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The antinociceptive action of etorphine in the dorsal horn is due to a direct spinal action and not to activation of descending inhibition

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1 Etorphine, microinjected into the brainstem or administered intravenously, inhibited the firing of dorsal horn neurones to noxious heat in spinal or non-spinal anaesthetized cats and in decerebrate, non-anaesthetized cats with intact spinal cords.

2 Small doses of etorphine sometimes caused facilitation, especially when the cord was intact, but this was invariably followed by inhibition at higher doses.

3 The ED_{50} for inhibition (mean 3.9 μg/kg) after microinjection into nucleus raphe magnus, nucleus reticularis magnocellularis or the lateral tegmental field was similar at all sites in anaesthetized, non-spinal cats.

4 The ED_{50} for microinjection was not increased by spinal transection in anaesthetized cats (mean ED_{50}, 2.6 μg/kg) and was similar to the ED_{50} in decerebrate, non-anaesthetized cats.

5 Intravenous administration was 2 to 3 times more effective than microinjection and the time course of inhibition was faster after intravenous administration than after microinjection.

6 It is concluded that etorphine inhibits dorsal horn neurones after microinjection or intravenous administration by a direct action on the spinal cord and not by activating a descending inhibition. After microinjection it rapidly enters the general circulation and subsequently distributes into the spinal cord.

7 It is also concluded that naloxone readily gains entry to the circulation from the brain because microinjection antagonized the effects of systemic etorphine on dorsal horn neurones in spinal cats.

Introduction

There is evidence that morphine acts directly on the spinal cord. The ionophoresis of morphine in the dorsal horn of the spinal cord (Calvillo, Henry & Neuman, 1974; Duggan, Hall & Headley, 1977; Belcher & Ryall, 1978; Davies & Dray, 1978) or the systemic administration of morphine or etorphine in spinal animals (Kitahata, Kosaka, Taub, Bonikos & Hoffert, 1974; LeBars, Menetrey, Conseiller & Besson, 1975; Yaksh, 1978) selectively attenuates the excitation of dorsal horn neurones by noxious stimulation. Intrathecal administration of morphine causes behavioural analgesia in experimental animals and man (Yaksh & Rudy, 1977; Wang, Nauss & Thomas, 1979). The effectiveness of morphine in reducing spinal polysynaptic reflexes is similar in both quadriplegic man and in normal volunteers (Willer & Bussel, 1980) and its effectiveness in reducing the excitation of dorsal horn neurones by noxious stimulation is unchanged (LeBars, Guilbaud, Chitour & Besson, 1980b) or only minimally decreased (Hanaoka, Ohtani, Toyooka, Dohi, Ghazi-Saidi, Taub & Kitahata, 1978) in spinal animals compared with animals with an intact spinal cord. All of these observations are consistent with the hypothesis that morphine acts predominantly at sites in the spinal cord to cause analgesia.

In contrast with the direct evidence for a spinal site of action, the evidence for the hypothesis that morphine causes analgesia by an activation of a descending system, originating in the brain stem and which is inhibitory to spinal nociceptive neurones, is based largely upon indirect evidence and is controversial. It is undeniable that the ionophoresis of opiates to brainstem neurones modifies their excitability (Bradley & Dray, 1974; Bramwell & Bradley, 1974; Davies & Dray, 1978), that the microinjection of opiates into various sites in the brainstem causes behavioural analgesia (Pert & Yaksh, 1974; Jacquet & Lajtha, 1976; Lewis & Gebhart, 1977; Dickenson, Oliveras & Besson, 1979; Levy & Proudfit, 1979;

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LeBars, Dickenson & Besson, 1980a; Rosenfeld & Stocco, 1980; Takagi, 1980; Azami, Llewelyn & Roberts, 1982) and that electrical stimulation of these regions inhibits the activity of dorsal horn neurones when they are excited by noxious stimulation (Engberg, Lundberg & Ryall, 1968; Oliveras, Besson, Guilbaud & Liebeskind, 1974; Fields, Basbaum, Clanton & Anderson, 1977; Guilbaud, Oliveras, Giésler & Besson, 1977; Willis, Haber & Martin, 1977; Belcher, Ryall & Schaffner, 1978; Duggan & Griersmith, 1979; Carstens, Klumpp & Zimmermann, 1980; Johnston & Davies, 1981). However, attempts to show directly that opiates increase descending inhibitory control of spinal neurones have not always been successful. Thus Duggan, Griersmith & North (1980) have shown that systemically administered morphine attenuates, rather than increases, tonic descending inhibition in cats and LeBars *et al.* (1980a) failed to demonstrate inhibition of dorsal horn neurones by microinjection to nucleus raphe magnus (NRM) of morphine in rats, employing doses which cause analgesia. In contrast, Bennett & Mayer (1979) found that microinjection of etorphine or morphine in the periaqueductal grey (PAG) in rats does inhibit the excitation of dorsal horn neurones in anaesthetized rats and Gebhart, Sandkühler, Thalhammer & Zimmermann (1982) have recently observed similar effects with morphine in cats. These actions of opiates have been attributed to an activation of a descending inhibitory control system in the spinal cord.

In this paper we present evidence to show that, at least for etorphine, this hypothesis is untenable. A preliminary account of this work has been communicated to the Physiological Society (Clark & Ryall, 1982).

Methods

Preparation

Experiments were carried out in cats of either sex, weighing 2.3 to 3.3 kg. Anaesthesia was maintained by intravenously administered pentobarbitone after induction either with halothane or with pentobarbitone administered in a dose of 35 mg/kg intraperitoneally. In most of the experiments a stable level of anaesthesia was maintained by an intravenous infusion of pentobarbitone at a rate determined in each experiment by monitoring the amount required over several hours before the start of record-

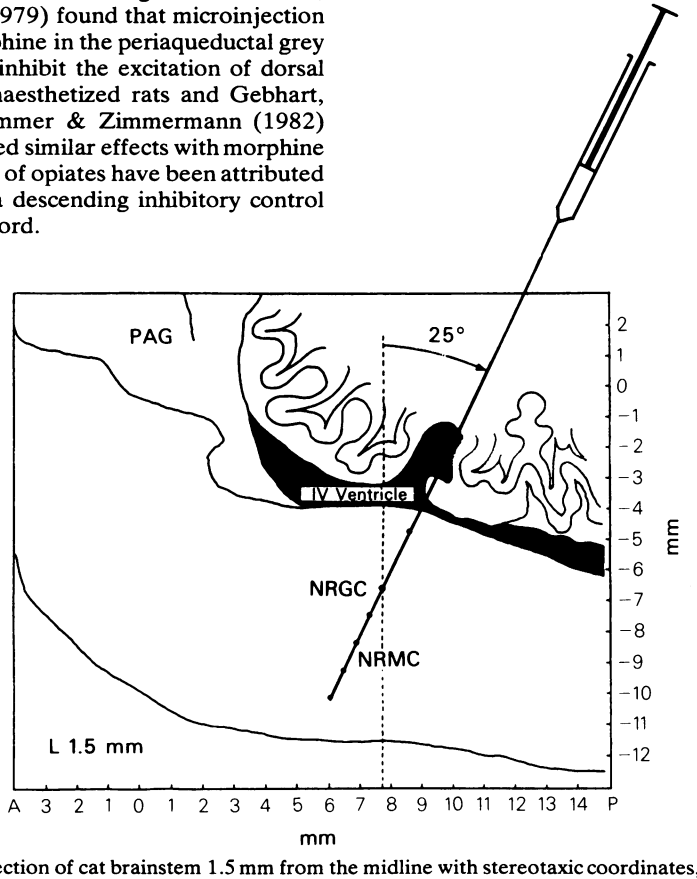

Figure 1 Sagittal section of cat brainstem 1.5 mm from the midline with stereotaxic coordinates, showing insertion of microinjection cannula at an angle of 25°. Nucleus reticularis magnocellularis (NRMC); nucleus reticularis gigantocellularis (NRGC); periaqueductal grey (PAG).

ing. The mean rate of infusion was 6.6 mg/h and varied from 4 to 15 mg/h in different cats. Thereafter, most cats were paralysed by the intravenous administration of gallamine triethiodide, repeated every 20 min, end-tidal CO_2 being maintained between 3.5–4.5%. Intra-rectal temperature was monitored by means of a thermistor and maintained at 38.5°C under feed-back control by heating blankets. Blood pressure was monitored from the right carotid artery.

The spinal cord was exposed by a laminectomy extending from the second to the seventh lumbar vertebrae and in some animals it was transected at the level of the second lumbar segment. After severing L6 to S1 ventral roots and opening the dura mater the cord was covered by a pool of warmed paraffin oil. Some cats were decerebrated by intercollicular transection of the brainstem and removal of the fore-brain under pentobarbitone anaesthesia. No further anaesthetic was administered and recording started 21 to 34 h later.

Recording

Extracellular recordings from single dorsal horn neurones responding to noxious heat stimulation of the ipsilateral (left) hind foot were obtained by means of tungsten microelectrodes. Such neurones also responded to light mechanical stimuli. Action potentials were discriminated from the background activity and counted by a rate-meter. The firing frequency was plotted on a chart recorder.

Stimulation

Noxious stimuli consisted of 30–45 s applications of radiant heat from a 100 W projector lamp with a parabolic reflector. The stimulus was applied to either the foot pad or, more usually, to a toe pad. The skin temperature was monitored by means of a thermistor placed in firm contact with the skin. An electronic feed-back circuit controlled the heat source to give a variable set maximum temperature at the thermistor. The skin temperature was plotted on the chart recorder. Most neurones responded to increases in skin temperature above 43°C with an increase in firing frequency which was greater as the temperature was increased to a maximum of 56°C. The temperature employed usually fell within the range 47 to 53°C (mean 51.2°C) and the stimulus was repeated automatically every 3 to 4 min. Stimuli within this range were painful to the experimenter's hand.

Electrical stimulation of the brainstem was employed in some decerebrate cats. The constant current stimuli were delivered through stainless steel monopolar microelectrodes and comprised trains of 12 stimuli at 300 Hz repeated twice per s. The pulse duration was 1 ms.

Stereotaxic coordinates

The sites chosen for microinjection or microstimulation were those at which electrical stimulation was the most effective in inhibiting the responses of dorsal horn neurones to noxious heat (R.O. Edeson & R.W. Ryall, unpublished data). These sites included nucleus raphe magnus (NRM: coordinates, P, 7.1; L, O: D, −7.9 mm), a site in the lateral tegmental field (LTF: coordinates, P, 7.7; L, 3; D, −6.5 mm) and the nucleus reticularis magnocellularis (NRMC: coordinates, P, 6.9; L, 2; D, −8.3 mm). Microinjections were also made into one additional region of NRMC (coordinates, P, 8.5; L, 1.5; D, −8.0 mm). The coordinates were determined from the atlas of Berman (1968). NRMC in the cat is equivalent to a site in the rat generally referred to as nucleus reticularis paragigantocellularis (Zorman, Hentall, Adams & Fields, 1981). Microinjection cannulae and stimulating electrodes were introduced at an angle of 25° towards the anterior plane (Figure 1). Microinjection sites were visualized by injecting a small (approximately 0.1 μl) quantity of pontamine sky blue (2% in 0.5 M sodium acetate) at the end of the experiment.

Drug administration

Substances were administered intravenously through a cannula in the cephalic vein or were microinjected. Etorphine hydrochloride (Reckitt & Colman Ltd.) was dissolved in 165 mM sodium chloride and microinjected from 3.12, 6.25 or, occasionally, 12.5 mM solutions in volumes usually within the range of 0.5 to 4 μl. Naloxone hydrochloride (Endo Laboratories) was microinjected from 183 mM solutions in 165 mM sodium chloride. Doses are expressed as μg/kg of the salts for ease of comparison with most of the data in the literature. Microinjections were made through a cannula of 0.48 mm o.d.

Experimental protocol

A dorsal horn neurone was located from which stable responses to noxious heat could be obtained for periods of more than 30 min. The microinjection cannula was then lowered to the selected coordinates and any change in response was noted. In animals with an intact spinal cord this procedure sometimes caused a brief excitation or inhibition of the dorsal horn cell. After a further interval to ensure that responses were stable, control microinjections of 165 mM saline were generally administered and rarely caused any effect. The procedure was then repeated with a cannula loaded with either etorphine or

naloxone. If an injection caused no effect within, usually, 15 to 20 min a subsequent injection of twice the previous amount was given. Attempts were made in every experiment to find two doses of etorphine, one of which produced less than and the other more than 50% inhibition of evoked activity. The dose required to produce 50% inhibition (ED_{50}) was determined by interpolation. At the end of most experiments the ED_{50} for intravenous administration was determined but in a few experiments the intravenous administration preceded microinjection with no difference in the results.

Results

Responses to noxious heat

Of those dorsal horn neurones which responded to the noxious heat stimulus most were excited but a few were inhibited. Only excitatory responses were studied in this investigation. In some neurones the excitatory response increased to a plateau which was maintained during the application of the stimulus but in others it declined to a variable degree and in some it temporarily increased immediately after the cessation of the stimulus. Many neurones displayed a decrease in successive responses to the stimulus before responses became stable although a few increased their firing rates with successive stimuli. On rare occasions the responses were stable over prolonged periods but then unaccountably declined: a return to stable firing rates could then sometimes be achieved either by transferring the stimulus to another location on the foot, which had previously been less effective, or by slightly raising the temperature at the same location.

There were no significant differences between background or evoked firing frequencies in anaesthetized spinal, anaesthetized non-spinal or in non-anaesthetized decerebrate cats: the mean background frequencies in these preparations were 9, 7 and 8 Hz respectively and the evoked firing frequencies were31, 37 and 43 Hz respectively.

Circulatory changes produced by the intravenous injection of either hexamethonium (Figure 2) or adrenaline, in doses sufficient to cause marked falls or increases in the arterial blood pressure did not

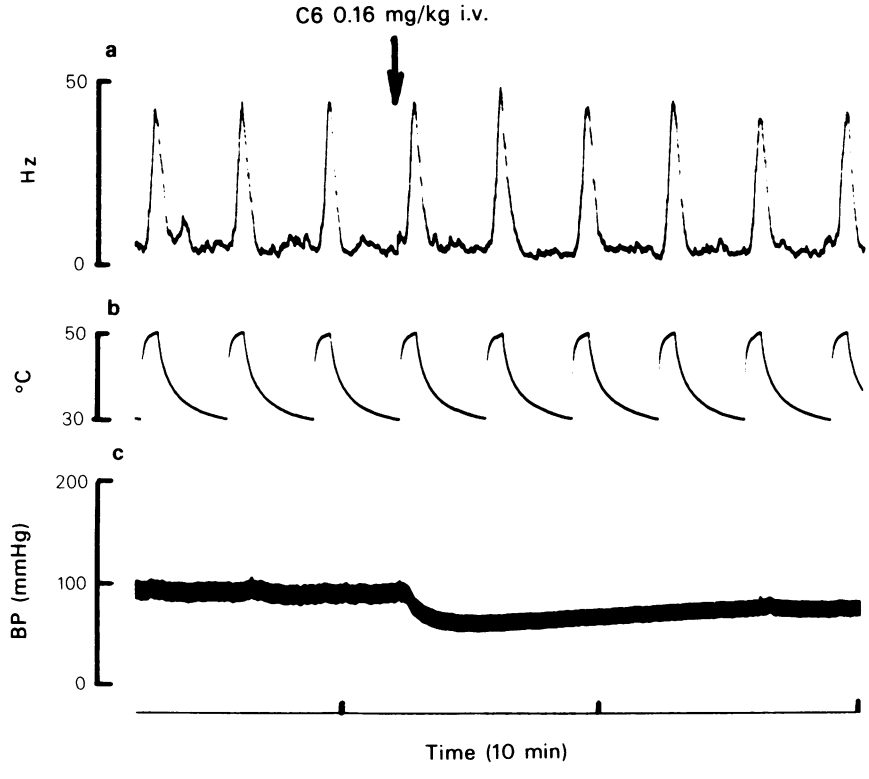

Figure 2 Spinal, anaesthetized cat. Hexamethonium (C6), 0.16 mg/kg intravenously at the arrow. (a) Effect of noxious heat on firing frequency in Hz of a dorsal horn neurone; (b) skin temperature (°C) of the toe pad at the heated site; (c) carotid artery blood pressure (mmHg).

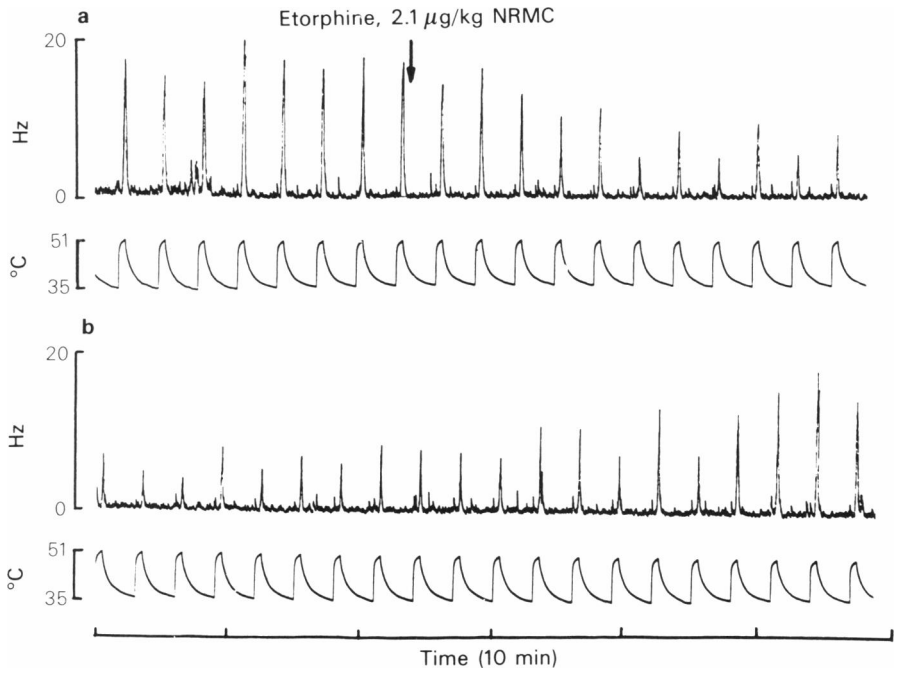

Figure 3 Spinal anaesthetized cat. (a) Inhibitory effect of etorphine (2.1 μg/kg) microinjected in a volume of 2 μl into NRMC at the arrow. (b) Continuation of records in (a) showing partial recovery. Upper records in (a) and (b): effect of noxious heat on the firing frequency in Hz of a dorsal horn neurone. Lower records in (a) and (b): skin temperature (°C) of the toe pad at the heated site.

affect the evoked responses to noxious heat. It is therefore unlikely that the hypotensive action of etorphine, frequently observed in these experiments, was responsible for the effects on dorsal horn neurones, a conclusion supported by the fact that the antinociceptive action was not invariably associated with hypotension. Lynn (1979, 1980) has previously concluded that the activation of peripheral nocicep-

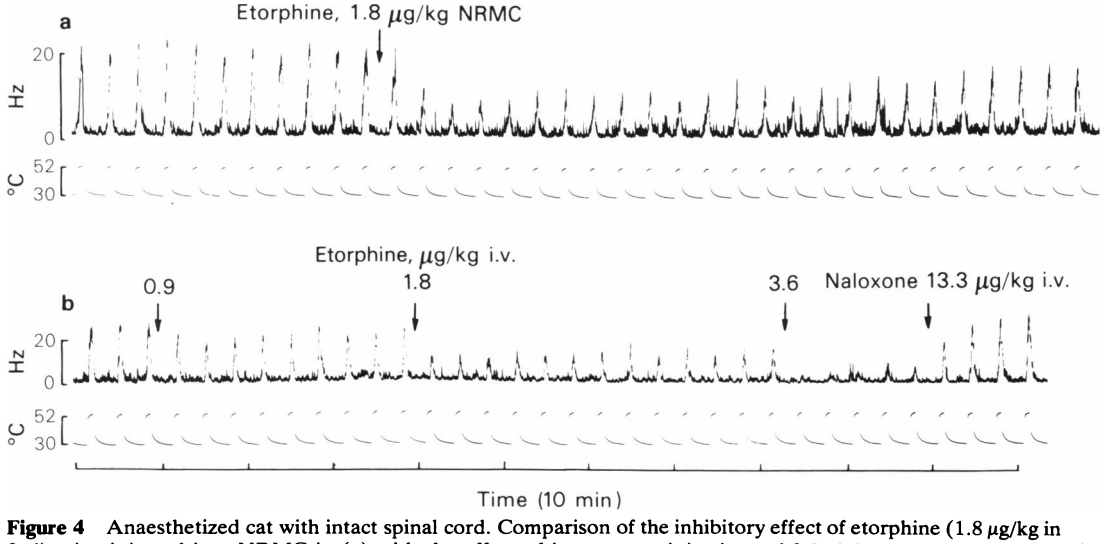

Figure 4 Anaesthetized cat with intact spinal cord. Comparison of the inhibitory effect of etorphine (1.8 μg/kg in 2 μl) microinjected into NRMC in (a) with the effect of intravenous injections of 0.9, 1.8 and 3.6 μg/kg in (b). Naloxone (13.3 μg/kg) injected intravenously at the end of the experiment. Coordinates as in Figure 3.

tors by heat stimuli is independent of local blood flow.

Microinjection of etorphine in anaesthetized cats

Etorphine was microinjected into the brainstem of 19 anaesthetized cats, 7 of which had been spinalized. Some examples of the effects obtained are illustrated in Figures 3–6. Inhibition of dorsal horn neurones was observed on all neurones (Figures 3, 4, 6). In all but one cat an estimate of the dose causing 50% inhibition (ED_{50}) of the nociceptive response was obtained. The individual values of the ED_{50} are shown in Table 1 and varied from 0.5 to 8.1 μg/kg. There were no obvious differences between the effects obtained by microinjection at different sites in NRM, LTF or NRMC. Therefore, no further distinction is made between these sites and the data obtained at different sites have been pooled for subsequent analysis.

There were no significant differences ($P > 0.05$) between the ED_{50}s obtained in 12 non-spinal cats compared with those in 7 spinal animals. In the former group the mean ED_{50} was 3.9 ± s.e.0.73 μg/kg compared with 2.6 ± s.e.0.84 μg/kg in the latter. The similarity of these ED_{50}s is consistent with the hypothesis that etorphine is absorbed into the circulation from its site of administration in the brainstem and subsequently enters the spinal cord to produce an antinociceptive effect. Qualitatively similar results were obtained in spinal and non-spinal cats, except that the microinjection of small doses of etorphine increased the responses to noxious heat in 4 of 12 cats with an intact spinal cord (Figure 5) but had a similar effect in only 1 of 7 spinal animals. A facilitation was the only effect observed by LeBars *et al.* (1980a) when morphine was microinjected in NRM in anaesthetized rats. In the present experiments, etorphine usually had no effect in small doses and caused a progressive increase in the degree of inhibition of evoked and background activity as the amount injected was increased.

The time of onset of inhibition, for inhibitions of 30–100% (mean 61%) of the control responses ranged from 5–15 min (mean 7.5 min) in different experiments. The maximum degree of inhibition was usually achieved within 10–40 min (mean 33 min). The duration of inhibition varied from about 40 min to more than 3 h in different cats and in 12 of 20 animals it exceeded 85 min.

Intravenous injection of etorphine in anaesthetized cats

The effect following an intravenous injection of etor-

Table 1 Inhibitory effects of etorphine microinjections on the activity of dorsal horn neurones in anaesthetized cats

		ED_{50} (μg/kg)				
		Microinjection (M)		*Intravenous* (I)		*Ratio* M/I
Experiment	Site	Intact	Spinal	Intact	Spinal	
1	LTF	7.7				
2	LTF	3.5				
3	NRM	<7.3*		<7.3*		1*
4	NRM	2.9		2.6		1.1
5	NRM	3.7		0.5		7.5
6	NRM	2.1		6.2		0.3
7	NRM	2.5		2.3		1.1
8	NRM		1.1		2.0	0.6
9	NRM		0.5			
10	NRM		3.4			
11	NRM		7.2		4.6	1.6
12	NRMC[1]	8.1		0.9		9.1
13	NRMC[1]	3.9		0.8		4.9
14	NRMC[1]	1.1		1.3		0.8
15	NRMC[1]	3.7		1.3		0.8
16	NRMC[2]	0.6		0.18		3.2
17	NRMC[2]	7.5		2.3*		3.2
18	NRMC[1]		3.3		1.9	1.8
19	NRMC[1]		1.7		1.2	1.4
20	NRMC[1]		1.6		0.4	3.9
Mean ± s.e.		3.9 ± 0.7	2.6 ± 0.8	1.8 ± 0.6	2.0 ± 0.7	2.7 ± 0.7

Experiments 7 and 17 were in the same cat. Stereotaxic coordinates as in Methods.
* These figures are omitted from the calculation of the means.

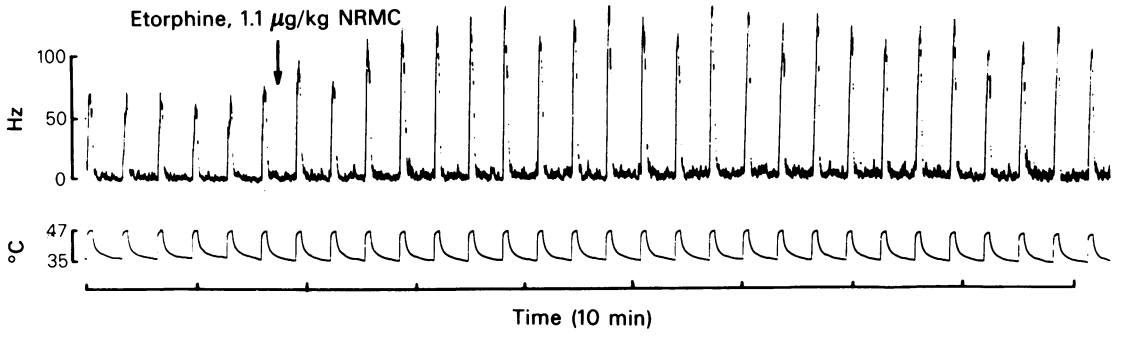

Figure 5 Anaesthetized cat with intact spinal cord. Facilitatory effect of etorphine (1.1 μg/kg in 1 μl) microinjected into NRMC. Larger doses caused inhibition. Coordinates as in Figure 3.

phine was observed in 10 cats with an intact spinal cord (Figure 4) and the ED_{50} was determined in 9 of them (Table 1). The ED_{50} was also determined in 5 spinal animals (Figures 6, 7) and it was similar to the ED_{50} obtained when the cord was intact ($1.8 \pm$ s.e.0.60 μg/kg and $2.0 \pm$ s.e.0.69 μg/kg respectively). The ratio of microinjected to intravenous ED_{50} in the same animal was estimated in 14 cats, 9 of which had intact spinal cords and 5 of which were spinalized. The ratio was usually greater than 1 and the mean was $2.7 \pm$ s.e.0.7. Thus microinjecion was less effective ($P < 0.02$) than intravenous administration on a μg/kg basis (Figure 6).

The time course of inhibition by etorphine was also 2 to 3 times more prolonged, for a similar degree of inhibition, for microinjection than it was for intravenous administration (Figure 6). The time of onset of inhibition for inhibitions of 35–100% (mean 53%) of the control firing frequency varied from 1 to 11 min (mean 2.8 min). The maximum degree of inhibition was achieved within 2–40 min (mean 12 min) and the duration of inhibition, measured to

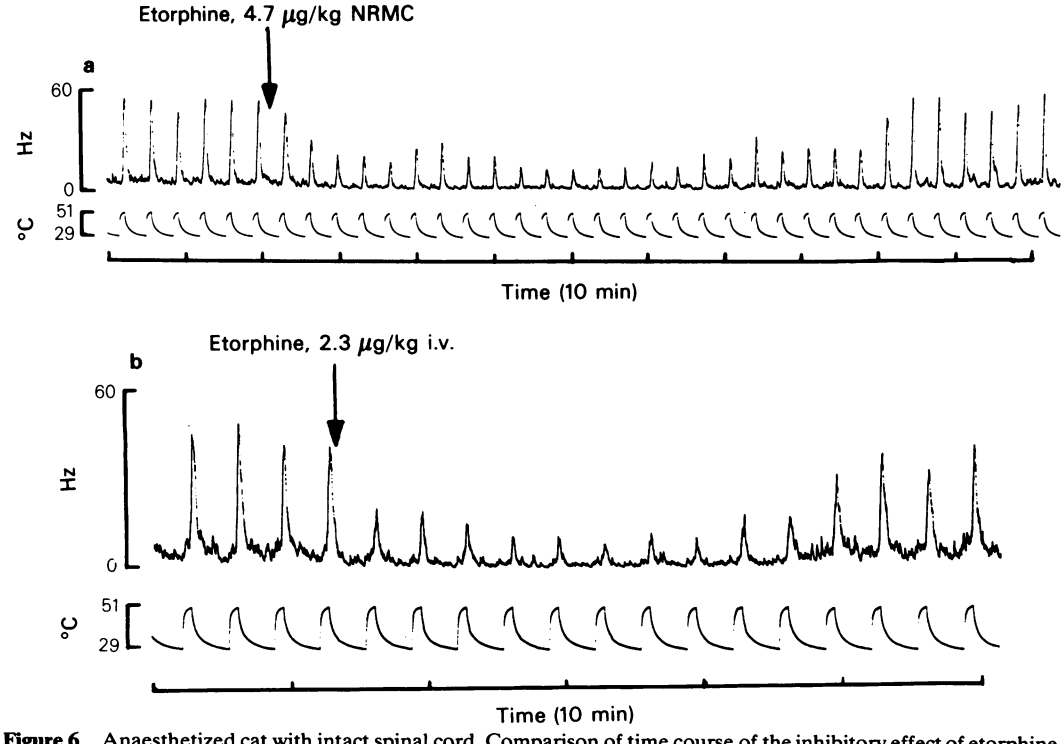

Figure 6 Anaesthetized cat with intact spinal cord. Comparison of time course of the inhibitory effect of etorphine (4.7 μg/kg in 4 μl) microinjected into NRMC in (a) with the effect of intravenous injection of 2.3 μg/kg in (b). Note the changes in scales in (a) and (b). Coordinates as in Figure 3.

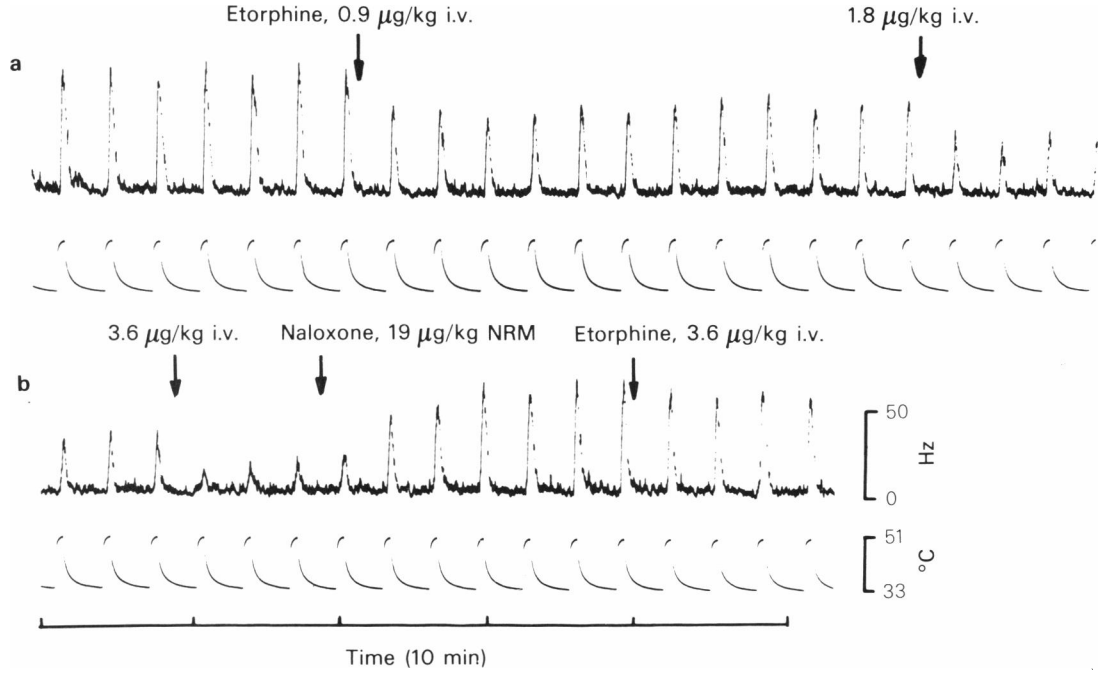

Figure 7 Spinal anaesthetized cat. The reversal by naloxone (19 μg/kg in 1 μl) microinjected into NRM of the inhibitory effects of etorphine injected intravenously in three successive doses of 0.9, 1.8 and 3.6 μg/kg. A subsequent administration of 3.6 μg/kg of etorphine had little effect. Records (a) and (b) are continuous. Coordinates as in Figure 3.

80 to 100% recovery, varied from 20 to 120 min (mean 48 min) and it exceeded 85 min in duration in only 2 of 14 cats.

The higher potency, faster onset and shorter duration after intravenous administration compared with microinjection (Figures 4, 6) is consistent with the conclusion that microinjected etorphine exerts its effect on the spinal cord only after it has first been absorbed into the blood.

The effect of naloxone in anaesthetized cats

The intravenous injection of 30–100 μg/kg of naloxone partially or fully counteracted the depressant effect of microinjected etorphine in all 5 experiments in which it was tested and 13–100 μg/kg counteracted the effect of intravenously administered etorphine in 7 of 8 cats (Figure 4).

Of more interest is the observation that the mic-

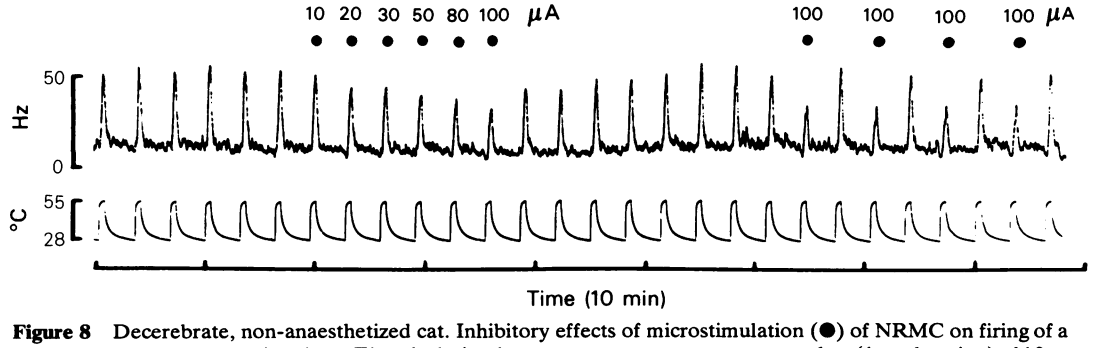

Figure 8 Decerebrate, non-anaesthetized cat. Inhibitory effects of microstimulation (●) of NRMC on firing of a dorsal horn neurone to noxious heat. Electrical stimulus parameters; constant current pulses (1 ms duration) of 10 to 100 μA in trains of 12 pulses at 300 Hz delivered every 0.5 s, starting 10 s before heat application and continuing throughout the period of heating. Coordinates as in Figure 3.

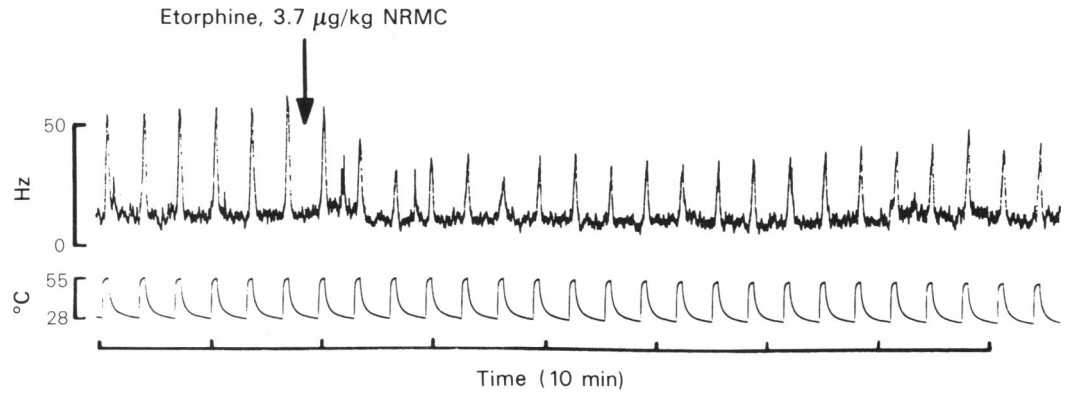

Figure 9 Decerebrate, non-anaesthetized cat. Inhibitory effect of etorphine (3.7 μg/kg in 4 μl) microinjected into NRMC at the arrow. Coordinates as in Figure 3.

roinjection of similar quantities of naloxone (19–50 μg/kg) counteracted the depressant effects of systemically administered etorphine in five cats with transected spinal cords. The effect of naloxone was maximal within a few minutes. Subsequent injections of etorphine in amounts up to four times those used before the administration of naloxone were ineffective or much less effective than before (Figure 7).

The antagonism of the depressant effect of etorphine by microinjected naloxone was therefore also due to an action on the spinal cord after the antagonist had first been absorbed into the circulation.

The effect of etorphine in decerebrate animals.

To investigate the possibility that pentobarbitone anaesthesia in the preceding experiments may have masked or prevented an activation by etorphine of descending pathways inhibitory to spinal nociceptive neurones, experiments were carried out in decerebrate cats in which no anaesthetic had been administered for 21 to 34 h. At this time in 4 of 5 cats there was little remaining decerebrate rigidity, withdrawal reflexes were present and the animals were breathing spontaneously with an end-tidal CO_2 of 3.5–5.5%. West & Wolstencroft (1982) have shown that decerebrate rigidity decreases and withdrawal reflexes increase over a similar time course in such animals.

Three normal animals were anaesthetized with pentobarbitone (35 mg/kg intraperitoneally) and the time course of recovery was noted. In confirmation of previous observations (R.W. Ryall, unpublished data) recovery of consciousness occurred within 3–6 h and 22 h after administration the animals appeared quite normal and showed no residual effects from the anaesthesia.

In one cat, radiolabelled pentobarbitone was administered slowly over a period of 14 min and the concentration of anaesthetic was determined at intervals over a 7.6 h period. At 7.6 h after administration the blood level had declined to 32% of the concentration present 15 s after the termination of administration. After an initial rapid decline to 48% over 2 h there was a slower linear phase with a half time of 9.3 h. Thus at 30 h the estimated residual concentration of pentobarbitone in the blood was 6% of the concentration at 15 s. The two phases of removal from the blood are attributed to redistribution followed by elimination and metabolism and are similar to, but more prolonged than, those observed in rats (Ossenberg, Peignoux, Bourdiau & Benhamou, 1975). These data indicate that the residual concentration of anaesthetic in the decerebrate cats was low relative to the concentrations required to cause anaesthesia and the behavioural experiments indicate that these low concentrations were unlikely to have caused any serious interference with control mechanisms originating from the brainstem.

Direct evidence that a descending inhibition of lumbar nociceptive neurones could be activated in decerebrate animals was also obtained. Electrical stimulation of NRMC at the end of three experiments, in which etorphine had first been administered, inhibited the nociceptive heat-evoked firing of dorsal horn neurones (Figure 8). With monopolar stimulation (300 Hz trains of pulses of 1 ms duration, 12 pulses per train, repeated at 0.5 s intervals) the threshold currents required to cause inhibition were 20 μA in each of two experiments and 80–150 μA in the third. In similar experiments in intact, anaesthetized cats (R.O. Edeson & R.W. Ryall, unpublished data), the mean threshold for inhibition elicited from NRMC was 34 μA.

Etorphine was microinjected into NRMC (Figure 9) in the five decerebrate animals. In two cats the ED_{50} values for inhibition of the excitation of dorsal horn neurones by noxious heat were 3.7 and 1.1 μg/kg. In the remaining three animals the ED_{50}

values were greater than 0.4, 0.9 and 4.4 μg/kg. Decerebrate non-anaesthetized animals were therefore no more sensitive to microinjected etorphine than were intact animals anaesthetized with pentobarbitone.

In 2 of 5 cats, low doses of etorphine caused a facilitation which was less apparent or absent at higher doses which caused inhibition.

Discussion

This study has clearly demonstrated that the microinjection of etorphine in microgram quantities into several sites in the brainstem of anaesthetized or decerebrate, non-anaesthetized cats inhibits the noxious heat-evoked excitation of dorsal horn neurones but that the mechanism does not involve an activation of descending inhibitory systems. The effect after microinjection is due to a direct depressant action of etorphine on the spinal cord after first entering the systemic circulation. There are three observations which support this hypothesis. Firstly, it is to be expected that if etorphine acted locally at the site of microinjection, then the amount administered should be far less than the amount required intravenously to produce the same effect. In fact, quantitative studies, based on ED_{50} determinations, revealed that the amount required to cause inhibition after microinjection was greater than the amount required after intravenous administration. Secondly, the time course of inhibition was faster after intravenous than it was after microinjection, indicating that there was an additional time-limiting factor after microinjection. Thirdly, and most importantly, the ED_{50} after microinjection was unchanged by complete transection of the spinal cord which ensured that there was no possibility of a change in descending control.

Herz & Teschemacher (1971) have shown that 20% of a dose injected intracerebrally passes into the blood within 5 min. Such a rate of entry into the circulation, coupled with some elimination, explains the differences in potencies and time courses for microinjection and intravenous administration.

Microinjected naloxone attenuated the action of systemically administered etorphine in spinal cats. Thus, naloxone also acted on the spinal cord after first entering the circulation. Etorphine is extremely lipid soluble, with an octanol/water partition coefficient of approximately 70 at pH 7.4 (Herz & Teschemacher, 1971). Naloxone is about thirteen times less lipid soluble than etorphine with a partition coefficient of 5.4 (Misra, Pontani, Vadlamani & Mule, 1976). Thus lipid solubility within this range is not a limiting factor for entry to the circulation.

Regardless of the route or site of administration or whether the cord was intact or transected or whether pentobarbitone was present or absent, as in decerebrate animals, the mean ED_{50} for etorphine lay between 1.8 and 3.9 μg/kg, with values in individual cats varying from 0.5 to 8 μg/kg. Usually, small inhibitory effects were evident at one half but not at one quarter of the ED_{50}. The amounts are similar, on a per kilogram basis, to those used by Bennett & Mayer (1979), who microinjected 0.2 to 0.5 μg of etorphine into the periaqueductal grey (PAG) in anaesthetized rats weighing 350–700 g. In their experiments more than 25% depression was observed on most, but not all dorsal horn cells. They attributed these effects to a direct activation of descending inhibitory pathways originating from PAG, but did not consider the possibility that etorphine acted on the spinal cord via the circulation.

It could be argued that our inability to observe an activation of descending inhibition by small amounts of microinjected etorphine was due to an inappropriate choice of sites for injection. However, the sites chosen were those at which focal electrical stimulation was particularly effective in inhibiting dorsal horn neurones (R.O. Edeson & R.W. Ryall, unpublished observations) and are similar to those where others have shown that the microinjection of morphine causes behavioural analgesia (see Introduction). Furthermore, direct projections from some of these sites to the spinal cord have been reported (Basbaum, Clanton & Fields, 1978). Even if there were sites in the brainstem more sensitive to etorphine than those which we have studied the similarity of the ED_{50}s in spinal and intact animals would suggest that such sites are unimportant for depression of dorsal horn responses when the opiate is given systemically.

There are a number of observations which suggest that the failure to activate descending inhibitory systems was not due to the presence of anaesthetic. The most convincing is the demonstration that microinjection of etorphine was no more effective in decerebrate, non-anaesthetized cats than it was in intact, anaesthetized preparations. This is supprted by the observation that electrical stimulation of the brainstem as readily inhibits noxious heat-evoked responses in anaesthetized cats (Belcher *et al.*, 1978; R.O. Edeson & R.W. Ryall, unpublished data) as it does in decerebrate cats. Finally, cold-block experiments (Duggan *et al.*, 1980) have shown that tonic descending inhibition is present in pentobarbitone-anaesthetized cats.

The amount of etorphine required to inhibit the noxious heat-evoked firing of dorsal horn neurones in anaesthetized cats was smaller than the amount required to cause behavioural analgesia in this species and is similar to the amounts required in a number of species, which vary slightly according to the species, route of administration and tests emp-

loyed (Blane, Bourra, Fitzgerald & Lister, 1967). It may therefore be concluded that the powerful spinal depressant action which we have observed both after systemic administration and after microinjection into the brain is a sufficient explanation of the analgesic action of this substance in conscious animals. This interpretation in no way excludes the possibility that etorphine may have additional supraspinal actions which may influence reflexes involving supraspinal structures or which may contribute to other aspects of the perceived quality of the pain experience. Indeed the ready penetration of the blood-brain barrier and the presence of opiate receptors in the brain render this highly probable.

The interpretations of microinjection studies with naloxone have relied upon the assumption that the action of the antagonist was restricted to sites at or near the site of injection. Our demonstration that small quantities of naloxone microinjected into the brain of spinal cats rapidly attenuated the depressant effect of etorphine on the spinal cord shows that, despite its lower lipid solubility, it too can exert its effects on the spinal cord after first entering the systemic circulation: the amounts of naloxone microinjected in this study are similar to the quantities reported to be effective in non-anaesthetized rats (about 20 μg/kg; Yaksh & Rudy, 1977; Dickenson *et al.*, 1979; Azami *et al.*, 1982).

The relevance of these conclusions regarding the site of action of etorphine to the site of action of the less lipid-soluble opiate, morphine, will be considered in detail in a subsequent publication. However, our preliminary data indicate that the antinociceptive effect of microinjected morphine on dorsal horn neurones may, in part, be attributed to a direct supraspinal activation of descending inhibition but that the amounts required are such that it is improbable that they are of consequence for analgesia produced by systemic administration. Such a conclusion supports those drawn by LeBars *et al.* (1980a, b) from experiments in rats and is compatible with observations that the effectiveness of morphine on spinal nociceptive transmission is only minimally changed by spinal transection in cats (Hanaoka *et al.*, 1978) and in quadriplegic man (Willer & Bussel, 1980).

The fact that opiates and antagonists can produce their effects after microinjection only after first entering the circulation introduces a note of caution in the interpretation of microinjection studies, particularly when relatively large quantities are administered, similar to those which are required intravenously.

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