Br. J. Pharmac. (1981), **73**, 435–442

OPIATE-LIKE ANALGESIC ACTIVITY IN GENERAL ANAESTHETICS

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1 The interaction of naloxone with various anaesthetics was studied both *in vivo* and *in vitro*.

2 Naloxone (10 mg/kg) did not significantly alter the anaesthetic duration of halothane, diethylether, ketamine, pentobarbitone or Althesin.

3 Naloxone (10 mg/kg) reduced the analgesic activity of nitrous oxide, ketamine and morphine in the rat tail-flick test. With the exception of pentobarbitone and Althesin, the other anaesthetic agents also induced analgesia but were not antagonized by naloxone.

4 Specific [^{3}H]-dihydromorphine binding was displaced by the opiates naloxone (IC_{50} = 7.6 nM), methionine-enkephalin (Met-enkephalin, IC_{50} = 40 nM) and morphine (IC_{50} = 54 nM). Similarly, displacement was observed with xylazine (IC_{50} = 9 μM), ketamine (IC_{50} = 130 μM) and Althesin (IC_{50} = 150 μM); other anaesthetics agents tested were inactive in mM concentrations.

5 Ketamine (IC_{50} = 200 μM) and xylazine (IC_{50} = 9.5 μM) were also capable of displacing specific [^{3}H]-D-Ala$_2$-enkephalin (D-Leu) binding, as were morphine (IC_{50} = 95 nM) and Met-enkephalin (IC_{50} = 40 nM).

6 On the stimulated guinea-pig ileum, Met-enkephalin and morphine inhibited the contractions, the IC_{50} values were 30 nM and 50 nM respectively. The anaesthetics ketamine (IC_{50} = 10 μM) and Althesin (IC_{50} = 8 μM) were active. Xylazine (IC_{50} = 12 nM) exhibited considerable potency in inhibiting the contractions on this preparation. Naloxone 0.5 μM produced a 1000 fold shift in the opiate dose-response curve but the anaesthetic responses showed only slight sensitivity to antagonism by naloxone.

7 The activity of Althesin was found to be due to the vehicle in which this anaesthetic is solubilised.

8 Whilst several anaesthetic agents showed analgesic activity, specific dihydromorphine binding displacement or guinea pig ileum inhibiting activity, these effects showed variable sensitivity to naloxone.

Introduction

General anaesthetics are considered to act non-selectively on biological membranes rendering ineffective synapses essential for the maintenance of consciousness. A non-selective mechanism of action suggests that all anaesthetic agents should affect biological functions in a similar fashion. However, this is not always the case. Anaesthetics exhibit differing abilities to depress respiration, produce hypotension, excitation and analgesia. Nitrous oxide and ketamine for example are capable of producing analgesia at concentrations inadequate to produce a loss of consciousness (Bovill, Clarke, Dundee, Pandit & Moore, 1971; Richards, Parbrook & Wilson, 1976), while barbiturate and steroid anaesthetics can lower the pain threshold (Morgan, Whitwain & Page, 1973).

Balmer & Wyte (1977) have shown that physostigmine reverses anaesthesia but not analgesia induced by ketamine. Other investigators have observed that the analgesic properties of certain general anaesthetics were anatagonzied by the opiate antagonist, naloxone (Finck, Ngai & Berkowitz, 1977). These observations when taken together could suggest that anaesthesia and analgesia are not produced by a single non-selective action but by more than one separate action.

From evidence obtained with the antagonist, naloxone, it has been suggested that anaesthetics can interact with the opioids-peptidergic system. The object of this study was to investigate the effect of various anaesthetic agents on different opioid test systems in order to test the hypothesis that some anaesthetic agents produce analgesia via an opioid mechanism. The term anaesthesia has been taken to mean the loss of consciousness, analgesia to mean the lack of response to an unpleasant stimulus, with or without the loss of consciousness, often called antinociception. Some preliminary reports of parts of this study have been given (Lawrence & Livingston, 1979; 1980).

0007-1188/81/060435-008 $01.00

Methods

In vivo *experiments*

The effect of naloxone on anaesthesia and analgesia Groups of six adult Wistar rats (300 g) were injected intraperitoneally with saline (0.9% w/v NaCl solution) or naloxone. Five minutes later, both the control and naloxone pretreated animals were injected with various anaesthetics. For ease of handling, the gaseous volatile anaesthetics were injected intraperitoneally in the form of a saturated solution of olive oil (Paton, 1974). Anaesthesia duration was measured as the time from the loss of the recovery of the righting reflex by an animal. The tail-flick method was used as a measure of analgesia (Sewell & Spencer, 1976). The test was performed by immersing the animal's tail in hot water at 58°C and the time for removal of the tail was measured. A 15 s maximum exposure time was permitted. The test was performed at 10 min intervals before and during anaesthesia.

In vitro *experiments*

The action of anaesthetics on opiate receptor binding The method of measuring opiate receptor binding followed was similar to that of Pert & Snyder (1973). Adult male Wistar rats were used. After decapitation the brain was rapidly removed and the cerebellum dissected off and discarded. The brain was homogenized in 40 volumes of ice cold Tris-HCl buffer (50 mM, pH 7.7 at 25°C), with a motor driven Teflon pestle; this homogenate was centrifuged at 40,000 *g* for 15 min and the pellet resuspended in fresh Tris-HCl buffer. This suspension was then preincubated at 37°C for 40 min and recentrifuged. After resuspension (1 g of tissue per 100 ml) aliquots of homogenate were incubated at 25°C for 30 min with different concentrations of anaesthetic agent and [^{3}H]-dihydromorphine (2 nM, sp. act. 65 Ci/mmol) and made up to 2 ml with Tris-HCl or 10 μM morphine. All tubes and each experiment were repeated in triplicate. Bound [^{3}H]-dihydromorphine was collected by vacuum filtration of Whatman GFB glass-fibre filters and washed immediately with 10 ml of ice cold Tris-HCl buffer. Filters were assayed for radioactivity on a Packard Tri-Carb scintillation spectrometer. These procedures were carried out in a darkened room to prevent degradation of the dihydromorphine.

Specific [^{3}H]-dihydromorphine binding was calculated by subtracting the binding in the presence from that obtained in the absence of morphine (10 μM). The specific binding in the presence of the various anaesthetics was expressed as a percentage inhibition of the total specific binding. Specific binding represented 60% of the total [^{3}H]-dihydromorphine binding. The effects of Met-enkephalin, morphine, ketamine and xylazine were studied in a similar manner on the binding of [^{3}H]-D-Ala$_2$-enkephalin (D-Leu) (1 nM, sp. act. 42 Ci/mmol) to rat brain homogenates which were prepared as previously described. The presence of Met-enkephalin (10 μM) was used as a measure of non-specific binding.

Anaesthetics on the transmurally stimulated guinea-pig ileum Guinea-pigs were stunned and bled. The ileum was removed and small muscle strips were suspended in an organ bath containing 5 ml of Krebs bicarbonate solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1, $K_2H_2PO_4$ 1.2, $NaHCO_3$ 25 and glucose 11.1, which was gassed continuously with 95% O_2 and 5% CO_2 and maintained at 37°C. The ileum was stimulated electrically with a supramaximal voltage, pulses of 1 ms duration supplied by a Grass SD9 stimulator at 0.1 Hz. Contractions were recorded via an isotonic transducer (1 g tension) on a Bryants pen recorder (Paton & Vizi, 1969).

Statistical analysis

All error bars represent s.e.mean. Student's *t* test was used for testing significance between groups.

Drugs

The drugs used in this investigation were naloxone hydrochloride kindly supplied by Endo Laboratories, morphine hydrochloride (Macfarlan-Smith Ltd.), Met-enkephalin (Peninsular Laboratories), ketamine hydrochloride (Parke-Davis), xylazine hydrochloride (Bayer), Althesin and Althesin chremophore (Glaxo), pentobarbitone (May and Baker), halothane (I.C.I.), diethylether (B.D.H.), nitrous oxide (B.O.C.), [^{3}H]-dihydromorphine and [^{3}H]-D-Ala$_2$-enkephalin (D-Leu) (Radiochemical Centre, Amersham).

Results

In vivo *experiments*

In these experiments the effects of naloxone treatment were observed on the analgesic and anaesthetic properties of the gaseous anaesthetic nitrous oxide, the volatile anaesthetics halothane and diethylether, the injectable agents ketamine, pentobarbitone, Althesin and xylazine, a non-narcotic sedative. Morphine was used as a control drug for comparison throughout all these experiments.

The histogram, Figure 1, shows the anaesthetic durations of the anaesthetic agents and the effect of the premedication with naloxone, 10 mg/kg. In all

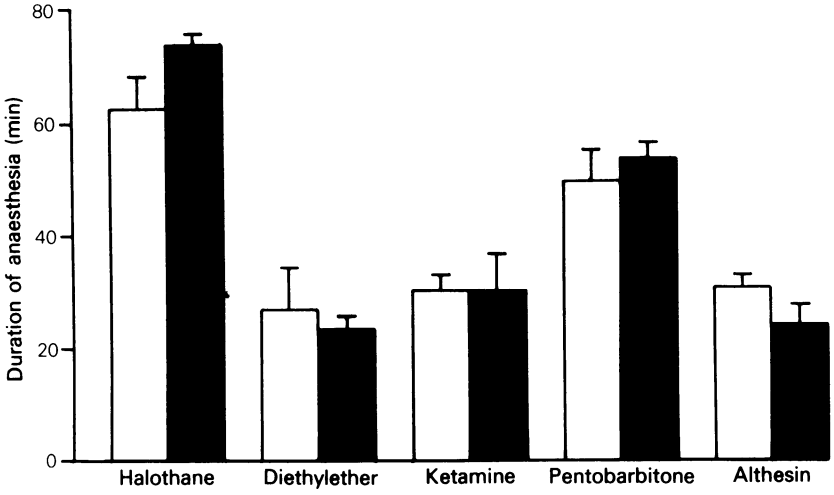

Figure 1 The effect of naloxone (10 mg/kg) premedication (solid columns) on the anaesthetic duration of halothane (1.5 ml/kg), diethylether (5 ml/kg), ketamine (100 mg/kg), pentobarbitone (30 mg/kg) and Althesin (40 mg/kg). Anaesthesia duration was measured by the righting reflex in groups of six animals. Vertical lines show s.e.mean.

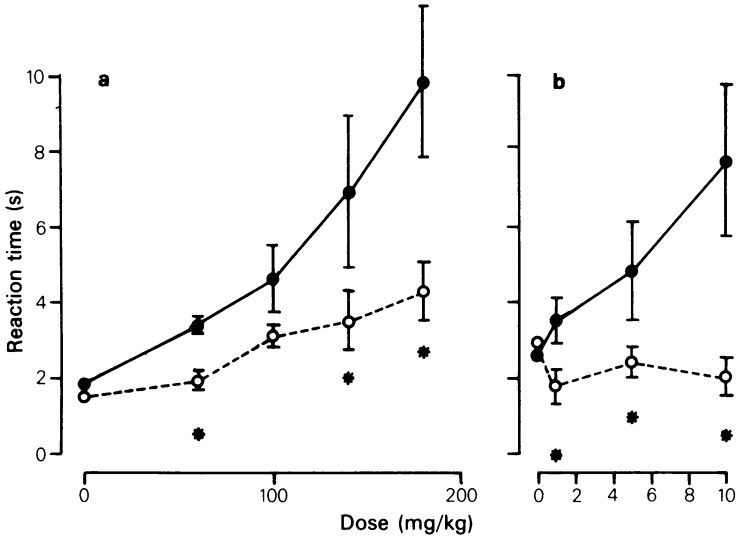

Figure 2 Dose-response curves for (a) ketamine (●) and (b) morphine (●) analgesia as measured by the tail-flick method. The open symbols show the effect of naloxone (10 mg/kg) on the ketamine and the morphine analgesia. Analgesia was tested 25 min after drug administration. Saline or naloxone were given 10 min before testing. Vertical lines show s.e.mean. * $P<0.05$ level of significance between control and naloxone-treated rats.

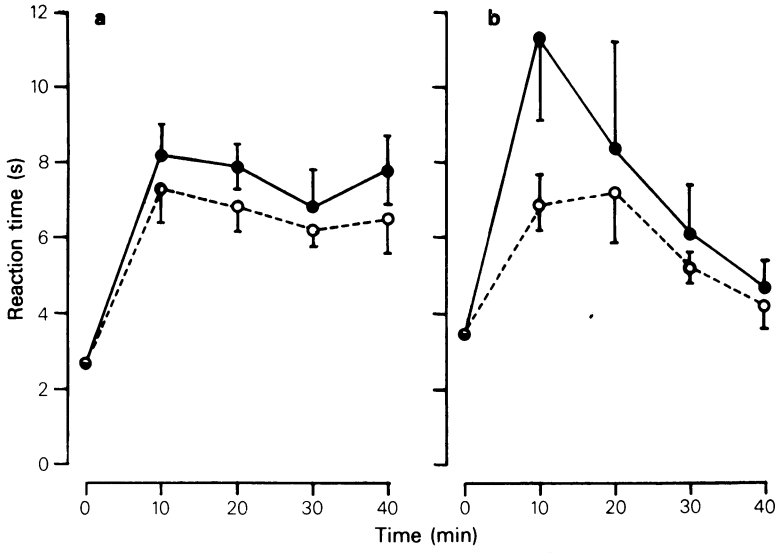

Figure 3 The effect of naloxone (10 mg/kg) premedication (○) on the analgesic activity of (a) halothane (1.5 ml/kg) (●) and (b) diethylether (5 ml/kg) (●) measured by the tail flick method every 10 min. Vertical lines show s.e.mean.

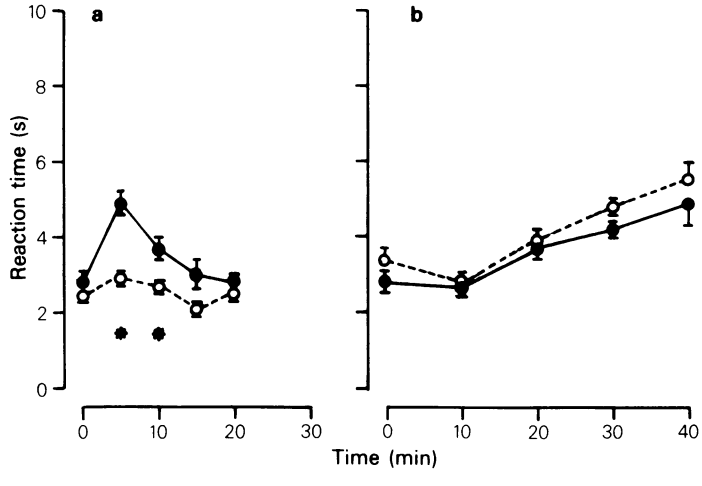

Figure 4 The effect of naloxone (10 mg/kg) premedication (○) on the analgesic activity of (a) nitrous oxide (4 ml/kg) (●) and (b) xylazine (20 mg/kg, ●) as measured by the tail-flick method. Vertical lines show s.e.mean. * $P<0.05$ level of significance between control and naloxone-treated rats.

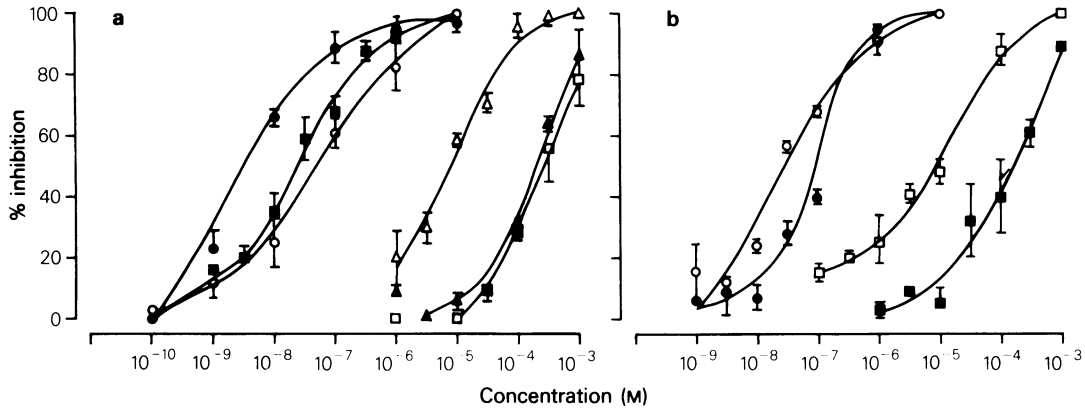

Figure 5 The effect of naloxone, morphine, xylazine, ketamine and Althesin on the specific [^{3}H]-dihydromorphine binding to the opiate receptor of the rat brain. [^{3}H]-dihydromorphine (2 nM) was incubated at 25°C for 30 min with rat brain homogenate in 2 ml of 50 mM Tris HCl (pH 7.7) in the presence of each agent. Bound activity was separated by filtration on GFB Whatman filters. Specifically bound radioactivity was the difference. (a) The effect of naloxone (●), Met-enkephalin (■), morphine (○) xylazine (△), ketamine (▲) and Althesin (□) on the specific [^{3}H]-dihydromorphine binding to rat brain homogenate. (b) The effect of Met-enkephalin (○), morphine (●), xylazine (□) and ketamine (■) on the specific [^{3}H]-D-Ala$_2$-enkephalin (D-Leu) binding to rat brain homogenate. Each point represents the mean of at least 3 experiments run in triplicate; vertical lines show s.e.mean.

cases the anaesthetic duration of the control and naloxone-treated groups were not significantly different. The anaesthetic nitrous oxide, xylazine and also morphine at the doses used failed to produce a loss of the righting reflex in the animals.

Dose-response curves for ketamine and morphine analgesia are shown in Figure 2a,b. In these experiments naloxone (10 mg/kg) was given 10 min before testing for analgesia and the test was performed 25 min after the administration of ketamine and morphine. Naloxone produced a significant reduction in the analgesic activity of both agents. However, the ketamine analgesia was not totally abolished by naloxone.

Halothane and diethylether both produced a marked analgesia which was not significantly reduced ($P > 0.05$) by the naloxone treatment (Figure 3a,b).

Nitrous oxide and xylazine produced an increase in the tail-flick reaction time without causing a loss of the righting reflex by the animal (Figure 4a,b). Nitrous oxide analgesia lasted only 20 min; this necessitated a 5 min rather than a 10 min testing interval. Naloxone significantly reduced the nitrous oxide analgesia but failed to alter that produced by xylazine. The barbiturate and steroid anaesthetics showed no alteration between control and naloxone-treated groups with neither group producing an increase in the tail-flick reaction time. The pad pressure test was used as a second analgesic test and similar results were obtained with all the anaesthetic agents.

In vitro *experiments*

Binding studies and isolated preparations permit studies on the direct receptor action of drugs. Morphine, Met-enkephalin and naloxone exhibited a characteristic inhibition of the specific ligand binding (Figure 5a,b). The concentration inhibiting 50% of the specific [^{3}H]-dihydromorphine binding (IC_{50}) was 54 nM for morphine, 40 nM for Met-enkephalin and 7.6 nM for naloxone. On specific [^{3}H]-D-Ala$_2$-enkephalin (D-Leu) binding morphine and Met-enkephalin had IC_{50} values of 95 nM and 40 nM respectively.

Nitrous oxide, halothane and diethylether did not influence specific [^{3}H]-dihydromorphine binding. However, the intravenous anaesthetics, with the exception of pentobarbitone, were all capable of displacing the specific [^{3}H]-dihydromorphine binding from the opiate receptor. The IC_{50} values for ketamine, xylazine and Althesin were 130 μM, 9 μM and 150 μM respectively. Although they were about a thousand times less potent than morphine and naloxone, the anaesthetic and opiate inhibition curves showed parallelism suggesting competition for a common site.

Ketamine and xylazine were also found to be capable of displacing the specific [^{3}H]-D-Ala$_2$-enkephalin (D-Leu) binding as shown in Figure 5b. Ketamine had an IC_{50} value of 200 μM while xylazine an IC_{50} value of 9.5 μM.

On the transmurally stimulated guinea-pig ileum,

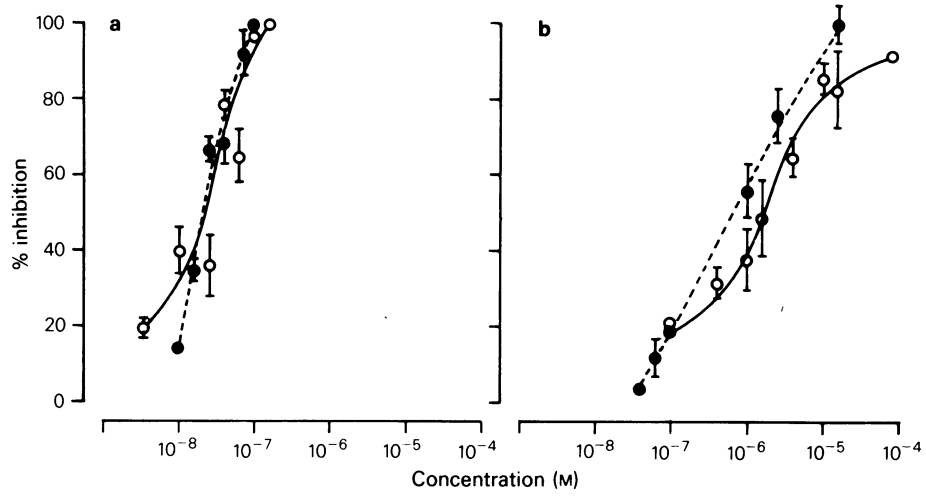

Figure 6 Shows the percentage inhibition by Met-enkephalin (●) and morphine (○) on the contractions of the transmurally stimulated guinea-pig ileum in the absence (a) and presence (b) of naloxone 0.5 μM. Vertical lines represent s.e.mean of 4 experiments.

Met-enkephalin and morphine both produced an inhibition of the contractions giving IC_{50} values of 30 nM and 50 nM respectively (Figure 6). In the presence of 0.5 μM naloxone a hundred fold rightward shift in the Met-enkephalin and morphine dose-response curves was observed. Nitrous oxide failed to inhibit the contractions, while both halothane and diethylether at high doses (> 1 mM) inhibited the contractions. These anaesthetics were not antagonized by naloxone.

The intravenous anaesthetics, ketamine (IC_{50} 10 μM), xylazine (IC_{50} 12 nM) and Althesin (IC_{50} 8 μM) were capable of inhibiting the transmurally stimulated guinea-pig ileum (Figure 7). Pentobarbitone also exhibited activity at high doses and this activity was not antagonized by naloxone. Naloxone (0.5 μM) produced a slight shift in the ketamine dose-response curve increasing the IC_{50} to 17 μM; xylazine and Althesin were not antagonized by naloxone. The solubilizing agent (polyoxyethylated castor oil) present in Althesin was tested on both the guinea-pig ileum and on binding studies, and was found to be responsible for all the activity shown by Althesin on these test systems.

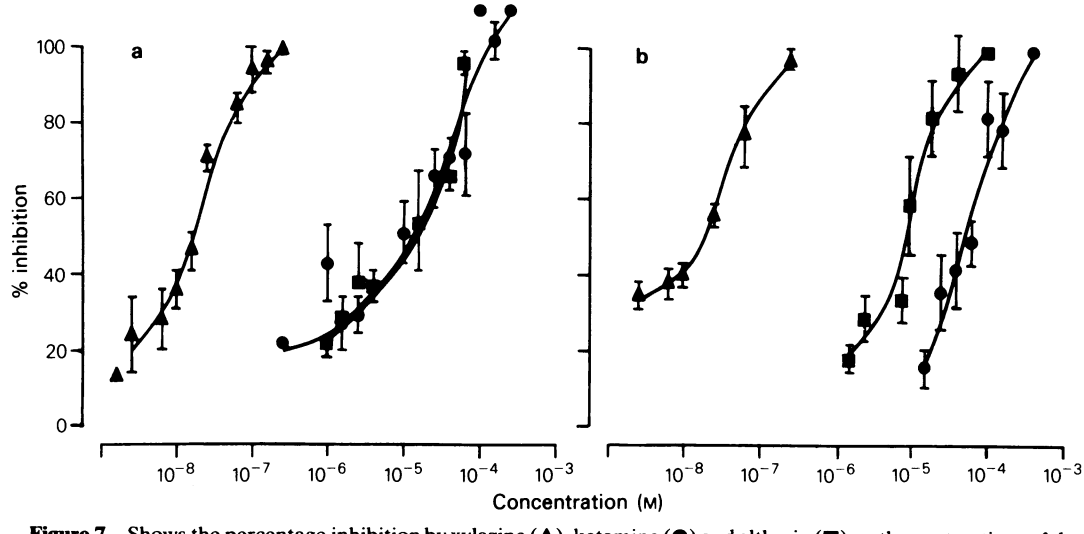

Figure 7 Shows the percentage inhibition by xylazine (▲), ketamine (●) and althesin (■) on the contractions of the transmurally stimulated guinea pig ileum in the absence (a) and presence (b) of naloxone 0.5 μM. Vertical lines represent s.e.mean of 4 experiments.

Discusion

In this study, naloxone antagonized the analgesic actions of nitrous oxide, confirming the results of Finck *et al.*, (1977). Although the inhalation anaesthetics halothane and diethylether caused analgesia this was not significantly reduced by naloxone. The unconventional method of administering these agents will have altered their distributional and kinetic characteristics and for this reason we cannot exclude the possibility that the action of these anaesthetics may be naloxone-sensitive under other conditions.

The structure of inhalation anaesthetic agents makes it unlikely that they would have direct opiate receptor activity. This was confirmed by the lack of effect of all three inhalation anaesthetics on specific [^{3}H]-dihydromorphine binding. Teschemacker & Haarmann (1977) concluded from the fact that general anaesthetics decreased the binding of [^{3}H]-naloxone and increased [^{3}H]-etorphine binding that anaesthetics may alter the conformation of the opiate receptor in favour of agonist binding. There was no evidence for increased dihydromorphine binding in the presence of any anaesthetic agent used in this study. Likewise, the inhibitions of the guinea-pig ileum produced by halothane and diethylether were due to non-specific effects rather than a direct action on opiate receptors. This was confirmed by the lack of naloxone antagonism and the high concentrations required to produce an inhibition.

Inhalational anaesthetics would appear to have a naloxone-sensitive analgesic action which is not due to a direct modifying action on the opiate receptor. While naloxone antagonism is regarded as a necessary criterion for opiate interaction, this alone is not sufficient to infer a narcotic mechanism (Sawynok, Pinsky & LaBella, 1979). By way of support for an opiate action in anaesthetically induced analgesia, Berkowitz, Finck, Hynes & Ngai (1979) have shown partial cross tolerance between nitrous oxide and morphine in rats. These investigators propose that nitrous oxide releases endogenous opiate peptides as part of its analgesic actions.

With the intravenous anaesthetic agents, a complicated picture emerged from this study. Pentobarbitone and Althesin both failed to produce analgesia. Xylazine and ketamine, in contrast, produced analgesia even in subanaesthetic doses and the ketamine effect was naloxone-sensitive. The antagonsim of the analgesia by naloxone would not appear to be due to the lowering of the anaesthetic level since no reduction in the anaesthetic duration was produced by naloxone premedication.

Binding studies showed that ketamine has greater ability in displacing the μ-receptor agonist dihydromorphine than the δ-receptor agonist D-Ala$_2$-enkephalin (D-Leu). Xylazine had equal potency in displacing both types of receptor binding. These two agents would appear to have direct effects on opiate receptors at concentrations which are achieved at normal anaesthetic doses.

Further evidence for a direct action came from the agonist activity which was displayed on the guinea-pig ileum. However, the sensitivity of ketamine and xylazine to naloxone antagonism was negligible when compared with the naloxone antagonism of the opioids morphine and Met-enkephalin. The atropine-like actions of ketamine (Maayani, Weinstein, Ben Svi, Cohen & Sokolovsky, 1974) could explain the potency and apparent naloxone-insensitive actions of this agent on the guinea-pig ileum. Xylazine, was outstanding in its ability to depress the guinea-pig ileum, where it was even more potent than the physiological agonist, Met-enkephalin. The anomalous results obtained with Althesin on the guinea-pig ileum and binding studies were, however, found to be wholely due to the action of the vehicle (polyoxyethylated castor oil) on these test systems.

The interpretation of the different agonist potencies of these anaesthetics on the various opiate test systems could lead to some interesting speculation with regard to their affinities for the different binding sites which have been reported for opiate-like compounds. From structure-activity relationships carried out by Kosterlitz & Waterfield (1975), it would be reasonable to predict that the compounds ketamine and xylazine could have opiate activity. However, further work is necessary to explain the contribution of this activity to the discrepancies observed between anaesthetic agents in producing analgesia.

D.L. is an M.R.C. scholar.

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(Received September 8, 1980.
Revised December 8, 1980.)