

The relative significance of spinal and supraspinal actions in the antinociceptive effect of morphine in the dorsal horn: an evaluation of the microinjection technique

S.L. Clark, R.O. Edeson¹ & R.W. Ryall

Department of Pharmacology, University of Cambridge, Hills Road, Cambridge, CB2 2QD

1 Large quantities of morphine injected directly into the brainstem of spinal anaesthetized cats inhibited the noxious heat-evoked excitation of dorsal horn neurones. The amounts required were similar to those that were required intravenously in cats with the spinal cord intact or transected.

2 When the spinal cord was intact the amount of morphine microinjected into the brainstem required to inhibit the excitation of dorsal horn neurones was about ten fold less than it was in spinal animals.

3 It is concluded that large, but not small doses of morphine microinjected into the brainstem can exert effects on the spinal cord after first entering the circulation. The effects of small doses are attributed to a local action in the brainstem which causes inhibition of spinal neurones either by activating descending inhibitory neuronal systems or by liberating endogenous substances which reach the spinal cord via the cerebro-spinal fluid.

4 The concentrations of morphine achieved at various distances from the site of injection by the microinjection of μg quantities and the time courses of the concentration changes were calculated from diffusion equations, assuming diffusion coefficients of 3 or $5 \times 10^6 \text{ cm}^2 \text{ s}^{-1}$. The curves obtained closely approximated those obtained experimentally.

5 The concentrations achieved at distances up to 2 mm from the site of injection of $10 \mu\text{g}$ of morphine were calculated to exceed 10^{-4} M and the time-courses of these concentration changes were compatible with the time course of inhibition of spinal neurones, or the production of analgesia after microinjection. Such concentrations are vastly in excess of those achieved in the brain after the systemic administration of morphine in analgesic doses.

6 It is concluded that the local effects in the brainstem produced by the microinjection of μg quantities of morphine have no relevance to the mechanism of analgesia produced by systemic administration.

Introduction

Opiates attenuate nociception in part by a direct action in the dorsal horn of the spinal cord. This has been demonstrated by systemic administration in spinal animals (Kitahata, Kosaka, Taub, Bonikos & Hoffert, 1974; Le Bars, Menetrey, Conseiller & Besson, 1975; Hanaoka, Ohtani, Toyooka, Dohi, Ghazi-Saidi, Taub & Kitahata, 1978; Le Bars, Guilbaud,

Chitour & Besson, 1980; Clark & Ryall, 1983) and in quadriplegic man (Willer & Bussel, 1980), by iontophoresis (Calvillo, Henry & Neuman, 1974; Duggan, Hall & Headley, 1977; Belcher & Ryall, 1978; Davies & Dray, 1978) and by intrathecal administration (Yaksh & Rudy, 1977; Wang, Nauss & Thomas, 1979). There is an additional hypothesis that systemically administered opiates activate descending systems originating in the brainstem and which are inhibitory to spinal nociceptive neurones. This

¹Present address: Department of Anaesthesia, Sir Charles Gairdner Hospital, Nedlands, Western Australia

hypothesis is based to a large degree upon the results obtained when morphine is microinjected into the brainstem. Thus, it has been shown that microinjections of morphine into the periaqueductal grey (PAG: Sharpe, Garnett & Cicero, 1974; Jacquet & Lajtha, 1976; Yaksh, Yeung & Rudy, 1976; Lewis & Gebhart, 1977), nucleus raphe magnus (NRM: Dickenson, Oliveras & Besson, 1979; Levy & Proudfit, 1979; Le Bars, Dickenson & Besson, 1980; Azami, Llewelyn & Roberts, 1982) and, nucleus reticularis paragigantocellularis (NRPG: Akaike, Shibata, Satoh & Takagi, 1978; Rosenfeld & Stocco, 1980; Azami *et al.*, 1982) in rats and nucleus reticularis gigantocellularis in cats (Chan, 1979) cause analgesia. The amounts injected are usually of the order of 5 µg. Only Takagi (1980) used much smaller quantities of morphine in the ng range, which he claimed were of relevance to the concentrations of morphine in the brain achieved by systemic administration of analgesic doses.

In opposition to the hypothesis that morphine activates descending inhibitory systems, Duggan, Griensmith & North (1980) demonstrated that intravenous morphine attenuated, rather than increased, tonic descending inhibition of dorsal horn neurones in cats and Le Bars, Dickenson & Besson (1980) failed to demonstrate an inhibition of dorsal horn cells by microinjection of morphine into NRM in rats. Nevertheless, apparently direct evidence for activation of descending inhibitory systems was obtained by Bennett & Mayer (1979) and Gebhart, Sandkühler, Thalhammer & Zimmermann (1982, 1983). Bennett & Mayer (1979) found that 4 to 16 µg of morphine or 0.25 or 0.5 µg of etorphine microinjected into PAG in rats inhibited the excitation of dorsal horn neurones by noxious stimulation in 55% or 82% respectively of the neurones tested. Similarly, Gebhart *et al.* (1982, 1983) found that 10 to 20 µg of morphine microinjected into PAG in cats inhibited the excitation of 14 of 18 neurones examined.

We have previously demonstrated (Clark & Ryall, 1982; 1983) that etorphine microinjections in the brainstem of cats inhibited the excitation of dorsal horn neurones with a mean ED₅₀ of 3.9 µg kg⁻¹ but that the effectiveness of etorphine was unchanged by spinal transection and was similar to or slightly less than the intravenous ED₅₀. The effect of etorphine microinjections was therefore attributed to entry to the circulation, with subsequent effects on the spinal cord.

In this paper we describe our results with morphine, which is much less lipid-soluble than etorphine, and show that the entry of morphine into the circulation after microinjection in the brainstem is insufficient to account for the depression of dorsal horn activity. However, we argue that the local concentrations of morphine achieved by microinjection

in this and most other studies exceed by several orders of magnitude the concentrations achieved by systemic administration and that the effects obtained after microinjection are unlikely to be of relevance to the mechanism of analgesia when opiates are administered by conventional methods.

Methods

Experiments were carried out in 39 cats of either sex weighing 2.2 to 3.7 kg (mean 2.8 ± s.e. 0.06 kg). Anaesthesia was maintained by pentobarbitone administered intravenously, either by continuous infusion at a slow rate or intermittently as required, after induction either by halothane or by 35 mg kg⁻¹ of pentobarbitone intraperitoneally. Three animals were decerebrated on the day before the experiment by intercollicular transection of the brainstem and removal of the fore-brain under pentobarbitone anaesthesia. No further anaesthetic was administered in these three experiments. In 31 animals, 3 of which were decerebrated, the spinal cord was intact. In the remaining 8 cats the cord was transected at the second lumbar segment.

Extracellular recordings were obtained by means of tungsten microelectrodes (12 MΩ) from dorsal horn neurones in L7 or L6 responding both to noxious radiant heat stimulation of ipsilateral foot pads and to light mechanical stimuli such as hair movement or light touch. The noxious heat stimulus (duration 20 to 40 s) was repeated automatically every 2 to 4 min and the firing frequency of the neurone under observation was plotted continuously on a pen recorder.

Morphine sulphate, or in four experiments the hydrochloride, was microinjected from 0.1 M solutions into three sites in the brainstem. These were the nucleus raphe magnus (NRM: stereotaxic coordinates, P, 7.1; L, 0; D, -7.9 mm), nucleus reticularis magnocellularis (NRMC: P, 6.9; L, 2; D, -8.3 mm) and the periaqueductal grey (PAG: P, 0; L, 1 or 2; D, 0 or 1 mm) (Berman, 1968). Morphine was also administered intravenously. All doses are expressed as µg of base. Means are indicated with standard errors. Full details of the experimental procedure have been described previously (Clark & Ryall, 1983).

Results

In our earlier study with etorphine (Clark & Ryall, 1982; 1983) we were able to determine doses which caused 50% inhibition (ED₅₀) of the evoked response on every cell tested because dose-response curves were reasonably easily obtained. In the pres-

ent study the effects of morphine were quite variable, even on the same cell, and it was not possible to determine ED₅₀s.

Table 1 therefore summarizes the data in a less satisfactory manner than if it had been feasible to obtain ED₅₀s. Nevertheless, a more precise quantitative estimate of the effectiveness of morphine would not have materially affected the conclusions which are drawn.

Microinjection into the brainstem of cats with an intact spinal cord

The experiments shown in Table 1 were carried out on 26 cats. Three of these were decerebrated under pentobarbitone anaesthesia on the day before the experiment and anaesthesia was subsequently discontinued (Clark & Ryall, 1983). The remaining experiments were carried out under continuous pentobarbitone anaesthesia. Since there were no obvious differences in the data obtained, the results have been pooled. In some cats, microinjections were made into more than one site.

The initial amount of morphine microinjected varied widely from 28–285 µg in different experiments. The amount injected was usually increased by a factor of three if no inhibition resulted from the first administration. In fourteen tests, the threshold amount of morphine required to cause inhibition ranged from 28 to 285 µg (mean 94.6 ± 27.5 µg). In the remaining experiments the range of doses employed was not sufficiently low for a threshold amount to be determined.

In 11 of 14 animals, microinjections of morphine (mean quantity injected, 121 ± 27 µg) to NRM

caused inhibition of dorsal horn neurones ranging from 20 to 80% (mean $47 \pm 6\%$). In 11 of 16 cats, injections to NRMC (mean quantity injected 137 ± 27 µg) caused a similar degree of inhibition ($57 \pm 11\%$). In 7 of 11 cats, morphine caused less inhibition ($21 \pm 4\%$) when injected into PAG but the administered dose was also lower (93 ± 28 µg). There were therefore no marked differences between the effectiveness of morphine microinjected at these three sites, and the mean effective dose at all sites was 120 ± 16 µg. This amount caused $44 \pm 5\%$ inhibition of the evoked excitation of dorsal horn neurones.

In those animals in which morphine produced no detectable inhibition (Table 1), the injected doses were similar to those employed in the other experiments in which morphine was effective.

Some examples showing the inhibitory effect of microinjected morphine are given in Figures 1 and 2. The evoked firing was completely abolished by the microinjection of morphine in NRMC in three experiments. The onset of the effect in Figure 1a was delayed about 6 min after the injection and recovery required a wait of about 2 h. In this cat, there was about 80% inhibition after an injection in NRM but only 20% inhibition after microinjection in PAG (not illustrated). Microinjection of 168 µg to PAG in another animal (Figure 1b) caused about 30% inhibition of the response after 4 min and recovery occurred within a further 10 min. A more prolonged inhibition was observed after microinjections into NRMC either when 168 µg was injected (not illustrated) or when 285 µg was microinjected (Figure 1b). In thirteen cats a much smaller quantity of morphine (28 µg) was injected into NRM, NRMC or PAG. There was no effect in six cats. In the remaining seven

Table 1 Inhibitory effects of morphine microinjections on the excitation of dorsal horn neurones by noxious heat

	<i>NRM</i>	<i>NRMC</i>	<i>PAG</i>
Number of cats tested	14	16	11
(A) Morphine: No. of cats in which inhibition observed	11	11	7
Amt. injected ¹ : range (µg)	28–285	28–285	28–171
: mean ± s.e. (µg)	121 ± 27	137 ± 27	93 ± 28
% inhibition : range (%)	20–80	20–100	10–40
: mean ± s.e. (%)	47 ± 6	57 ± 11	21 ± 4
Amt. injected : mean ± s.e. µg (all sites)		120 ± 16	
% inhibition : all sites		44 ± 5	
(B) Morphine: No. of cats in which no inhibition observed	3	5	4
Max. Amt. injected ² : range (µg)	56–149	28–285	28–171
: mean ± s.e. (µg)	116 ± 30	148 ± 42	92 ± 32

¹The amount injected is the smallest amount injected which caused an inhibition in a particular experiment.

²The maximum amount injected is the maximum quantity administered in any experiment in which morphine failed to cause inhibition.

NRM = nucleus raphe magnus; NRMC = nucleus reticularis magnocellularis; PAG = periaqueductal grey.

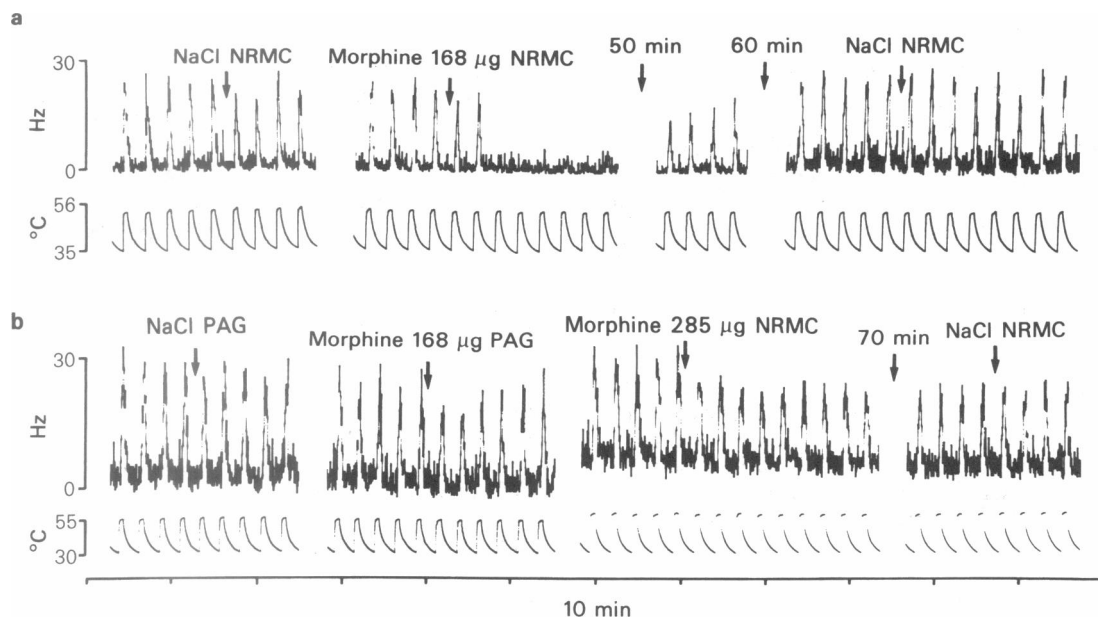


Figure 1 Inhibitory effect of morphine microinjection in two cats (a and b) on excitation of dorsal horn neurones by radiant heat stimulation of nociceptors in the toe pads. Morphine completely abolished the responses when microinjected into NRMC in (a) and recovery was prolonged. Control microinjections of isotonic saline had no effect. In (b), microinjection of morphine to PAG caused a short-lasting inhibition and injection to NRMC caused a more prolonged effect. Intervals between records are shown where appropriate. Upper records show frequency of firing (Hz) of the dorsal horn neurones and lower records show skin temperatures at the heated sites (°C).

cats inhibitions of 10 to 60% (mean $28 \pm 7\%$) were observed (Figure 2a). It is not possible to give precise estimates of the duration of inhibition when recovery was not observed because there was often a slow, progressive, spontaneous decline of the responses over a period of hours for which adequate allowance could not be made.

The effects produced by insertion of the microinjection cannula, or by the injection of saline at the three sites in the brainstem are shown in Table 2. In three-quarters of the animals, cannula insertion into

NRMC (Table 2) caused an inhibition of evoked responses which was usually mild, transitory in nature and not repeatable at the same site. Repeating the procedure at the same site was rarely effective as may be seen from a comparison of the two rows of data in Table 2. Changes in dorsal horn activity due to insertion of the cannula in NRM or PAG were observed more rarely. On three of the four occasions on which saline microinjections caused an effect, the cannula had been inserted into NRMC. Morphine was not administered until the response pattern had

Table 2 Inhibitory effects of cannula insertion or control saline microinjections (0.5 to 5 μ l) on the excitation of dorsal horn neurones by noxious heat in animals with an intact spinal cord

	<i>Cannula insertion</i>			<i>Saline injection</i>		
	<i>NRM</i>	<i>NRMC</i>	<i>PAG</i>	<i>NRM</i>	<i>NRMC</i>	<i>PAG</i>
Inhibition: No. of cats ¹	2/11	15/20	4/10	1/13	3/16	0/9
Inhibition: No. of trials ²	2/27	22/60	5/30	1/16	3/22	0/10

¹The number of animals in which saline injection or cannula insertion caused inhibition of evoked responses as a fraction of the number of animals tested.

²The number of trials in which inhibition was observed as a fraction of the total number of trials. There were repeated trials at the same site in some animals.

Abbreviations as for Table 1.

stabilized after insertion of the cannula and the effects of morphine were readily distinguished from the rather uncommon and briefer effects produced by saline microinjections.

Microinjection into the brainstem of cats in which the spinal cord was transected

The microinjection of morphine in amounts up to 285 μg , the maximum doses injected in cats with an intact spinal cord, into the NRMC in four spinal cats failed to inhibit the evoked responses of dorsal horn neurones in any experiment. It was similarly inactive in an additional four cats in which the injections were made into NRM. However, in all experiments the injection of much larger amounts (mean $1015 \pm 224 \mu\text{g}$) inhibited the dorsal horn neurones by

$28 \pm 5\%$. These effects were much more prolonged in time course than were the effects observed when the cord was intact. They are therefore attributed to the passage of morphine from the injection site into the circulation from which the drug entered the spinal cord to produce inhibitory effects.

The intravenous administration of morphine

Morphine was administered intravenously to 17 cats with an intact spinal cord and to 3 animals in which the cord was transected.

Morphine attenuated the responses of dorsal horn neurones to noxious stimulation in every experiment (Figure 2(b), (c)). When the cord was intact the doses employed to demonstrate inhibition varied from 0.15 to 8.6 mg (mean $3.2 \pm 0.6 \text{ mg}$). The evoked excitation

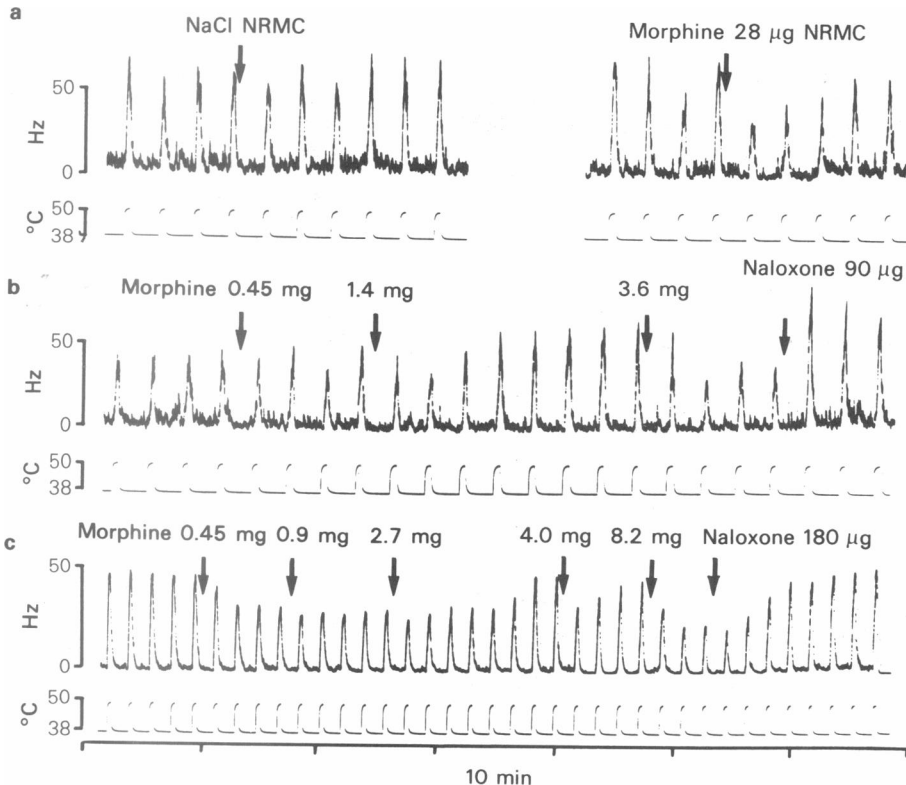


Figure 2 Inhibitory effect on noxious heat evoked responses of a dorsal horn neurone of a small quantity of morphine microinjected into NRMC in (a) compared with the effect of intravenous injections of much larger quantities in the same animal in (b). The inhibitory effect in (b) was reversed by the intravenous injection of naloxone. Acute tolerance caused by intravenous administration in another animal is shown in (c). After injections of 0.45, 0.9 and 2.7 mg of morphine, a further 4 mg caused no more inhibition than was first observed with 0.45 mg and the effect was shorter-lasting. An additional 8.2 mg caused further inhibition which was reversed by intravenous administration of naloxone. Coordinates as in Figure 1.

of dorsal horn cells was inhibited by $65 \pm 7\%$ in these experiments. In five of these experiments the minimum effective dose of morphine was determined to be 2.1 ± 0.7 mg, causing inhibition of $58 \pm 10\%$. In three spinal cats injections of 0.2, 0.9 and 7.4 mg of morphine caused inhibitions of 15, 50 and 60% respectively. The sensitivity of spinal animals to morphine was therefore of the same order as was the sensitivity of cats with an intact spinal cord.

The inhibitory effects of intravenously administered morphine were rapid in onset and usually prolonged.

Although acute tolerance to the effect of repeated morphine administrations was not systematically studied, this was clearly evident in the experiment illustrated in Figure 2c.

The concentration achieved in microinjection experiments

Since microinjected morphine was more effective when the spinal cord was intact than it was when the cord was transected, it seemed probable that at least part of the inhibition observed with the cord intact could be attributed to a local action of morphine in the brainstem. The following analysis was carried out to evaluate the likely concentrations of morphine which would be achieved at the injection site in order to determine the relevance of these local actions to the analgesia produced by systemically administered morphine.

The microinjection of a quantity of morphine within a reasonably brief space of time could be treated as an instantaneous point source (Jaeger, 1965) from which the drug would diffuse to adjacent regions with kinetics determined from diffusion equations (Jaeger, 1965).

The relevant equations are:

$$(1) \quad C_{\max} = 0.073 M/r^3$$

where C_{\max} is the maximal concentration attained at a distance r from the point source and M is the amount injected.

Assuming that the amount of morphine base injected is $10 \mu\text{g}$, the dotted line in Figure 3 shows how the maximum concentration achieved declines with distance from the injection site. It is apparent that the maximum concentration achieved exceeds 10^{-4} M at distances up to 2 mm from the injection site.

$$(2) \quad t_{\max} = r^2/6D$$

where t_{\max} is the time taken to achieve C_{\max} at distance r ; D is the diffusion coefficient.

The solid lines in Figure 3 show the variation in t_{\max} with distance for two values of D . Figure 4 shows the relationship between t_{\max} and D at different distances from the source. The value of the diffusion coefficient is related to molecular weight. By interpolation from published data on the diffusion coefficients of a wide

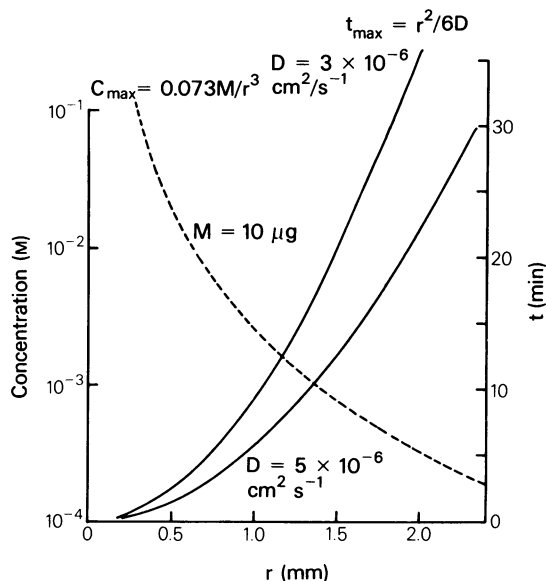


Figure 3 Theoretical maximum molar concentrations (C_{\max}) achieved at various distances (r) from the site of administration of a fixed quantity ($M = 10 \mu\text{g}$) of morphine are shown by the broken line. The solid lines show the times (t) at which the maximum concentrations are obtained for two values of the diffusion coefficient ($D = 3$ or $5 \times 10^{-6} \text{cm}^2 \text{s}^{-1}$).

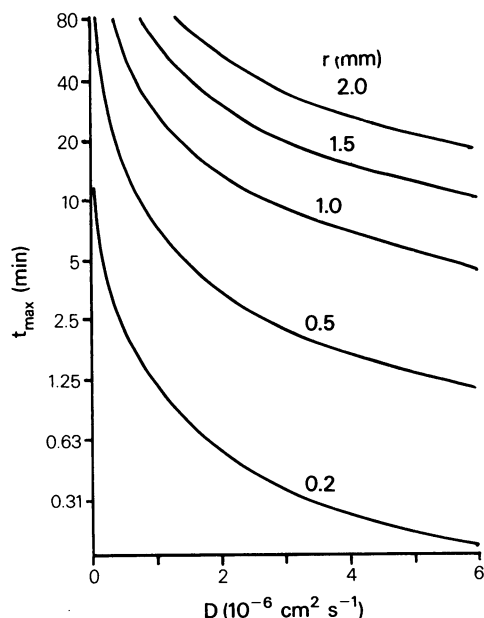


Figure 4 The relationship between the time at which the maximal concentration is achieved (t_{\max}) and the diffusion coefficient (D) at varying distances (r) from the site of administration. The curves are derived from the expression $t_{\max} = r^2/6D$.

range of substances (Figure 5), a reasonable value for the diffusion coefficient of morphine would be in the region of 3 to $5 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$.

It can be seen in Figure 3 that at distances up to about 2 mm from the injection site, the time at which the maximum concentration would be achieved is less than 30 min and at distances up to 1 mm would be in the region of 10 min for the two values of D selected.

The profiles of concentration changes with time at different distances and with diffusion coefficients of 3 or $5 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ are shown in Figure 6 and are derived from equation 3.

$$(3) C = \frac{M}{8(\pi Dt)^{1.5}} \times e^{-r^2/4Dt}$$

where C is the concentration at time t and distance r from the injection site.

These curves show that, as might be expected intuitively, the concentration falls rapidly at locations

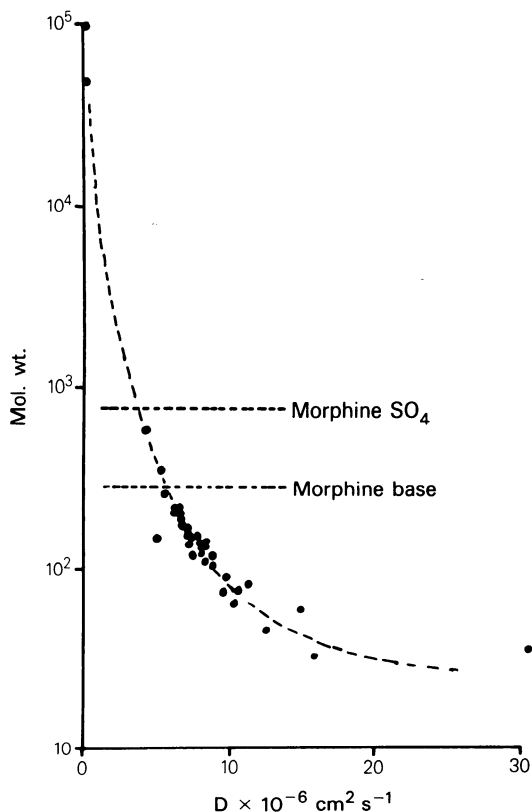


Figure 5 The relationship between molecular weight (Mol. wt.) and the diffusion coefficient (D). Each point represents a different substance. Values taken from Jaegar (1965), Diem & Lentner (1975) and West (1977).

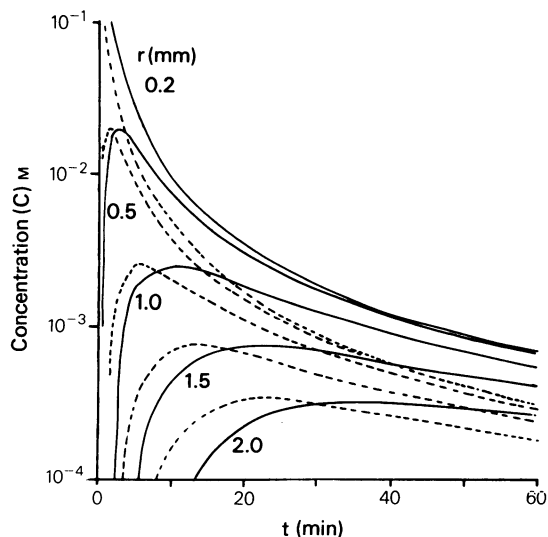


Figure 6 Relationship between molar concentration (C) and time after injection (t) at different distances (r). The solid lines show the curves obtained with a diffusion coefficient (D) of $3 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$. The broken lines show the curves obtained when $D = 5 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$. The lines were calculated from the expression

$$C = \frac{M}{8(\pi Dt)^{1.5}} \times e^{-r^2/4Dt}$$

where $M = 10 \mu\text{g}$.

close to the injection site but rises more slowly at distant sites and that after about 20 min the concentration declines rather similarly at all distances but remains above 10^{-4} M for more than 1 h.

These calculations make the assumption that the diffusion coefficient in the brain is in the region of 3 to $5 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$. A re-interpretation of the data presented by Lomax (1966) confirms that this assumption is reasonably correct. Lomax microinjected radiolabelled morphine into the brain and measured the radioactivity present in alternate $100 \mu\text{m}$ sections at different times after the injection both in the living animal and in post mortem brains. The data which he obtained are averaged and are shown in Figure 7(b). Figure 7(a) and (c) show the distributions calculated for diffusion coefficients of 3 and $5 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$. The correspondence between the experimental and the theoretical curves is quite striking and shows that the results obtained by Lomax are explicable in terms of diffusion from the site of administration.

Lomax (1966) also determined the amount of radioactivity remaining in these brain samples at various times after injection. The theoretical amount remaining within 2 mm of the injection site was calculated by summing the amounts present in consecutive 0.2 mm shells up to 2 mm from the injection site at

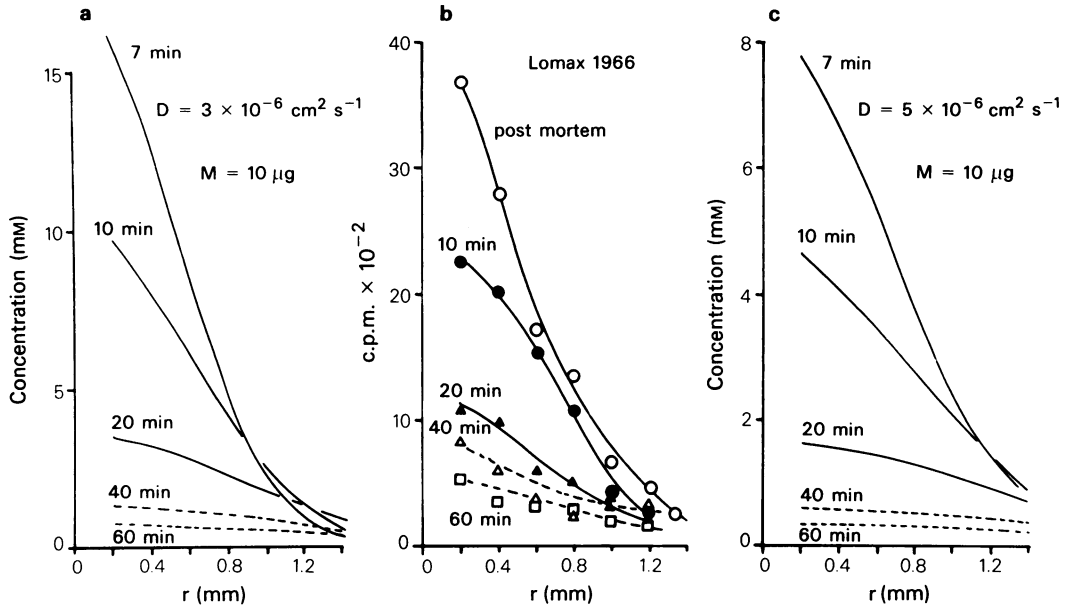


Figure 7 A comparison of theoretically derived millimolar concentration versus distance curves for two values of the diffusion coefficient (a and c) with experimental values calculated from data given by Lomax (1966), at 7, 10, 20, 40 and 60 min after injection. The experimental curves in (b) were obtained by the injection of tritiated morphine in live animals except for the curve labelled 'post mortem' in (b) which was obtained within a few minutes after the microinjection in dead animals.

different times after injection. The results are shown by the solid lines in Figure 8, alongside the experimental data obtained by Lomax (1966). It is therefore evident that a large proportion of the morphine disappearing from the region around the injection site is lost to the surrounding brain regions where the concentrations were presumably too small to be detected by the technique used by Lomax. However, a fraction is also lost into the circulation (Herz & Teschemacher, 1971). This fraction represents about 3% of the injected amount in 5 min and could account for part of the small difference between our theoretical data and those obtained by Lomax. Clearly, such a small fraction lost into the circulation would not materially affect our calculations.

Discussion

The injection of morphine into the brainstem of cats with the spinal cord transected inhibited the excitation of convergent dorsal horn neurones evoked by noxious stimulation of the hind foot. The amounts of morphine required to produce this effect were in the mg range and were similar to those that caused inhibition when injected intravenously in animals either with the spinal cord intact or with it transected. The amounts required are similar to those employed intravenously in spinal and non-spinal cats in a much

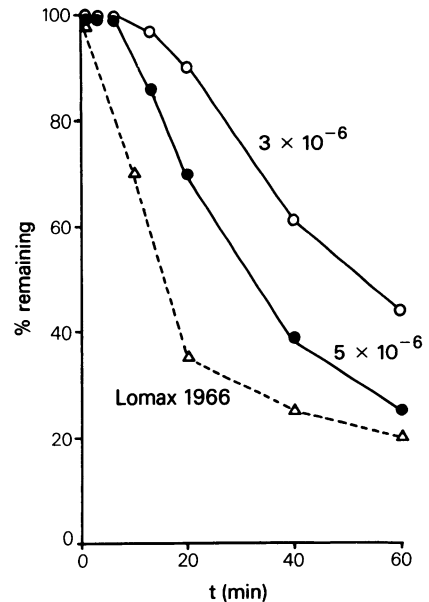


Figure 8 Comparison of theoretically determined percentage of morphine remaining within 2 mm of the injection site for two values of the diffusion coefficient (see text) at different times after injection (t) with experimentally-determined percentages taken from Lomax, 1966. The difference between theoretical and experimental curves may be due to absorption into the blood (see text).

larger series of experiments (Hanaoka *et al.*, 1978).

Thus morphine, like the more lipid-soluble analgesic etorphine (Clark & Ryall, 1982; 1983), when injected into the brain in amounts comparable to those required intravenously appears to be able to enter the circulation and affect the spinal cord directly.

Unlike etorphine, which was no more effective when microinjected into cats with an intact spinal cord than it was when the cord was transected, morphine was about ten times more effective after microinjection when the cord was intact. These experiments indicate that, in addition to the direct effects upon the spinal cord, morphine can also exert local effects in the brainstem which indirectly inhibit spinal neurones. The inhibition of spinal neurones could be due either to the activation of descending inhibitory neuronal systems, which can easily be demonstrated by electrical stimulation of the brain (see Edeson & Ryall, 1983, for references) or to the local release of endogenous substances which rapidly reach the spinal cord via the cerebro-spinal fluid (Ohlsson, Fu, Jones, Martin & Dewey, 1982).

In the present study an average inhibition of 44% was achieved by the microinjection of 120 μg into the brainstem. The average weight of the animals was 2.8 kg and the amount injected was therefore 43 $\mu\text{g kg}^{-1}$ which is comparable to the quantities microinjected into the PAG in rats by Bennett & Mayer (1979), with results similar to those obtained in the present study at three different sites. There were no marked differences between the effects of morphine microinjected into PAG, NRM or NRMC and at all three sites electrical stimulation inhibits spinal neurones (Edeson & Ryall, 1983).

In a more recent study (Gebhart *et al.*, 1982; 1983), 10–20 μg of morphine microinjected into PAG in cats caused an average of 43% inhibition of evoked responses of dorsal horn neurones in 14 of 18 sites. Although the doses employed were somewhat smaller than in the present study, the effects were quite similar. When smaller amounts (5 μg) were injected into NRM in rats no inhibition of dorsal horn activity was observed (Le Bar *et al.*, 1980a).

Thus the present results agree with others in demonstrating that morphine microinjected into various areas of the brainstem does exert a local effect resulting in the inhibition of nociceptive dorsal horn neurones and that this effect could be the mechanism by which opiates produce analgesia when *microinjected* in μg quantities into the brain (see Introduction). In contrast to the conclusions drawn by other workers from such microinjection experiments we believe that the concentrations attained at the site of administration following microinjection are several orders of magnitude higher than those which are achieved following the systemic administration of analgesic doses. It follows that the effects produced

by microinjection have no relevance to the mechanism of analgesia after *systemic* administration.

The brain concentration of morphine after systemic administration has been determined in several laboratories. Concentrations ranging from 100 to 500 ng g^{-1} (corresponding to molar concentrations of about 0.3 to $1.5 \times 10^{-6} \text{M}$) have been reported in the majority of studies (Herz & Teschemacher, 1971; Patrick, Dewey, Spaulding & Harris, 1975; Hipps, Eveland, Meyer, Sherman & Cicero, 1976; Ngai, Berkowitz, Yang, Hempstead & Spector, 1976).

The calculations made from the diffusion equations in this paper show that concentrations in excess of 10^{-4}M would be achieved by 10 μg of microinjected morphine at distances up to 2 mm from the injected site. Such concentrations are vastly in excess of even the highest reported values for the brain concentration after systemic administration. Since the analgesia resulting from morphine microinjections is said to be critically dependent upon the position of the microinjection cannula (Pert & Yaksh, 1974; Yaksh *et al.*, 1976) the effects must be localized within, say, 1 mm from the orifice, where the expected maximal concentration is likely to be of the order of 10^{-3}M when quantities of about 10 μg are injected: this maximal concentration is independent of the diffusion coefficient and is directly related to the amount injected and would be reduced only slightly by the small amount entering the circulation (Herz & Teschemacher, 1971).

The calculated distribution of morphine around the injection site, assuming a diffusion coefficient of 3 or $5 \times 10^{-6} \text{cm}^2 \text{s}^{-1}$, agreed well with the kinetic studies performed by Lomax (1966). It is therefore possible to draw further conclusions from the diffusion calculations which show that the time course of analgesia or of the depression of dorsal horn neurones corresponds to the time course which could be predicted if morphine were acting over a radius of approximately 1 mm from the injection site.

It has been observed in these experiments and in those of Bennett & Mayer (1979) and those of Gebhart *et al.* (1982, 1983) that the inhibitory effect of microinjected morphine takes several minutes to develop. A similarly long time to maximum analgesia after microinjection (5 to 30 min) has often been reported (Yaksh *et al.*, 1976; Dickenson *et al.*, 1979; Oley, Cordova, Kelly & Bronzino, 1982; Bryant, Olley & Tyers, 1982; Azami *et al.*, 1982). Such a long time to maximum effect can be explained on the basis that a considerable volume of tissue needs to be reached by morphine before the full effect is achieved. The diffusion equations show that at a distance of 2 mm from the injection site, a maximal concentration would be achieved about 20 min after the injection. However, it is again emphasized that the concentrations at such distances are still extremely high. Alternatively, it is possible that locally very

high concentrations of morphine liberate endogenous peptides which are transported to distant sites of action in the cerebro-spinal fluid (Ohlsson *et al.*, 1982).

Naloxone usually antagonizes the effects of morphine microinjections but it is not necessary to propose that this antagonism is achieved by a direct competition between morphine and naloxone at specific opiate receptors at the injected site. There are two additional possibilities. First, if morphine at high concentrations non-specifically liberates opioid peptides which are transported in the cerebro-spinal fluid to distant sites such as the spinal cord (Ohlsson *et al.*, 1982), then naloxone may antagonize the effect of the released peptide, rather than the direct and non-specific action of morphine. Second, morphine at high concentrations may non-specifically excite descending neuronal systems which are inhibitory to spinal neurones via enkephalinergic synapses and naloxone could be acting upon receptors at these synapses. These possibilities apply equally, whether naloxone is administered intravenously or is microinjected because, after microinjection, the antagonist can reach pharmacologically active concentrations in the blood (Clark & Ryall, 1983).

There is one notable exception to the generalization that the concentration of morphine achieved at the site of microinjection after the administration of μg quantities is far too high to be of relevance to the mechanism of analgesia: Takagi (1980) and Akaike *et al.* (1978) showed that the injection of 2 ng of morphine in NRPG in the rat caused analgesia. The local concentrations achieved by such microinjections would be 5000 fold lower than those achieved after 10 μg was injected. Takagi (1980) argues, on a basis different from that employed in the current study, that the low concentrations after the injection of ng quantities are similar to those obtained by systemic administration. His data cannot necessarily be construed as evidence for direct activation of descending inhibitory systems by low concentrations of morphine because, unlike most other investigators, he employed a test of analgesia clearly involving supraspinal reflex pathways (Zorman, Hentall, Adams & Fields, 1981). If the NRPG is an

essential component in this pathway then the specific effect exerted by low concentrations of morphine could be analogous to the specific effect of low concentrations of morphine on nociceptive spinal reflexes.

We therefore conclude that, at present, the only sites at which morphine has been clearly demonstrated to exert a specific anti-nociceptive action at low concentrations is directly upon spinal reflex arcs and possibly upon supraspinal reflexes involving supraspinal structures. The apparent activation of descending inhibitory systems after microinjection is probably only present when extremely high local concentrations are achieved and these effects would have no relevance to the mechanism of analgesia after systemic administration.

The conclusions regarding the high local concentrations of morphine which are achieved by the microinjection of μg quantities may have relevance to other studies in which the technique is being used increasingly to investigate the central actions of drugs with diverse properties. For example, the technique has been used in studies on the action clonidine (Struyker Boudier & Van Rossum, 1972), α -methylnoradrenaline (De Jong, Nijkamp & Bohus, 1975), ACTH (Jacquet & Wolf, 1981), α -MSH (Walker, Akil & Watson, 1980), glutamic acid (Urca, Nahin & Liebeskind, 1980; Dampney, Goodchild, Robertson & Montgomery, 1982), 5-hydroxytryptamine (Llewelyn, Azami & Roberts, 1983), angiotensin (Epstein, Fitzsimons & Rolls, 1970), benzodiazepines (Mantegazza, Parenti, Tam-miso, Vita, Zambotti & Zouta, 1982), baclofen (Levy & Proudfit, 1979), nicotine (Llewelyn, Azami, Grant & Roberts, 1981) and neurotensin (Kalivas, Gau, Nemeroff & Prange, 1982). In all of these studies, the concentrations of substances achieved by microinjection were likely to have been exceedingly high.

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