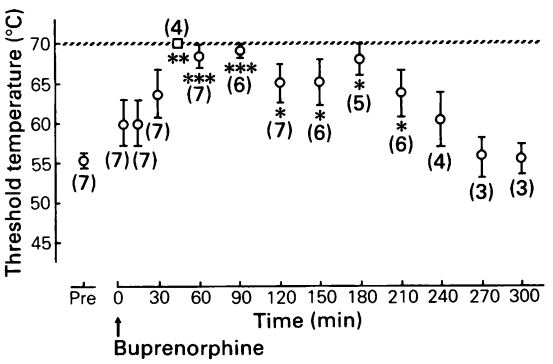


**Figure 1** The effect of buprenorphine (6 $\mu g\,kg^{-1}$ i.v.) on the thermal response threshold. The response threshold (°C) is plotted against time (min). Buprenorphine was injected at time 0. The pre-test reading (Pre) is the mean value of an average of 3–4 determinations for each of 6 animals over a 45–60 min period before drug injection. Each point represents the mean value for $n$ animals and vertical lines indicate s.e.mean. The value of $n$ is shown in parenthesis. The broken line represents the maximal thermal threshold. ***$P < 0.01$, **$P < 0.02$, *$P < 0.05$, determined using the Chi-squared test.

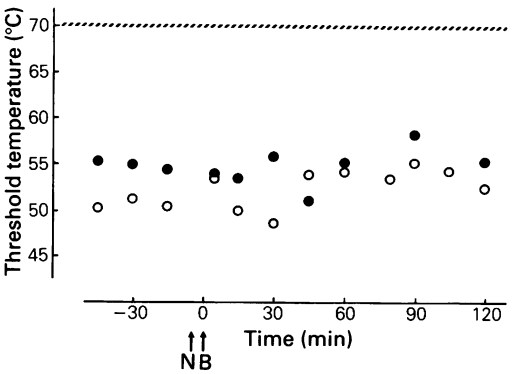


**Figure 2** The effect of buprenorphine (B, 6 $\mu g\,kg^{-1}$, i.v.) on the thermal response threshold after pretreatment with naloxone (N, 0.2 $mg\,kg^{-1}$, i.v.). The response threshold (°C) is plotted against time (min). Naloxone was injected 5 min before buprenorphine. Buprenorphine was injected at time 0. Each point represents an individual determination. The broken line represents the maximal thermal threshold. (●) Sheep 201, (○) sheep 218.

(Figure 2). Readings were normally taken until values fell to pre-test levels.

Saline injections did not alter the temperature at which the sheep responded to the stimulus.

*Mechanical nociceptive testing*

Buprenorphine (6 $\mu g\,kg^{-1}$) did not alter the response threshold from a control value of 180 ± 40 dial units (equivalent to 3.06 ± 0.68 N) except for a decrease in threshold at 5 min (Figure 3). Prior treatment with naloxone did not affect the response threshold (Figure 4). Subsequent injection with xylazine (50 $\mu g\,kg^{-1}$) increased the response threshold to maximum, and indicated that the test system was in order and that the opioid antagonist had no effect on the $\alpha_2$-adrenoceptor mediated antinociceptive effect. Buprenorphine (3 $\mu g\,kg^{-1}$) similarly did not alter response threshold.

Saline injection had no effect on control responses.

*Behavioural responses*

Injection of buprenorphine produced marked excitement. This excitement was slow in onset, with an obviously altered behaviour pattern recognisable only after 5–15 min. The sheep began to chew intensely at the box, pen or any ropes or cables within reach and bleated occasionally. Head movements were rapid and frequent. This excited behaviour often made it difficult to obtain regular readings in the thermal test and consequently the number of readings at particular time points in the results varies somewhat. Increased

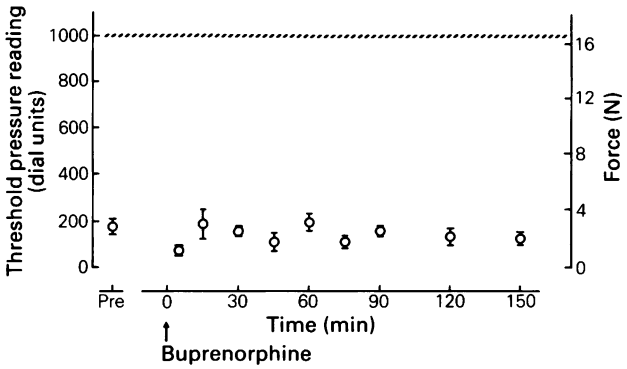


**Figure 3** The effect of buprenorphine (6 μg kg$^{-1}$, i.v.) on the mechanical response threshold. The response threshold (dial units or N) is plotted against time (min). Buprenorphine was injected at time 0. The pre-test reading (Pre) is the mean value of an average of 4 determinations for each animal over a 45–60 min period before drug injection. Each point represents the mean value for 5 animals and vertical lines indicate s.e.mean. The broken line represents the maximum mechanical threshold.

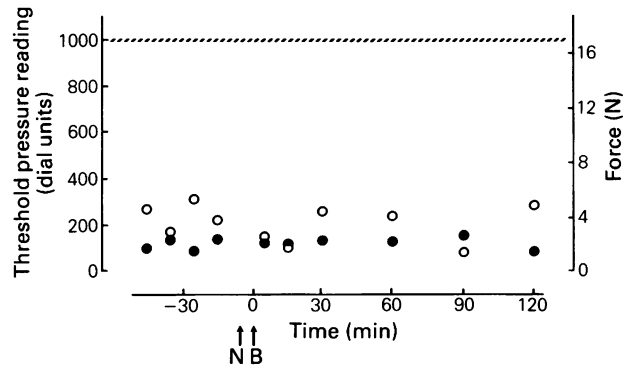


**Figure 4** The effect of buprenorphine (6 μg kg$^{-1}$, i.v.) on the mechanical response threshold after pretreatment with naloxone (0.2 mg kg$^{-1}$, i.v.). The response threshold (dial units or N) is plotted against time (min). Naloxone (N) was injected 5 min before buprenorphine (B). Buprenorphine was injected at time 0. Each point represents an individual determination. The broken line indicates the maximum mechanical threshold. (●) Sheep 201, (○) sheep 210.

locomotor activity was apparent when the sheep were in a larger pen, but this was not as readily visible when they were in a wooden box which restricted their movement. All the above signs were present for 120–180 min. Two hundred and ten minutes after drug injection all animals appeared calm once again.

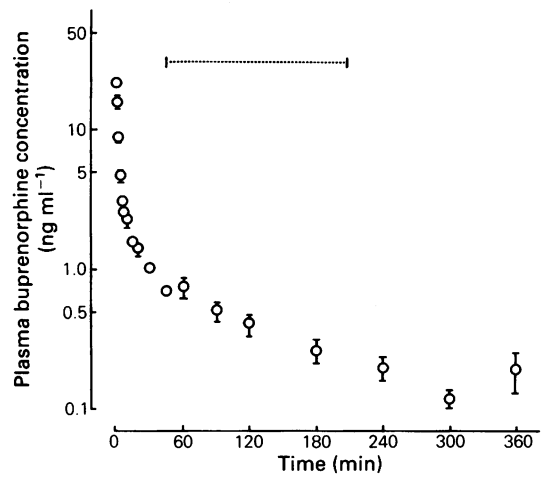


**Figure 5** Plasma levels of buprenorphine after intravenous administration of buprenorphine (6 μg kg$^{-1}$) to sheep. Concentration in ng ml$^{-1}$ has been plotted on a logarithmic scale against time in min after injection. Each point represents the mean values from 6 animals and vertical lines indicate s.e.mean. The mean weight of the sheep was 62.0 ± 3.3 kg. The broken line represents the time during which analgesia was present following thermal nociceptive testing.

*Plasma levels of buprenorphine*

Figure 5 shows the mean plasma levels of buprenorphine following i.v. injection (6 μg kg$^{-1}$) in six animals. There was an initial rapid decline in plasma concentration of the drug followed by two slower phases. All curves for each individual were best fitted to a triexponential decay pattern. Table 1 presents the individual results for the rate constants $\pi$, $\alpha$, and $\beta$ and maximum concentrations P, A and B and the half-time $t_{1/\pi}$, representing an initial fast distribution phase ranging from 0.242 min to 1.374 min. This was followed by a second slower distribution phase with a range in half-times ($t_{1/\alpha}$) from 1.6 min to 16.0 min. The elimination half-lives ($t_{1/\beta}$) varied widely from 44 to 350 min. It is interesting to note than one particular sheep (denoted 186) had consistently slower half-lives.

Vc was small in all animals, range 0.036–0.272 l kg$^{-1}$, one sheep (211) having a particularly low value of 0.036 l kg$^{-1}$. $Vd_{(area)}$ was relatively large (mean value of 4.644 l kg$^{-1}$) and clearance rates were relatively fast (mean value of 29.7 ml kg$^{-1}$ min$^{-1}$).

## Discussion

The injection of buprenorphine (6 μg kg$^{-1}$, i.v.) into sheep reduced sensitivity to a painful thermal

**Table 1** Individual pharmacokinetic constants for sheep determined after an intravenous injection of buprenorphine (6 μg kg$^{-1}$)

| Sheep | $\pi$ (min$^{-1}$) | $\alpha$ (min$^{-1}$) | $\beta$ (min$^{-1}$) | $P$ (ng ml$^{-1}$) | $A$ (ng ml$^{-1}$) | $B$ (ng ml$^{-1}$) |
|---|---|---|---|---|---|---|
| 156 | 1.542 | 0.196 | 0.007 | 33.527 | 7.479 | 1.427 |
| P911 | 0.963 | 0.098 | 0.006 | 22.830 | 3.208 | 0.973 |
| 186 | 0.505 | 0.043 | 0.002 | 19.143 | 2.640 | 0.297 |
| P820 | 1.445 | 0.226 | 0.012 | 27.875 | 10.502 | 1.107 |
| 211 | 2.870 | 0.424 | 0.016 | 141.452 | 20.465 | 2.907 |
| 218 | 2.247 | 0.298 | 0.013 | 42.988 | 6.480 | 1.216 |
| Mean ± s.e.mean ($n$ = 6) | 1.595 ± 0.349 | 0.214 ± 0.056 | 0.009 ± 0.002 | 48.136 ± 19.000 | 8.462 ± 2.675 | 1.321 ± 0.354 |

| Sheep | $t_{1/2\pi}$ (min) | $t_{1/2\alpha}$ (min) | $t_{1/2\beta}$ (min) | $Vc$ (l kg$^{-1}$) | $Vd_{(area)}$ (l kg$^{-1}$) | $Cl_\beta$ (ml kg$^{-1}$ min$^{-1}$) |
|---|---|---|---|---|---|---|
| 156 | 0.450 | 3.533 | 103.885 | 0.141 | 3.250 | 22.8 |
| P911 | 0.720 | 7.092 | 116.780 | 0.222 | 4.574 | 27.4 |
| 186 | 1.374 | 16.041 | 350.135 | 0.272 | 12.107 | 24.2 |
| P820 | 0.480 | 3.065 | 60.021 | 0.152 | 3.165 | 38.0 |
| 211 | 0.242 | 1.634 | 43.997 | 0.036 | 1.343 | 21.5 |
| 218 | 0.308 | 2.324 | 54.756 | 0.116 | 3.423 | 44.5 |
| Mean ± s.e.mean ($n$ = 6) | 0.596 ± 0.170 | 5.615 ± 2.224 | 121.596 ± 47.2 | 0.157 ± 0.034 | 4.644 ± 1.552 | 29.7 ± 3.822 |

For explanation of abbreviations used see Methods section.

stimulus. Although this antinociceptive activity was slow in onset, it lasted for 3½ h after injection. Surprisingly the drug was without influence on mechanically-induced pain. The reason for this difference is unclear. It is well documented that the drug displays marked antagonist properties at high doses, which causes its dose-response curve to be bell-shaped in rodents (Dum & Herz, 1981). It was therefore decided to give buprenorphine at a lower dose (3 μg kg$^{-1}$) to one sheep, and to pretreat two further sheep with naloxone before drug administration, in an attempt to determine if the lack of effect in the mechanical test was attributable possibly to a drug 'overdosage'. If this were the case in the sheep, the effect recorded would have lain on the right hand side of the bell-shaped dose-response curve. Measurements were continued for up to 2½ h after drug administration, and no signs of analgesia developed in this period. Nevertheless, the remote possibility that antinociception was present at times later than this, cannot be discounted completely. In any event, neither treatment altered the lack of effect of buprenorphine in the mechanical test system.

Martin *et al.* (1976) classified buprenorphine as a μ-selective opioid, as have other workers (Cowan *et al.* 1977). However, Richards & Sadée (1985) suggested it had equal affinity for both μ and κ sites. Tyers (1980) found that buprenorphine was much more potent against non-heat stimuli (paw pressure, writhing test) and on this basis suggested that the drug was predominantly a partial agonist at κ-sites. A year earlier Bryant & Tyers (1979) had shown that buprenorphine injected intrathecally was inactive on both hot plate and paw pressue tests unless the dose exceeded that which was active after subcutaneous injection, unlike morphine which was antinociceptive in both tests at low doses. This led the authors to suggest that this was further evidence supporting the hypothesis that buprenorphine's antinociceptive activity is mediated via a non-μ-receptor. Cowan *et al.* (1977) demonstrated that buprenorphine displayed antinociceptive activity in the rat tail pressure test and that the dose-response relationship was linear. But buprenorphine did not antagonize the antinociceptive actions of morphine in the same test system, while blocking its activity in the rat tail flick test, this can be explained by the proposition that buprenorphine is a low efficacy agonist, acting as a full agonist in the rat tail pressure test, but as a partial agonist/antagonist in the rat tail flick test which has a smaller effective receptor reserve.

It is possible that there are species differences in the number and distribution of receptors which account for the different activity of buprenorphine in the rat, dog and sheep. Alternatively, opiate receptors may be involved in a varying degree in mediating suppression of thermal and mechanical responses. The activity of buprenorphine in the thermal test system in sheep and its complete reversal by naloxone suggest that this

effect may be mediated at the $\mu$-receptor, although the dose of naloxone used was high and may have blocked $\kappa$- and $\delta$-receptors as well. A dose-response study of the drug using both nociceptive testing regimes would probably elucidate whether there are relative differences between the species, and antagonist studies using low doses of naloxone would confer more receptor selectivity to the effect. It is possible that higher doses of buprenorphine could have increased the mechanically evoked threshold response in the sheep. This would be especially possible if this test system were inherently more painful, since it does not necessarily follow, that the dose-response curve for both nociceptive test systems will be identical. In 1982, Sadée *et al.* suggested that the rat tail pressure test was a less intense stimulus than electrical stimulation, thus explaining buprenorphine's considerable potency in this test. It is therefore, conceivable that the two tests used demonstrated the low efficacy agonist nature of buprenorphine.

The bell-shaped dose-response curve for buprenorphine in rodents (Cowan *et al.*, 1977; Dum & Herz, 1981) suggests that the drug has a range of concentrations at which it produces a maximum antinociceptive effect, and that above or below this range it is less effective and the time at which tests are made may well influence the magnitude of the response recorded. Cowan *et al.* (1977) and Tyers (1980) did not monitor the drug's activity over a time course, but carried out nociceptive testing at only 30 min after injection of the drug, but this study in sheep indicates that at 30 min after injection of the drug ($6\,\mu g\,kg^{-1}$), no significant increase in response threshold can be recorded. Dum & Herz (1981) measured antinociceptive effects in rats over a time course with three different dose rates given subcutaneously. The two lower doses (0.1 and $0.5\,mg\,kg^{-1}$) produced a maximum effect about 1 h after injection (which is in agreement with results in the sheep), while the higher dose ($5\,mg\,kg^{-1}$) produced two peak effects at 30 min and 4½ h. It is difficult to compare data obtained following subcutaneous injection to work carried out after i.v. administration, but the overall shape of the antinociceptive curve for buprenorphine in the sheep was similar to that at the lower dose rates given by the above authors to rats. The long latent period (45 min) probably reflects the slow receptor kinetics displayed by the drug (Hambrook & Rance, 1976), which could result in different concentrations occuring in the plasma and CNS.

The assay method for buprenorphine proved to be reliable and sensitive, and compared very favourably in sensitivity with that described by Bartlett *et al.* (1980).

It is possible that later time points may represent a combination of buprenorphine and its N-dealkylated metabolite, as the antiserum used cross-reacted equally well with both drugs. However, this possibility was considered unlikely, as this metabolite is not produced in detectable quantities in man (Moore, personal communication) or dogs (Bartlett *et al.*, 1980); although Blom & Bondesson (1985) detected norbuprenorphine in plasma a few hours after an i.v. injection of 0.6 mg in one volunteer, using a gas chromatographic-mass spectrometric method to separate and identify the parent drug and its metabolite.

The wide variation in elimination half-lives ($t_{1\beta}$) found in the 6 sheep suggested that more sampling points may be required for accurate determination of the parameter. However, the low plasma levels detected at the later time points are close to the sensitivity limit of the assay.

The decline of buprenorphine levels in plasma after i.v. injection followed a triexponential pattern for each individual animal, similar to that described in man (Bullingham *et al.*, 1980). Bullingham *et al.* (1980) determined the pharmacokinetics of buprenorphine following i.v. injection of a standard dose of 0.3 mg to patients undergoing surgery. These people had all received a variety of other drugs before buprenorphine, and the authors collected samples for only 3 h. The elimination half-life lay between 2 and 3 h, with initial half-times of 2.1 min and 11.2 min, respectively. After surgery, the procedure was repeated, this did little to alter $t_{1\pi}$, $t_{1\alpha}$ and $t_{1\beta}$, but increased clearance rates by 30%, from $901\,ml\,min^{-1}$ to $1275\,ml\,min^{-1}$. These authors suggested that there was no relationship between plasma level and pharmacological effect, because of the long duration of effect of the drug in man. However, they did not continue sampling over a period of time corresponding to the presumed duration of effect of the drug.

The mean onset of analgesia following administration of buprenorphine ($6\,\mu g\,kg^{-1}$ i.v.) in the sheep was 45 min. At this time, the mean plasma concentration was $697\,pg\,ml^{-1}$. Analgesia persisted for a further 165 min; response thresholds were back to normal 240 min after injection of the drug when mean plasma concentration of the drug was $189\,pg\,ml^{-1}$.

The responses of sheep to injections of buprenorphine thus show a most interesting spectrum of antinociceptive activity with apparently no analgesia to mechanical stimulation, despite the fact that $\alpha_2$-adrenoceptor agonists were approximately equipotent in the two tests (Livingston *et al.*, 1986) and the opioids pethidine and fentanyl were effective, albeit briefly, in the mechanical tests, as antinociceptives (Nolan *et al.*, 1987).

The pharmacokinetics of buprenorphine in the sheep indicate that the drug is rapidly distributed in the body and although the half-lives varied between animals they appear to be similar to those found for man. It is also most interesting to note that there was no correlation between plasma levels of the drug and

its analgesic activity during the initial period following administration.

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## References

BARLOW, R.B. (1983). *Biodata Handling With Microcomputers*. Amsterdam: Elsevier/North Holland Biomedical Press.

BARTLETT, A.J., LLOYD-JONES, J.G., RANCE, M.J., FLOCKHART, J.R., DOCKRAY, G.J., BENNETT, M.R.D. & MOORE, R.A. (1980). The radioimmunoassay of buprenorphine. *Eur. J. Clin. Pharmac.*, **18,** 339–345.

BLOM, Y. & BONDESSON, U. (1985). Analysis of buprenorphine and metabolites in plasma and urine by selected-ion monitoring. *J. Chromatog.*, **338,** 89–98.

BRYANT, R.M. & TYERS, M.B. (1979). Antinociceptive actions of morphine and buprenorphine given intrathecally in conscious rats. *Br. J. Pharmac.*, **66,** 472–473P.

BULLINGHAM, R.E.S., McQUAY, H.J., MOORE, A. & BENNETT, M.R.D. (1980). Buprenorphine kinetics. *Clin. Pharmac. Ther.*, **28,** 667–672.

CONE, E.J., GORODETZKY, C.H., YOUSEFNEJAD, D. & DARWIN, W.D. (1985). 63Ni electron-capture gas chromatographic assay for buprenorphine and metabolites in human urine and feces. *J. Chromatog.*, **337,** 291–300.

COWAN, A., LEWIS, J.W. & MacFARLANE, I.R. (1977). Agonist and antagonist properties of buprenorphine, a new antinociceptive agent. *Br. J. Pharmac.*, **60,** 537–545.

DUM, J.E. & HERZ, A. (1981). *In vivo* receptor binding of the opiate partial agonist, buprenorphine, correlated with its agonistic and antagonistic actions. *Br. J. Pharmac.*, **74,** 627–633.

HAMBROOK, J.M. & RANCE, M.J. (1976). The interaction of buprenorphine with the opiate receptor: lipophilicity as a determining factor in drug-receptor kinetics. In *Opiates and Endogenous Opioid Peptides*, ed Kosterlitz, H.W. pp. 295–301. Amsterdam: Elsevier/North Holland Biomedical Press.

KAMERLING, S.G., DEQUICK, D.J., WECKMAN, T.J. & TOBIN, T. (1986). Dose-related effects by ethylketazocine on nociception, behaviour and autonomic responses in the horse. *J. Pharm. Pharmac.*, **38,** 40–45.

KAMMERLING, S.G., WECKMAN, T.J., DEQUICK, D.J. & TOBIN, T. (1985). A method for studying cutaneous pain perception and analgesia in horses. *J. pharmac. Meth.*, **13,** 267–274.

LIVINGSTON, A., NOLAN A. & WATERMAN, A. (1986). A comparison of the thermal and mechanical antinociceptive activities of $\alpha_2$ adrenoceptor agonists in sheep. *Proc. I.U.P.S.*, **XVI,** 572.

MARTIN, W.R., EADES, C.G., THOMPSON, J.A., HUPPLER, L. & GILBERT, P. (1976). The effects of morphine- and nalorphine-like drugs in the non-dependent and morphine-dependent chronic spinal dog. *J. Pharmac. exp. Ther.*, **197,** 517–532.

NOLAN, A., LIVINGSTON, A., MORRIS, R. & WATERMAN, A. (1987). Techniques for comparison of thermal and mechanical nociceptive stimuli in the sheep. *J. Pharmac. Meth.*, **17,** 39–50.

PIPPI, N.L. & LUMB, W-V. (1979). Objective tests of analgesic drugs in ponies. *Am. J. Vet. Res.*, **40,** 1082–1086.

RICHARDS, M.L. & SADÉE, W. (1985). In vivo opiate receptor binding of oripavines to $\mu$, $\delta$ and $\kappa$ sites in rat brain as determined by an ex vivo labelling method. *Eur. J. Pharmac.*, **114,** 343–353.

SADÉE, W., ROSENBAUM, J.S. & HERZ, A. (1982). Buprenorphine: differential interaction with opiate receptor subtypes in vivo. *J. Pharmac. exp. Ther.*, **223,** 157–162.

TYERS, M.B. (1980). A classification of opiate receptors that mediate antinociception in animals. *Br. J. Pharmac.*, **69,** 503–512.

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