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EVIDENCE FOR AN ACTION OF MORPHINE AND THE ENKEPHALINS ON SENSORY NERVE ENDINGS IN THE MOUSE PERITONEUM

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1 A modification of the abdominal constriction test in mice has been developed, and used to study the antinociceptive effects of morphine and several related drugs. In most experiments, acetic acid (0.6% i.p.) was used as the nociceptive stimulus, and in a few cases, acetylcholine (3.2 mg/kg i.p.) was used. When the abdominal constriction response had reached a maximum, the drugs under test were given intraperitoneally, and their ability to decrease the number of abdominal constrictions was determined, beginning immediately after its administration. The aim of this study was to investigate the possibility that morphine and its congeners may produce an antinociceptive effect by an action within the peritoneum.

2 It was found that morphine was an extremely potent antinociceptive agent in this modified test, with an ID_{50} of 5.4×10^{-9} mol/kg (1.54 μg/kg). Codeine and pentazocine were about 40 times less active and oxymorphine was about twice as potent as morphine. Met- and Leu-enkephalin were also potent but their action diminished very rapidly with time. Ketocyclazocine was the most potent substance tested, and had an ID_{50} value of 1.26×10^{-10} mol/kg (0.036 μg/kg). All the drugs tested produced their maximal effect within 1 or 2 min of administration.

3 Pretreatment of the mice with naloxone caused a dose-dependent shift to the right of the dose-response curve to morphine. The pAx plot was linear over part of the range, with a slope of − 1.02 and the 'apparent pA_2' value was 6.14. Naloxone was much less effective in antagonizing Met-enkephalin, and caused a slight potentiation of ketocyclazocine and pentazocine and of cocaine, which was used for comparison.

4 Pretreatment of mice with morphine, 3 h earlier, caused a marked tolerance to a subsequent dose of morphine, and a potentiation of the antagonist potency of naloxone. However, there was little cross-tolerance between morphine and Leu-enkephalin.

5 It is concluded that morphine and its congeners can produce an antinociceptive effect by an action within the mouse peritoneum, presumably by interacting with one or more types of opioid receptors which may be situated on sensory nerve endings.

Introduction

In 1977, Bentley, Copeland & Starr used the abdominal constriction test in mice to study the antinociceptive effects of various α-adrenoceptor agonist drugs and to compare these effects with that of morphine. They concluded that some of the α-adrenoceptor agonists produced their antinociceptive effects by an action within the peritoneum. The drugs tested in this earlier study were given by subcutaneous injection and, thus, would be partly subject to detoxification processes and excretion before reaching their postulated site of action in the peritoneum. In order to reduce these interfering effects and to obtain a more accurate estimate of the relative potencies of the different substances, the antinociceptive test has been modified so that the test drugs are given by intraperitoneal injection after the constriction response has reached a maximum. In the present study, results from this modified method are described which indicate that morphine and several related substances, as well as Met- and Leu-enkephalins can produce a marked antinociceptive action by an effect within the peritoneum and that this effect involves opioid receptors similar to those which are known to exist in the central nervous system. Some of these results were presented to the Australian Society for Clinical and Experimental Pharmacology, 1979.

Methods

The majority of experiments were conducted on male Balb-C mice, although in a few tests female Balb-C

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and male Swiss mice were used. All these animals were obtained from the Monash University Central Animal House, and were held overnight, with access to food and water, in the departmental animal room. All mice were within the weight range 25–35 g.

For most of the experiments, the antinociceptive test used was a modification of the abdominal constriction test (Siegmund, Cadmus & Lu, 1957; Collier, Dinneen, Johnson & Schneider, 1968) and for most experiments 0.6% acetic acid, 1.0 ml/100 g body weight injected intraperitoneally was used as the noxious stimulus (Koster, Anderson & de Beer, 1959). The test drugs were administered intraperitoneally, 6 min after the acetic acid. By this time, the constriction rate had reached a maximum. The number of constrictions was counted for either four successive 1 min periods, or more usually for two periods of 2 min, beginning immediately after injection of the test drug. The mice were randomised into groups of three and were caged individually for counting the constrictions. Control mice received 0.9% w/v NaCl solution (saline). To reduce effects of diurnal variation in response, all doses (including the control groups) were given in random order. From these data dose-response curves were constructed using results from 18 mice for each point on the curve.

Morphine and the two enkephalins were also tested after intravenous administration. Six min after receiving the acetic acid injection, the mice were placed in a small cage to restrain movement, and the antinociceptive drugs were given intravenously in a volume of 0.2 ml. Control mice received saline. The number of writhes was then counted for two periods of 2 min, beginning immediately after the intravenous injection. Dose-response curves were not constructed; only one or two doses of the drugs were given to provide an approximate estimate of potency.

In some experiments, acetylcholine 1.76×10^{-5} mol/kg (3.2 mg/kg i.p) was used as the noxious stimulus (Collier *et al.*, 1968). Since this substance induces writhing within a few seconds after its administration, drugs under test were mixed with the acetylcholine solution and given simultaneously with it. The abdominal constrictions were then counted immediately during two successive periods of 2 min.

A few experiments were performed, using the conventional abdominal constriction test, to compare the effects of levorphanol and dextrorphan with the two techniques. The drugs were administered subcutaneously 15 min before the acetic acid, and the number of writhes was counted for two periods of 2 min, beginning 6 min after the acetic acid injection.

When the antagonist drug naloxone was used, it was injected subcutaneously 15 min before the acetic acid. The agonist drugs were then given 6 min after the acetic acid and the procedure outlined above was followed. The control values were obtained from mice which had received the antagonist followed by saline (i.p.), given 6 min after the acetic acid.

The studies on the development of tolerance to morphine were conducted in female Balb-C mice. These were given a priming dose of morphine, 5.0×10^{-8} or 5.0×10^{-6} mol/kg subcutaneously. Three hours later, the mice were randomized into groups of three, and dose-response curves to morphine were constructed as described above, again using 18 mice for each point on the curves.

Where cross-tolerance between morphine and the enkephalins was studied, male Balb-C mice were used. These were given a priming dose of 5.0×10^{-6} mol/kg morphine (s.c.) and 3 h later dose-response curves were constructed to Leu- and Met-enkephalin given (i.p.) 6 min after the acetic acid. ID_{50} values were calculated from figures from the second 2 min period.

In experiments on the change of potency of naloxone following pretreatment with morphine, again female Balb-C mice were used. Dose-response curves to morphine alone, and in the presence of naloxone, 3×10^{-6} mol/kg subcutaneously were constructed in mice pretreated 3 h previously with either saline, or morphine 5×10^{-8} mol/kg. The naloxone was given 15 min before the test doses of morphine. The dose-ratios generated by naloxone in saline- and morphine-pretreated mice were determined.

Calculation of results

For each 1 or 2 min period, the mean number of constrictions for the appropriate control group of mice was calculated. The number of constrictions of each mouse in the three drug-treated groups was subtracted from this mean control value, thus providing figures showing the reduction in constriction caused by the drug treatment. Using a method based on linear regression as described by Colquhoun (1971), with the aid of a computer program, the ID_{50} values and their 95% confidence limits were calculated for each drug used and a dose-response curve plotted.

When naloxone was used, the control value was calculated from values obtained from mice which received the antagonist drug subcutaneously followed by acetic acid and then saline intraperitoneally. Values obtained with mice receiving the agonist drugs were used as described above and dose-response curves were plotted. These data were subjected to a 3-point, 1-way Anova analysis, again using a computer program, to provide potency ratios together with their 95% confidence limits.

Drugs

The following drugs were used: acetylcholine chloride (Sigma), atropine sulphate (Koch Light),

Table 1 Antinociceptive potency of morphine and other related compounds, and lignocaine and cocaine, estimated at successive 1 or 2 min intervals following their intraperitoneal administration

Drug	*ID_{50} with 95% confidence limits (mol/kg)*			
	1st min	2nd min	3rd min	4th min
Morphine	4.94(4.55–5.29) $\times 10^{-9}$	5.88(5.43–6.41) $\times 10^{-9}$	6.16(5.57–6.65) $\times 10^{-9}$	5.29(4.90–5.67) $\times 10^{-9}$
Morphine (Balb C, female)	–	5.50(5.11–5.99) $\times 10^{-9}$	–	6.09(5.50–6.69) $\times 10^{-9}$
Morphine (Swiss, male)	–	5.29(5.08–5.53) $\times 10^{-9}$	–	5.04(4.83–5.67) $\times 10^{-9}$
Normorphine	–	1.28(1.24–1.35) $\times 10^{-8}$	–	1.32(1.26–1.37) $\times 10^{-8}$
Codeine	–	1.95(1.85–2.08) $\times 10^{-7}$	–	1.94(1.82–2.08) $\times 10^{-7}$
Oxymorphone	–	2.59(2.46–2.72) $\times 10^{-9}$	–	2.49(2.36–2.65) $\times 10^{-9}$
Levorphanol	–	2.06(1.94–2.21) $\times 10^{-9}$	–	2.36(2.17–2.58) $\times 10^{-9}$
Dextrorphan	–	1.78(1.69–1.89) $\times 10^{-8}$	–	1.82(1.73–1.91) $\times 10^{-8}$
Ketocyclazocine	–	1.32(1.25–1.39) $\times 10^{-10}$	–	1.28(1.20–1.36) $\times 10^{-10}$
Pentazocine	–	2.29(2.03–2.39) $\times 10^{-7}$	–	2.47(2.37–2.58) $\times 10^{-7}$
Cocaine	–	2.31(2.02–2.71) $\times 10^{-7}$	–	2.25(1.99–2.65) $\times 10^{-7}$
Lignocaine	–	1.61(1.50–1.72) $\times 10^{-7}$	–	1.35(1.29–1.42) $\times 10^{-7}$

cocaine hydrochloride (Macfarlan Smith), codeine phosphate (Wellcome), dextrorphan tartrate (Roche), ketocyclazocine hydrochloride (Wyeth), lignocaine base (Astra), levorphanol tartrate (Roche), Leu-enkephalin and Met-enkephalin (Reckitt & Coleman), morphine hydrochloride (T. & H. Smith), naloxone hydrochloride (Endo), normorphine base (Reckitt & Coleman), oxymorphone hydrochloride (Endo), papaverine hydrochloride (Drug Houses of Australia) and pentazocine base (Winthrop).

Stock solutions of the two enkephalins were made up in saline at a concentration of 1×10^{-5} mol/l. Pentazocine and normorphine were dissolved in the minimum amount of 0.1 N HCl, and then diluted with distilled water to give a concentration of 1 mg/ml. All the other drugs were made up in saline at concentrations of 1 mg/ml. These were stored at 4°C, and diluted appropriately with saline immediately before use. All intraperitoneal injections were given in a volume of 0.1 ml per 100 g body weight.

All drug doses are expressed as mol/kg, and as μg/kg, of the free base.

Results

It was found that an intraperitoneal injection of saline given 6 min after the acetic acid injection had no detectable effect on the abdominal constriction response. Nevertheless, in all experiments, mice which had received acetic acid followed by saline (i.p.) were used as controls.

The modified method was found to be very sensitive to the analgesic drugs tested. The ID_{50} of morphine given intraperitoneally, estimated for the first 2 min period, was 5.4×10^{-9} mol/kg (1.54 μg/kg), which is about 500 times less than when estimated by the conventional method. Oxymorphone was about twice as potent as morphine, with an ID_{50} value of 2.6×10^{-9} mol/kg (0.78 μg/kg), while ketocyclazocine was the most potent substance tested (ID_{50} 1.32×10^{-10} mol/kg (0.038 μg/kg)). Normorphine was a little less potent than morphine (ID_{50} 1.29×10^{-8} mol/kg (3.51 μg/kg)), while codeine and pentazocine had about one-fortieth of the potency of morphine, with ID_{50} values of 1.96×10^{-7} mol/kg (58.8 μg/kg) and 2.29×10^{-7}

Table 2 Antinociceptive potencies of Met- and Leu-enkephalin, estimated at three successive 2 min intervals following intraperitoneal administration

Drug	*ID_{50} with 95% confidence limits* (mol/kg)		
	1st 2 min	2nd 2 min	3rd 2 min
Met-enkephalin	$1.59(1.07-2.02) \times 10^{-8}$	$6.29(5.79-6.78) \times 10^{-8}$	$8.02(7.55-8.58) \times 10^{-8}$
Leu-enkephalin	$1.20(0.81-1.62) \times 10^{-8}$	$7.57(7.13-8.03) \times 10^{-8}$	$9.63(8.91-10.46) \times 10^{-8}$

mol/kg (65.4 μg/kg) respectively (Table 1). Met- and Leu-enkephalin were also tested for antinociceptive activity. It was found that both these substances depressed writhing almost immediately following injection but their effect was transient, so that ID_{50} values measured at successive 2 min intervals became progressively larger. During the first 2 min period, the highest dose of both enkephalins often completely suppressed writhing, and the lower doses produced very variable results. By the second 2 min period, the writhing responses were much less variable, and therefore figures for this period are generally used. For Leu-enkephalin, the ID_{50} value estimated for the second 2 min period was 7.57×10^{-8} mol/kg (42.09 μg/kg) and for Met-enkephalin 6.29×10^{-8} mol/kg (36.11 μg/kg) (Table 2).

To examine the stereospecificity of this antinociceptive action, levorphanol and dextrorphan were tested. It was found that levorphanol had an ID_{50} value of 2.06×10^{-9} mol/kg (0.53 μg/kg) while dextrorphan was about 8 times less potent (ID_{50} value 1.79×10^{-8} mol/kg (4.59 μg/kg)). This gave an isomeric dose-ratio of 8.7, which was lower than expected. Therefore the two isomers were compared, by the conventional abdominal constriction method, where the drugs are administered subcutaneously 15 min before the acetic acid. It was found that the ID_{50} for levorphanol was 3.199×10^{-7} mol/kg, and for dextrorphan, 1.046×10^{-5} mol/kg. This gave an isomeric dose-ratio of 29.87.

The most notable finding was the speed at which these substances produced their effect with the modified method, which, in the case of morphine, was maximal in the first minute after administration. Thus the ID_{50} values measured over four successive 1 min periods varied very little from each other, and that for the first minute was actually the lowest. When sufficiently high doses of morphine were given (above 1.4×10^{-8} mol/kg) the writhing was immediately and completely suppressed. With the other drugs, which were measured at successive 2 min intervals, a similar result was found. When acetylcholine was used as the nociceptive stimulus, morphine was mixed with this drug and given simultaneously. The writhes again were counted immediately after the intraperitoneal injection. The ID_{50} value for morphine (first 2 min) was 5.85×10^{-9} mol/kg (1.67 μg/kg). This is not significantly higher than when acetic acid was used as the nociceptive agent, and again emphasizes the great speed with which the drug acts.

An experiment was conducted to determine the duration of the antinociceptive effect of morphine. Mice were pretreated with either a single dose of 5.26×10^{-9} mol/kg (1.5 μg/kg) morphine or saline, (i.p.); 24 min later they were injected with 0.6% acetic acid and 6 min after this, the abdominal constrictions were counted. It was found that there was a considerable suppression of the constriction response in the morphine-treated mice.

Cocaine and lignocaine were also tested by the modified method to compare the effect of local anaesthetics. Both of these substances were considerably less potent than morphine. Cocaine had an ID_{50} value of 2.31×10^{-7} mol/kg (70.0 μg/kg) while lignocaine was somewhat more potent (ID_{50} value 1.60×10^{-7} mol/kg (37.7 μg/kg)).

Both cocaine and lignocaine produced their effects very rapidly, but differed from morphine in having somewhat flatter dose-response curves.

Intravenous administration of drugs

An estimate was made of the potency of morphine and the enkephalins following intravenous injection. It was found that all three drugs produced equally rapid depression of the writhing response when given either by intraperitoneal or intravenous injection but the potency was much lower by the latter route, especially for the enkephalins. Morphine caused a dose-dependent depression of writhing. At 1.33×10^{-6} mol/kg (0.5 mg/kg) intravenously it caused a complete block of the writhing response for the first 2 min period, though this depressant effect diminished in the second 2 min period, when there was only an 84% depression. Morphine at 6.65×10^{-7} mol/kg, caused a 61.6% reduction in writhing, and at 3.33×10^{-7} mol/kg, a 24.6% depression occurred (first 2 min period). Higher doses of the enkephalins were necessary to depress the writhing. Met-enkephalin at 1.55×10^{-5} mol/kg (10 mg/kg) intravenously caused only a 31.5% reduction, and

Leu-enkephalin at 7.88×10^{-6} mol/kg (5mg/kg) caused a 68.8% reduction.

Effects of atropine and papaverine

The possibility existed that the antinociceptive action of the analgesic drugs was due to an ability to block intestinal spasms which might be caused by acetylcholine or acetic acid. Therefore, the effects of atropine and papaverine were tested.

It was found that, when acetic acid was used as the noxious stimulus neither atropine 5.8×10^{-6} mol/kg (1.66 mg/kg) nor papaverine 6.6×10^{-6} mol/kg (2.5 mg/kg) given intraperitoneally 6 min after the acetic acid depressed the writhing response at all. The mean number of writhes in the control saline-treated mice was 9.22 ± 0.28, for the atropine-treated mice, 10.78 ± 0.46, and for the papaverine-treated mice, 9.67 ± 0.47 (s.e.mean, $n = 9$). However, after 1.3×10^{-5} mol/kg (5.0 mg/kg) papaverine, the mean number of writhes was reduced to 3.0 ± 0.29 during the first 2 min period, but by the second 2 min, the mean value had increased to 8.22 ± 0.89. Papaverine 6.7×10^{-6} mol/kg intraperitoneally was also without significant depressant effect when acetylcholine was used as the noxious stimulus. The mean number of writhes for the control mice was 7.1 ± 0.39, and for those treated with papaverine 6.4 ± 0.38. However, atropine 5.8×10^{-6} mol/kg completely prevented the writhing produced by acetylcholine. In addition, it was found that when a dose-response curve to morphine was constructed in mice which had been pretreated with atropine 5.8×10^{-6} mol/kg subcutaneously given 15 min before the acetic acid, the ID_{50} value for morphine was 4.77×10^{-9} mol/kg (1.36 μg/kg) which is only slightly lower than in saline-pretreated mice.

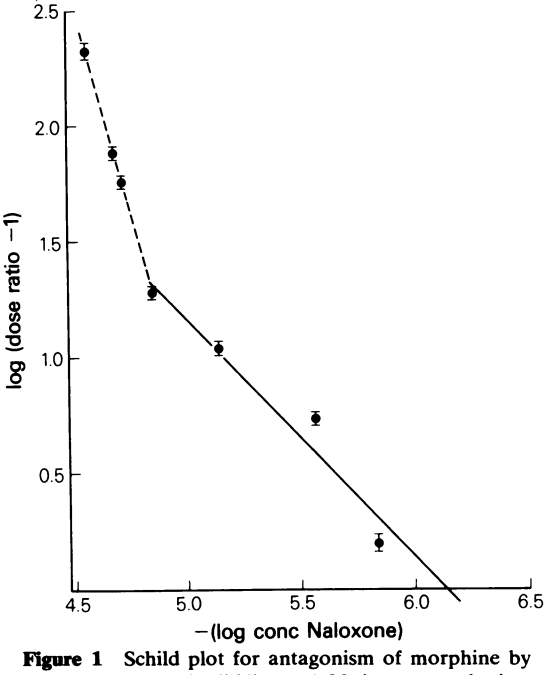

Figure 1 Schild plot for antagonism of morphine by naloxone. Slope of solid line, −1.02; intercepts abscissa to give a pA_2 value of 6.14.

Interaction with naloxone

Mice were pretreated with various doses of naloxone, given subcutaneously 15 min before the acetic acid. Six min after the acetic acid, they were injected intraperitoneally with either saline or morphine and dose-response curves were constructed as described above. It was found that naloxone given to saline-pretreated mice caused no significant change in the writhing response but nevertheless, the values from these mice were always used as the control values. Naloxone caused a dose-dependent shift of the dose-response curves to morphine to the right (Table 3). Analysis of variance showed that none of these curves deviated significantly from parallelism with the control curve obtained with morphine only. When log (dose-ratio −1) was plotted against log concentration of naloxone (Schild plot) it was found that, up to a concentration of 1.5×10^{-5} mol/kg of naloxone a straight line graph was obtained, with a slope of −1.02 and an intercept of 6.14. However, above this dose of naloxone the gradient was apparently steeper. Normorphine was also antagonized by naloxone. Only one single dose of the antagonist was tested (2.94×10^{-5} mol/kg), and this produced a dose-ratio of 15.13, which is close to that obtained with morphine.

In contrast to morphine, the anti-nociceptive effects of pentazocine and ketocyclazocine were not antagonized by naloxone, 1.5×10^{-5} mol/kg but in fact were slightly potentiated, so that the values were now 2.06×10^{-7} mol/kg (58.65 μg/kg) and 1.08×10^{-10} mol/kg (0.031 μg/kg). It is interesting to note that Kosterlitz & Hughes (1975) found that naloxone was a less effective antagonist of the mixed agonist-antagonist drugs such as nalorphine than of morphine. The effect of cocaine also was slightly potentiated by naloxone (3.0×10^{-5} mol/kg) and in the presence of this drug, the ID_{50} value was 1.85×10^{-7} mol/kg (62.97 mg/kg). Met-enkephalin was also antagonized by naloxone; ID_{50} values were estimated during the second 2 min periods, before and after naloxone (1.5×10^{-5} mol/kg s.c.) 15 min before the acetic acid injection. It was found that the ID_{50} value was increased from 6.3×10^{-8} mol/kg (36.11 μg/kg) to 1.4×10^{-7} mol/kg (79.25 μg/kg) after naloxone, and the dose-ratio was calculated to be 2.19, which is considerably lower than with morphine.

Table 3 Dose-ratios to morphine, normorphine, Met-enkephalin, pentazocine, ketocyclazocine and cocaine given intraperitoneally to mice pretreated with various doses of naloxone, given subcutaneously 15 min previously

Agonist drug	*Concentration of naloxone* (mol/kg)	*Dose-ratio with 95% confidence limits*
Morphine	1.48×10^{-6}	2.59(2.43–2.76)
	2.95×10^{-6}	5.09(4.75–5.45)*
	7.36×10^{-6}	11.98(11.31–12.68)
	1.48×10^{-5}	20.44(19.32–21.62)
		19.50(18.35–20.70)*†
	2.0×10^{-5}	58.39(54.41–62.58)
	2.2×10^{-5}	67.83(62.95–72.89)
	2.95×10^{-5}	210.49(199.14–222.54)
Normorphine	1.48×10^{-5}	15.13(14.26–16.05)
Met-enkephalin (2nd 2 min period)	1.48×10^{-5}	2.42(2.21–2.65)
Pentazocine	1.48×10^{-5}	0.81(0.77–0.86)
Ketocyclazocine	2.95×10^{-5}	0.92(0.85–0.996)†
Cocaine	2.95×10^{-5}	0.71(0.67–0.76)†

* Swiss mice

† The dose-response curves for these combinations deviated significantly from parallelism with the control.

Tolerance

For this part of the investigation, female Balb-C mice were used. In these animals the ID_{50} value of morphine was 5.50×10^{-9} mol/kg (1.57 μg/kg) (see Table 1).

Mice were pretreated with a single dose of morphine, either 5×10^{-8}, or 5×10^{-6} mol/kg (s.c.); 3h later, there was no sign of residual analgesia, since mice pretreated in this way produced the same number of abdominal constrictions in response to acetic acid (i.p.) as did control mice. However, when a dose-response curve to morphine was constructed using the pretreated mice, it was found that the ID_{50} value had increased by 29.1 and 1030 times, respectively. Thus a high degree of tolerance was produced following a single dose of morphine.

The effect of pretreatment with morphine on the potency of Leu-enkephalin was also tested. Mice were pretreated with morphine, 5.0×10^{-6} mol/kg (s.c.), 3 h before receiving the acetic acid injection. Leu-enkephalin was given 6 min later, and the ID_{50} value was calculated for the second 2 min period. It was 1.10×10^{-7} mol/kg, which is only 1.45 times greater than in control mice.

The effect of morphine pretreatment on the antagonistic potency of naloxone was also studied. As mentioned above, pretreatment with morphine caused considerable tolerance to this drug, so that the dose-response curve was shifted to the right. Therefore, it was necessary to use mice so treated as controls in the estimation of the potency of naloxone.

It was found that, in saline-pretreated mice, naloxone (3×10^{-6} mol/kg s.c.) given 15 min before the acetic acid injection, increased the ID_{50} value of morphine from 5.5×10^{-9} to 4.8×10^{-8} mol/kg (1.57 to 13.8 μg/kg) i.e. a dose-ratio of 8.8. However, if the mice were pretreated with morphine (5×10^{-8} mol/kg) 3 h before giving the naloxone, it was found that this same dose of naloxone now increased the ID_{50} value of morphine from 1.27×10^{-7} mol/kg (36.2 μg/kg) to 2.2×10^{-5} mol/kg (6.28 mg/kg) a dose-ratio of 173; that is, the potency of naloxone had been increased 19.7 times.

Discussion

The modified abdominal constriction test is remarkably sensitive and reliably detected morphine in doses down to 2.45×10^{-9} mol/kg (0.7 μg/kg). This is a greater than 500 fold increase in sensitivity over the conventional method. Dose-response curves to antinociceptive drugs can be rapidly constructed and ID_{50} values with acceptably small limits of error can be calculated. Codeine and pentazocine were both about 40 times less potent than morphine, while oxymorphine was about twice as potent. Ketocyclazocine was by far the most potent substance tested with an ID_{50} value of 1.26×10^{-10} mol/kg.

The local anaesthetics, cocaine and lignocaine were also active in the microgram dose range with ID_{50} values of 2.31×10^{-7} and 1.60×10^{-7} mol/kg, respectively. The dose-response curves of both substances were somewhat flatter than that of morphine.

It is interesting to note that Luduena (1957) found that lignocaine was about 2.5 times more potent than cocaine on the rabbit spinal cord.

Both Met- and Leu-enkephalin were also potent antinociceptive agents in this test but unlike the other analgesic drugs, their action was very brief, presumably because of rapid breakdown by peptidases in the peritoneal fluid (see Hughes, 1975). This appears to be the first time that significant antinociceptive activity has been reported for these substances when given other than directly into the central nervous system.

Morphine and the two enkephalins were also tested after intravenous administration. By this route, morphine was about 100 times less effective than after intraperitoneal injection and the enkephalins showed an even greater reduction in potency.

It seems likely that the analgesic drugs produced their antinociceptive effects by an action within the peritoneum, for two reasons. First, the maximal effect is seen within 1 to 2 min following injection of the drug; it seems improbable that the drug could have diffused into the central nervous system and reached equilibrium in this short interval. Secondly, the drugs produced their antinociceptive effects at doses far too low to be effective when given by subcutaneous or even intravenous injection. It is therefore, suggested that the site of action may be on sensory nerve endings within the peritoneum, where possibly the generation of the pain impulses may be depressed by the drugs. Ferreria (1978) showed that when microgram doses of morphine were injected into a rat paw previously made hyperalgesic by pretreatment with prostaglandin E_2 (PGE_2), there was a marked reduction in the hyperalgesia. This antinociceptive effect lasted for 2.5 h. Met-enkephalin was about five times less potent than morphine and had a shorter duration of action, while Leu-enkephalin had about one-tenth of the potency of morphine. Surprisingly, Ferreira (1978) found that naloxone also showed antinociceptive activity.

The mechanism of action of the analgesic drugs in the mouse peritoneum is not entirely clear. It is unlikely to be due to a conventional local anaesthetic action, since naloxone is such as effective antagonist of morphine but causes a slight potentiation of cocaine. An anti-spasmodic action also seems unlikely, since atropine and papaverine, at doses about 1000 times higher than the ID_{50} for morphine did not depress the abdominal constriction response at all. Also, in mice pretreated with atropine 5.8×10^{-6} mol/kg, the antinociceptive potency of morphine was only slightly enhanced. It also seems unlikely that morphine acts to suppress the production of the noxious substances which are believed to be released by acetic acid (Collier *et al.*, 1968) since, when acetylcholine was used as the nociceptive stimulus, the ID_{50} value of morphine was not significantly different from that obtained with acetic acid.

It seems probable, therefore, that morphine and its congeners act via a receptor mechanism and this suggestion is supported by several observations. First, naloxone caused a dose-dependent parallel shift to the right of the dose-response curve of morphine. A Schild plot gave a straight line with a slope of -1.02 with an 'apparent pA_2' value of 6.14. Secondly, pretreatment with a single subcutaneous injection of morphine caused the appearance of a very considerable degree of tolerance to a subsequent intraperitoneal injection of morphine. Thirdly, pretreatment with a single dose of morphine caused a marked increase in the antagonistic potency of naloxone, measured 3 h later. This is presumably the same phenomenon reported by Tulunay & Takemori (1974) who used the conventional abdominal constriction test. Fourthly, a degree of stereospecificity was observed, with levorphanol being about 8 times more potent than dextrorphan, though this differential is less than when the conventional abdominal constriction method is used.

This suggestion of an opioid receptor in peripheral tissue, possibly on sensory nerve endings, is consistent with the work of Ferreira (1978), who postulated the presence in the rat paw of an opioid receptor. However, because of the unexpected effect of naloxone in his experiments, he believed this peripheral receptor to be different from that in the central nervous system. Since the present study demonstrated that naloxone acts as an antagonist in the mouse peritoneum, there must also be some difference between the receptor postulated by Ferreira, and that in the mouse peritoneum. It seems likely too that the receptors in the peritoneum differ from those in the central nervous system.

The exact nature of the receptors involved in these antinociceptive effects is not entirely clear, but there is reason to believe that more than one type is present (Lord, Waterfield, Hughes & Kosterlitz, 1977). The very high activity of ketocyclazocine, a specific agonist for κ-receptors (Martin, Eades, Thompson, Huppler & Gilbert (1976)) strongly suggests that these receptors are present. Naloxone showed no antagonist activity whatsoever against ketocyclazocine, nor against pentazocine, which is also a mixed agonist-antagonist. Kosterlitz, Lord & Watt (1972) have previously shown that the antagonist potency of naloxone is lower against the agonist actions of compounds with dual agonist-antagonist actions than against morphine. The two enkephalins were considerably less potent than morphine, though because they are so unstable *in vivo* (Hughes 1975) the ID_{50} values quoted in this study certainly underestimate their true activity. It was also found that naloxone was much less effective in antagonizing Met-enkephalin than morphine, which is in agreement with the finding of Kosterlitz & Hughes (1975). This, together with the finding that there was negligible

cross-tolerance between morphine and Leu-enkephalin suggest the presence of specific receptors to the enkephalins. However, Waterfield, Hughes & Kosterlitz (1976), who used isolated organs, observed a complete cross-tolerance between morphine and Met-enkephalin; therefore it may be that atypical δ-receptors are present in the mouse peritoneum. On the other hand, Büscher, Hill, Römer, Cardinaux, Closse, Hauser & Plesse (1976) suggested that Leu-enkephalin acted as a mixed agonist-antagonist, so it is possible that such a compound would have less action on a typical μ-receptor than morphine, and therefore might show less cross-tolerance.

Morphine in the present study, was a very potent antinociceptive agent, and normorphine was only a little less active. These substances have a high affinity for the μ-receptor in the guinea-pig isolated ileum (Waterfield, Smokcum, Hughes, Kosterlitz & Henderson, 1977), and the present findings suggest the presence of a similar receptor in the mouse peritoneum. However, the pA_2 value calculated for naloxone in the present study (6.14) is an order of magnitude lower than that reported by Hayashi & Takemori (1971), who used the conventional writhing technique. This could indicate that the receptors are not typical μ-receptors, although, if more than one type of receptor was present in the mouse peritoneum, one of which had a low affinity for naloxone, the lower pA_2 value might be expected. The inflection in the Schild plot also seems consistent with the possibility of multiple opioid receptors.

It is not clear whether these receptors play any part in the antinociceptive effects of analgesic drugs given by other than the intraperitoneal route, or whether they have any normal physiological function. The observation that intravenous morphine acts so rapidly may suggest that even when given by this route, the drug reaches the receptors within the peritoneum, although larger doses are necessary than when intraperitoneal administration is used. It seems probable, however, that the receptors might respond to circulating endorphins or enkephalins (Clement-Jones, Lowry, Rees & Besser, 1980). Irrespective of this, the technique described provides a new method of studying the properties of opioid receptors *in vivo* under conditions where barriers to diffusion of drugs and metabolic interference are minimal. Further work is at present under way to investigate the interactions of opioids, α-adrenoceptor agonists, and α-antagonists on these receptors.

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