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EFFECT OF MORPHINE ON ADRENERGIC TRANSMISSION IN THE MOUSE VAS DEFERENS: ASSESSMENT OF AGONIST AND ANTAGONIST POTENCIES OF NARCOTIC ANALGESICS

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1 Morphine inhibits the electrically evoked (0.1-0.15 Hz, 1 ms) contractions of the longitudinal muscle of the mouse vas deferens but not of the rabbit, guinea-pig, rat, cat, hamster or gerbil. This effect is stereospecific and is antagonized by naloxone or naltrexone.

2 Normorphine is equiactive with morphine but its effects are more rapid in onset and decline.

3 In the mouse vas deferens, the resting outflow of tritium-labelled catecholamines is unaffected by morphine. The electrically evoked outflow is depressed by morphine or normorphine in a dose-dependent manner. The ID_{50} for inhibition of contraction and for depression of outflow is 0.5 μM.

4 The relative agonist potencies of compounds without antagonist component (codeine, pethidine, morphine, normorphine, heroin, levorphanol, Ba-20227, etorphine) show good correlation with the relative agonist potencies determined in the guinea-pig ileum and for analgesia in man.

5 For compounds with dual agonist and antagonist properties, the dose-response curves for agonist activity are shallow. When the lowest concentrations giving a depression of the contraction of the mouse vas deferens are used, a good correlation is obtained with the guinea-pig ileum.

6 The relative antagonist potencies of naloxone, nalorphine, levallorphan and cyclazocine agree well with those obtained in the guinea-pig ileum; these, in turn, correlate well with the values obtained in the morphine-dependent monkey.

7 The fact that the agonist effects of drugs with dual agonist and antagonist action show little or no dependence on concentration, makes the mouse vas deferens particularly suitable for the assay of antagonist activity.

8 As an assay preparation, the mouse vas deferens is less robust and consistent in its responses than the guinea-pig ileum.

Introduction

Henderson, Hughes & Kosterlitz (1972) have shown that contractions of the mouse vas deferens elicited by stimulation of the intramural nerves are inhibited by low concentrations of morphine. This effect was antagonized by naloxone and thus appeared to be mediated by specific morphine receptors. It was also shown that the inhibition of the contractions was associated with a depression of the evoked noradrenaline output as measured by bioassay. The aims of this paper are, first, to confirm and extend by radiochemical techniques the finding that noradrenaline output is depressed by morphine. Secondly, the mouse vas deferens was examined as a possible model for the prediction of the agonist and antagonist properties of narcotic analgesic drugs and the results compared with the findings obtained on the guinea-pig ileum (Kosterlitz & Watt, 1968; Kosterlitz, Lord & Watt, 1972; Kosterlitz, Waterfield & Berthoud, 1974). Some of the results have been described at a meeting of the British Pharmacological Society (Hughes, Kosterlitz & Leslie, 1974).

Methods

Experimental procedures

Albino mice of the T/O inbred strain, weighing 30-35 g, were killed by stunning or by exposing

them to an atmosphere of CO_2. Both vasa deferentia were dissected out as a single unit connected by the tissue at the junction with the seminal vesicle. They were carefully stripped of adhering fat, connective tissue and blood vessels, and then gently pressed to expel their seminal contents.

For the investigation of the relationship between contraction and noradrenaline output and the effect of drugs on this relationship, five pairs of vasa were mounted in an organ bath of 3 ml (Hughes, 1972). When contractions only were examined, one pair of vasa was used. Longitudinal contractions were recorded by attaching the upper end of the preparation to a light spring connected to an isometric transducer. The tissues were bathed in modified Krebs solution containing ascorbic acid (0.1 mM) and sodium edetate (0.027 mM), gassed with 95% O_2 and 5% CO_2. A tension of 100-150 mg was applied to single preparations and 200-300 mg to multiple preparations. The intramural nerves were stimulated with rectilinear pulses (1 ms duration, supramaximal voltage) passing between a platinum point source at the bottom and a ring (10 mm wide) of platinum foil fixed to the top of the organ bath.

For the assay of agonist and antagonist potencies isotonic contractions were recorded by means of a photoelectric transducer. Only a single vas deferens was used and the tension was 100 mg. The organ bath and the electrode arrangements were similar to those already described but the Krebs solution did not contain ascorbic acid and sodium edetate.

Estimation of noradrenaline output

The tissues were incubated at 35° C, either for 45 min in Krebs solution containing (−)-[7-^{3}H]-noradrenaline (100 ng/ml, 40 μCi/μg = 6.8 Ci/mmol) or for 2 h in Krebs solution containing L-[3,5-^{3}H]-tyrosine (0.25 μg/ml, 221 μCi/μg = 40 Ci/mmol). After incubation, the tissues were rinsed for 30 min in fresh Krebs solution, changed every 5 minutes. They were then mounted in the organ bath as described above and washed for a further hour. In each experiment a resting sample of the total bathing fluid was collected for 4 min prior to a stimulation period of 4 min at 0.25 Hz. The catecholamines and their deaminated metabolites were isolated on short alumina columns (Boadle-Biber, Hughes & Roth, 1970); the columns were eluted with 1.5 ml of 0.15 M perchloric acid. The radioactivity was estimated in 0.5 ml of the eluate by liquid scintillation spectrometry. The results were corrected for a column recovery of 72%.

Assessment of agonist and antagonist potencies of narcotic analgesics

The method was similar to that used for the guinea-pig ileum (Kosterlitz & Watt, 1968; Kosterlitz *et al.*, 1974). The concentration of the drug which causes 50% inhibition of the evoked longitudinal contraction (ID_{50}) was used to characterize agonist activity and the dissociation or equilibrium constant (K_e) to characterize antagonist activity.

Determination of ID_{50} Values of ID_{50} were determined either from log dose-response curves or more often by the 'single dose' method. When the latter method was used, the preparation was set up and stimulated at 0.1 Hz for 20 min, the bath fluid being renewed several times. When the size of the contractions had become constant, two successive dose-response curves for normorphine were constructed, the exposure time to reach maximum effect being 60-90 s and the dose cycle 7 minutes. Fifteen minutes after the last dose of normorphine had been washed out, the compound to be tested was added. The dose varied according to its agonist activity; an amount was chosen to give a depression of the contraction of 20-40%.

If the slope of the dose-response curve of the compound under test was similar to that of normorphine, the value of ID_{50} was obtained by extrapolation. While in the guinea-pig ileum this method was used for agonists and for compounds with dual agonist and antagonist activities, this was not possible in the mouse vas deferens. As will be shown in the results section, the dose-response curves of the great majority of compounds with dual action is very shallow so that extrapolation is not permissible. To obtain a measure of the agonist potencies of these compounds, the lowest concentration of drug was found which gave a depression of about 20%. The concentration was then compared to the concentration of normorphine which gave the same depression and the potency ratio, nM normorphine/nM compound X, calculated.

Determination of K_e The value of K_e was obtained from $K_e = a/(DR-1)$, where a is the concentration (nM) of the compound to be tested and DR the dose-ratio, determined as follows (Figure 1). After the preparation had been exposed to the test compound as described for the 'single dose' method for the determination of ID_{50}, the concentration of normorphine (N_1) that would have caused the same depression was read off the mean of the second and third dose-response curves for normorphine obtained at the beginning and end of the experiment, respectively.

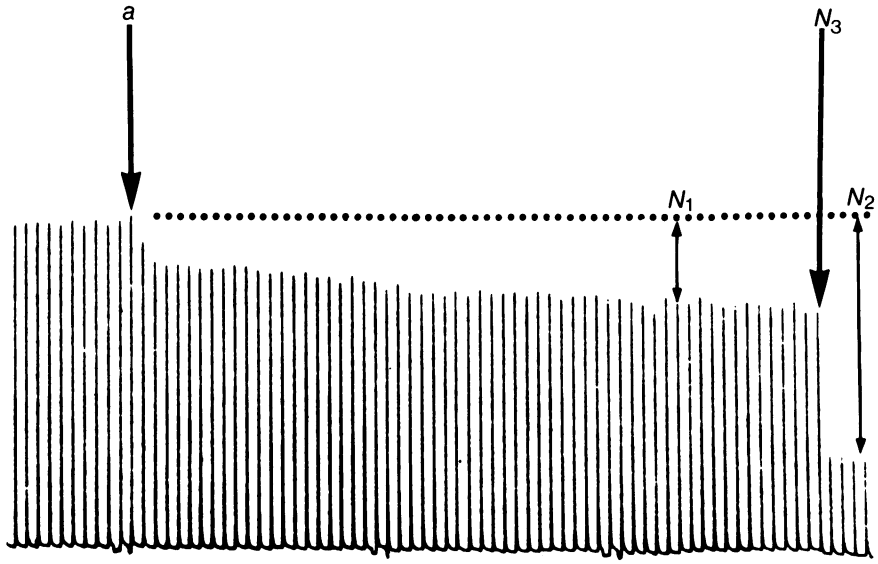

Figure 1 Measurement of the antagonist activity of a compound with dual agonist and antagonist actions, by determination of its equilibrium constant, K_e. Mouse isolated vas deferens. Isotonic recording of the longitudinal contractions induced by supramaximal electrical field stimulation at 0.1 Hz. The test drug was added at the arrow marked *a* and produced a depression of the twitch equal to a depression caused by normorphine in concentration N_1. After exposure of 10 min, normorphine was added to give a concentration of N_3; the total depression was equal to a reduction in the size of the twitch which would have been caused by normorphine in a concentration N_2 sr, in the absence of the test drug. $DR = N_3/(N_2 - N_1)$; $K_e = a/(DR-1)$.

After the vas deferens had been exposed to the test drug for 5-10 min normorphine was added to the bath to give a concentration of N_3. The normorphine concentration (N_2) which would have a depressant effect equal to the combined actions of the test drug and the normorphine present in the bathing fluid was read off the mean dose-response curves for normorphine. The test drug was then washed out and normorphine added at intervals of 7 min until the sensitivity of the vas deferens to normorphine had returned to near its original level. If there was a change in sensitivity of more than 30%, the experiment was discarded. Finally, a third dose-response curve for normorphine was constructed. The values for N_1 and N_2 were then extrapolated from the mean of the second and third dose-response curves. The dose-ratio was then $DR = N_3/(N_2 - N_1)$. N_3 should reduce the residual contraction by at least 20% and the total inhibition should not be more than 80%.

Solutions and drugs

The composition of the modified Krebs solution was (mM): NaCl 118, KCl 4.75, $CaCl_2$ 2.54, KH_2PO_4 0.93, $NaHCO_3$ 25, glucose 11.

The tritiated compounds, (−)-[7-^{3}H]-noradrenaline and L-[3,5-^{3}H]-tyrosine (Radiochemical Centre, Amersham) were purified on alumina columns before use.

The following drugs were used: adenosine triphosphate (Sigma); Ba-20227 (1-diethylaminoethyl-2-*p*-methoxybenzyl-5-nitro-benzimidazole hydrochloride, Ciba); codeine phosphate (B.P.); (−)-cyclazocine ((−)-α-2-cyclopropylmethyl-5,9-dimethyl-2′-hydroxy-6,7-benzomorphan, Sterling-Winthrop); (−)-cyclorphan hydrochloride ((−)-N-cyclopropylmethyl-3-hydroxy-morphinan, Dr M.D. Gates); dextrorphan tartrate (Roche Products); etorphine hydrochloride (Reckitt & Colman); dopamine hydrochloride (Koch-Light); heroin (diamorphine hydrochloride, B.P.); hexamethonium bromide (May & Baker); hyoscine hydrobromide (B.P.); levallorphan tartrate and levorphanol tartrate (Roche Products); lysergic acid diethylamide (Sandoz); morphine hydrochloride (B.P.); nalorphine hydrobromide (Burroughs Wellcome); naloxone hydrochloride and naltrexone hydrochloride (N-cyclopropylmethylnoroxymorphone) (Endo Laboratories); noradrenaline bitartrate (Winthrop); normorphine hydrochloride (Dr E.L. May); pethidine hydro-

chloride (Roche Products); phentolamine hydrochloride (Ciba); prostaglandin E_2 (Upjohn).

The stock solutions of the drugs were made in distilled water and that of cyclazocine in water plus the calculated amount of HCl. The concentrations are given as nM, μM or mM.

Results

Effects of narcotic analgesics on contractor responses to nerve stimulation

Morphine inhibited the contractions of the mouse vas deferens but no such action could be discerned in preparations from the rabbit, guinea-pig, rat, cat, hamster or gerbil. The effect was best seen when the mouse vas deferens was continuously stimulated at 0.1 or 0.15 Hz and the bath fluid was Krebs solution without magnesium ions. In Krebs solution containing 1.4 mM Mg^{++} the size of the contractions was only 40-60% of that in Mg^{++}-free solution; more importantly, the responses to morphine and other agonists were not consistent since the twitch tended to decline after repeated exposures to the drugs even when the tissues were washed vigorously between exposures. Dose-dependent inhibitions of the twitch were obtained with a series of narcotic analgesic agonists. Normorphine was used as reference because its action was rapid in onset and decline and it could be given at 5 min intervals without any apparent tachyphylaxis (Figure 2a,b). Morphine and the other agonists investigated had a slower onset and decline of action and the dose-cycle had to be lengthened to 15 min to avoid the development of tachyphylaxis. Naloxone (10-500 nM), which *in vivo* and *in vitro* is an antagonist without significant agonist activity (Blumberg & Dayton, 1974; Kosterlitz *et al.*, 1974), had no effect of its own on the twitch but reversed the inhibitory effects of the agonists (Figure 2b). Naltrexone, the N-cyclopropylmethyl analogue of naloxone, which has very weak agonist activity but as an antagonist is 3-4 times more potent than naloxone (Blumberg & Dayton, 1974; Kosterlitz *et al.*, 1974), completely antagonized the inhibitory effects of morphine and normorphine in the mouse vas deferens.

Table 1 shows the concentrations of various narcotic agonists required to depress the twitch by 50%; these ID_{50} values were calculated from dose-response curves obtained as shown in Figure 2. In each experiment at least two dose-response curves were obtained for the test drug, interspersed with dose-response curves for either morphine or normorphine. From the mean

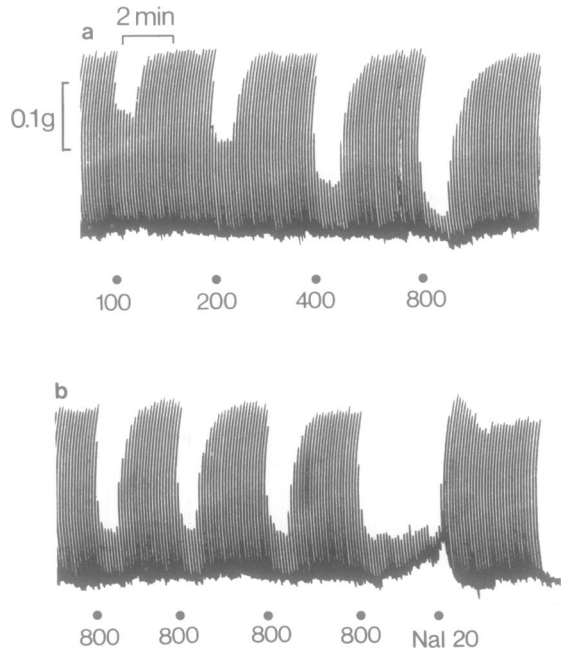

Figure 2 Mouse vas deferens. Effects of normorphine and naloxone on the longitudinal contractions evoked by electrical field stimulation (1 ms, 0.15 Hz, supramaximal voltage). (a) Effects of different concentration of normorphine (nM). (b) Absence of tachyphylaxis to normorphine (800 nM) and reversal by naloxone (Nal, 20 nM). Calibrations: tension, 50 mg; time, 2 minutes.

values for morphine and normorphine it follows that the mouse vas deferens is 6-7 times less sensitive than the guinea-pig ileum. The depressant effects of the narcotic agonists are stereospecific; it was found that the (+)-isomer of 3-hydroxy-*N*-methylmorphinan, dextrorphan, had only 0.1% of the potency of the (−)-isomer, levorphanol. The rank order of potency in the mouse vas deferens is in good agreement with that in the guinea-pig ileum; additional evidence in this respect will be given below.

It should be noted that the strength of the electrical stimulation markedly influences the ID_{50} values of the narcotic agonists. When the current was reduced from a just supramaximal strength of 100 mA to 30 mA, the ID_{50} value for normorphine was reduced from 440 ± 42 nM to 120 ± 30 nM.

A number of compounds unrelated to narcotic analgesic drugs were investigated in order to confirm that the naloxone-induced reversal of the narcotic agonist action was indeed specific.

Adenosine triphosphate (ID_{50}, 150 mM), noradrenaline (ID_{50}, 10 μM), dopamine (ID_{50}, 3 μM), prostaglandin E_2 (ID_{50}, 10 μM), and lysergic acid diethylamide (ID_{50}, 0.14 μM) all inhibited the contractions elicited by electrical stimulation but these effects were not reversed by naloxone in concentrations (10-500 nM) that readily antagonized the inhibitory actions of the narcotic agonists.

Treatment of the vas deferens with phentolamine (30 μM) increased the twitch height by 30-80% and abolished the inhibitory actions of noradrenaline and lysergic acid diethylamide but did not reduce the effects of morphine or normorphine.

Effect of narcotic analgesics on noradrenaline output evoked by nerve stimulation

In order to exclude the possibility that ganglionic synapses might play a role in the action of morphine, the following experiments were carried out in the presence of hexamethonium (28 μM). It had previously been established that neither hexamethonium nor hyoscine (2.3-230 μM) affected the contractions elicited by nerve stimulation, nor did they modify the inhibitory effect of morphine on the twitch (Henderson *et al.*, 1972).

The resting outflow of ^{3}H-labelled catecholamines (after incubation with [^{3}H]-tyrosine, 1 expt, or with [^{3}H]-noradrenaline, 2 expts) was unaffected by morphine (1 μM). However, the stimulated output of labelled catecholamines was reversibly depressed by either morphine or normorphine. Figure 3 shows the marked inhibition of output from a vas deferens pre-incubated with [^{3}H]-tyrosine. The effect was readily reversed by washing the morphine out of the bath. When the tissue was treated with naloxone (50 nM) in the absence of morphine, there was no change in the stimulated output and a subsequent addition of morphine to the bath was without effect.

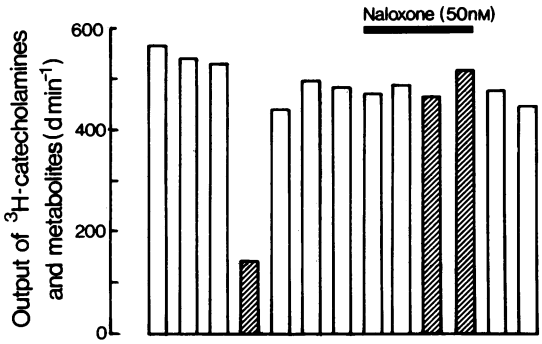

Figure 3 Effect of morphine and naloxone on the output of newly synthesized catecholamines from the mouse vas deferens. The tissues were labelled with [^{3}H]-tyrosine as described in the methods section. Columns represent the output of ^{3}H-catecholamines and their deaminated metabolites after electrical stimulation at 0.25 Hz for 4 min every 15 min; the resting output of the preceding 4 min period has been deducted. Exposure to morphine (800 nM, shaded columns) reduced the output by 73%. Treatment with naloxone (50 nM, horizontal bar) did not affect the output of ^{3}H-catecholamines but the effect of morphine was abolished in the presence of the antagonist.

Table 1 The relative potencies of narcotic analgesic drugs in the mouse vas deferens and the guinea-pig ileum

Drug	*Mouse vas deferens ID_{50} (nM)*		*Relative agonist potencies (morphine or normorphine = 1) Mouse vas deferens*	*Guinea-pig ileum*
Ba-20227	4.3 ± 0.02	(4)	108	53
Levorphanol	168 ± 23	(5)	2.8	7.3
Heroin	350 ± 36	(4)	1.3	2.0
Normorphine	440 ± 42	(9)	1.0	1.0
Morphine	492 ± 53	(7)	1.0	1.0
Pethidine	16,000 ± 3,000	(5)	0.029	0.064
Codeine	55,000 ± 800	(5)	0.008	0.007

The ID_{50} values are the means ± s.e. mean obtained from dose-response curves; the number of observations are given in parentheses. The relative agonist potencies in the guinea-pig ileum are from Kosterlitz & Watt (1968) and Kosterlitz *et al.* (1974). The ID_{50} values for morphine and normorphine do not differ significantly; the combined means are 463 (n = 16) for the mouse vas deferens and 70.5 (n = 12) for the guinea-pig ileum, giving a ratio of 6.5.

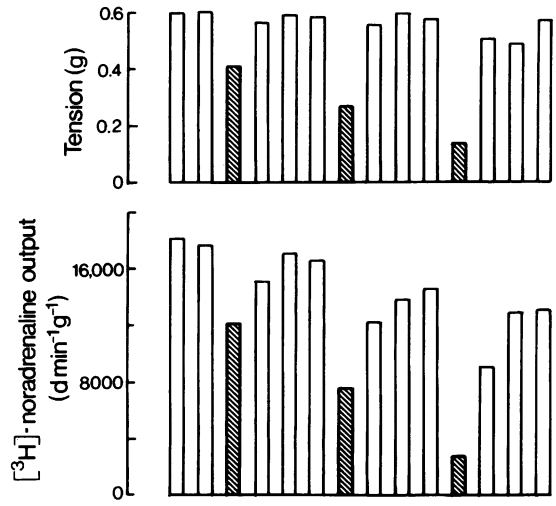

Figure 4 Effect of graded normorphine concentrations on the output of [^{3}H]-noradrenaline and contractions of the mouse vas deferens. The tissues were labelled with [^{3}H]-noradrenaline as described in the methods section. The Krebs solution contained phentolamine (30 μM) throughout the experiment. The upper panel shows the tension developed during stimulation (0.25 Hz for 3 min every 10 minutes). The lower panel shows the output of [^{3}H]-noradrenaline (including deaminated metabolites) during stimulation after deduction of the resting output for the preceding 3 minutes. Normorphine (shaded columns, 300, 600 and 1200 nM) caused a parallel reduction in both the output of [^{3}H]-noradrenaline and in the mechanical response.

The effects of morphine or normorphine on output were related to the dose. Figure 4 shows an experiment in which the vasa deferentia were first incubated with tritiated noradrenaline and then treated with phentolamine (3.1 μM), which increased the stimulated output threefold compared to outputs from untreated tissues and thus made possible the investigation of effects of a range of normorphine doses. Normorphine (300, 600 and 1200 nM) reduced the output by 35%, 60% and 80%, respectively, and the contractions by 32%, 53% and 76%. From four of these experiments it was calculated that the ID_{50} for inhibition of the contraction by normorphine was 510 ± 30 nM whilst the ID_{50} for inhibition of the output was 465 ± 58 nM. The difference between these values was not significant.

Assessment of agonist potency of compounds with dual agonist and antagonist actions

No difficulties were encountered in the assessment of the agonist potencies of compounds without antagonist component, either by dose-response curves (Table 1) or by the 'single dose' method.

As far as compounds with dual agonist and antagonist actions are concerned, their agonist potencies can be readily assessed in the guinea-pig ileum because the dose-response curve of their depressant action on the contractile responses has a slope near to that of agonists without antagonist component (Kosterlitz & Watt, 1968). Only when the agonist activity is small in comparison to the antagonist activity, is the dose response shallow and an estimate of the ID_{50} value becomes impossible (Kosterlitz *et al.*, 1972; 1974).

However, in the mouse vas deferens, all compounds which show antagonist activity have shallow dose-response curves for their agonist action. An example is given in Figure 5, showing dose-response curves for levorphanol and its N-allyl analogue, levallorphan. In an attempt to improve the slope, the interval between doses was increased from 7-40 min (Figure 5b) but without effect. It follows from these findings that the agonist potency of levallorphan relative to levorphanol was at its maximum when a low concentration was used. Therefore, with a compound with dual agonist and antagonist action, the lowest concentration was determined which gave a depression of about 20% of the evoked contraction. For the calculation of the relative agonist potency, the value obtained was related to the equiactive concentration of normorphine read from a dose-response curve. The values obtained agree within a factor of 1.5-3 with those found in the guinea-pig ileum (Table 2).

When the values of the relative agonist potencies of compounds with or without antagonist component were related to the relative potencies obtained in the guinea-pig ileum, the correlation coefficient was $r = 0.967$ ($n = 12$) and the regression line $y = 2.25x - 33.8$; the mean value of the ratios, relative potency in the vas deferens/relative potency in the ileum, did not differ significantly from unity (1.08 ± 0.21, $n = 12$). To assess the significance of the deviation of the slope from unity and of the constant from zero, it was necessary to establish whether or not the standard deviations of the potencies differed significantly over the wide range examined. Analysis showed that the standard deviations were closely correlated to the potencies ($r = 0.995$ for the ileum and $r = 0.997$ for the vas deferens). Since the ratios, standard deviation/potency, were not significantly correlated to the potencies ($r = 0.349$ for the ileum and $r = 0.142$ for the vas deferens), it was concluded that a logarithmic transformation would be suitable. This treatment would also give more equal weight to the widely

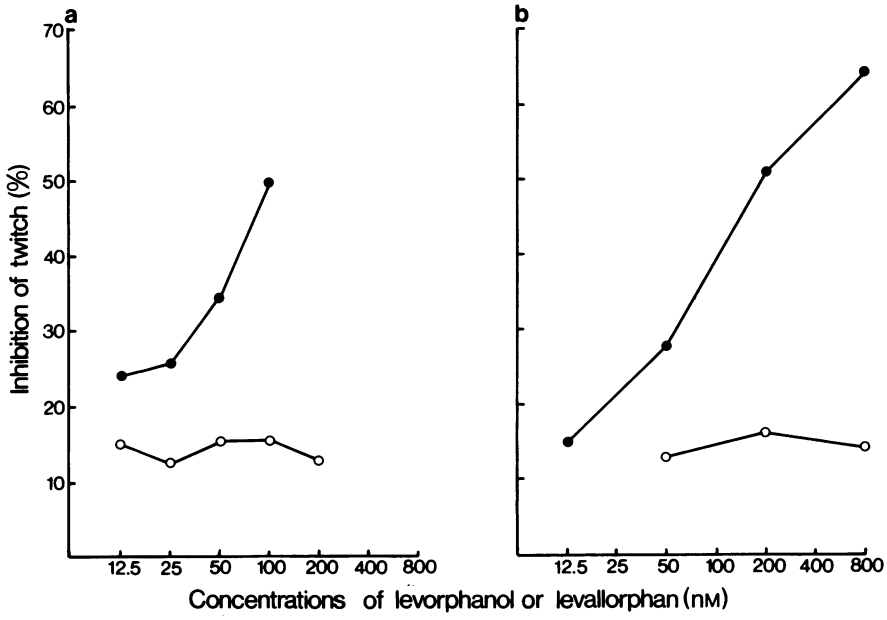

Figure 5 Dose-response curves for the inhibition of the evoked longitudinal contraction of the mouse vas deferens. Abscissae, concentration of levorphanol (•) or levallorphan (○); ordinates, inhibition of contraction (%). Dose-cycle 7 min in (a) and 40 min in (b).

varying potencies which had been obtained from log concentration-response curves.

After logarithmic transformation, the correlation coefficient for the relative potencies in the ileum and vas deferens was $r = 0.979$ which was similar to the value found for the non-transformed data. The slope of the regression line (Figure 6) was now close to unity (b = 1.02) and the constant near to zero (a = 0.07); these findings suggest that the values obtained in the two preparations vary in direct proportion to each other. When the relative potencies obtained in the mouse vas deferens, without codeine and Ba 20227, were compared to the relative potencies in human analgesia, r was 0.960 ($n = 10$) for the non-transformed data and 0.944 after logarithmic transformation. The slope of the regression line for the logarithmic data was, however, different from unity (b = 0.72, a = 0.01). This deviation was not surprising because any direct proportionality would have been modified by the effects of drug distribution, biotransformation and excretion. Similar values ($r = 0.926$,

Table 2 The relative agonist potencies of compounds with dual agonist and antagonist actions in the mouse vas deferens and the guinea-pig ileum

Drug	*Relative agonist potencies (morphine or normorphine = 1)*	
	Mouse vas deferens	*Guinea-pig ileum*
(−)-Cyclorphan	141 ± 11.1 (4)	240
(−)-Cyclazocine	45.4 ± 1.3 (3)	35
Levallorphan	8.6 ± 1.0 (2)	16
Nalorphine	0.92 ± 0.04 (3)	2.8

The relative agonist potencies are the means ± s.e. mean; the number of observations are given in parentheses. The relative agonist potencies in the guinea-pig ileum are from Kosterlitz & Watt (1968) and Kosterlitz *et al.* (1974).

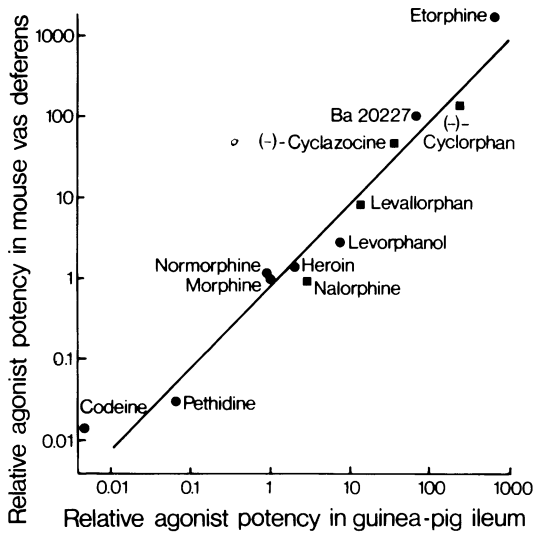

Figure 6 Correlation between the relative potencies of narcotic analgesics in the guinea-pig ileum and the mouse vas deferens. Abscissae, relative agonist potency in guinea-pig ileum (morphine = 1); ordinates, relative agonist potency in mouse vas deferens (morphine = 1). (•), Drugs with no significant antagonist action, (▪) drugs with dual agonist and antagonist actions. The values are plotted on a logarithmic scale. Correlation coefficient $r = 0.979$ ($n = 12$). The line has been drawn from $y = 1.02x - 0.07$. The values in the guinea-pig were obtained from Kosterlitz & Watt (1968), Kosterlitz *et al.* (1974) and Kosterlitz & Waterfield (1975). The values in the mouse vas deferens were determined by the 'single dose' method, with the exception of pethidine, levorphanol and Ba 20227 which were taken from Table 1.

b = 0.79, a = 0.03) have been found for the relationship between guinea-pig ileum and human analgesia (Kosterlitz & Waterfield, 1975).

Assessment of antagonist potency

The antagonist potency was determined by estimation of the dissociation or equilibrium constant K_e. The results obtained on four compounds are given in Table 3. Naloxone is an antagonist without significant agonist properties. Nalorphine, levallorphan and cyclazocine show agonist activities in both the mouse vas deferens (Table 2) and the guinea-pig ileum (Kosterlitz & Watt, 1968). Cyclorphan is a very potent agonist. Its antagonist properties cannot be demonstrated in the guinea-pig ileum; before a concentration is reached sufficient to produce a suitable dose-ratio, the contraction is completely inhibited by the agonist action (Kosterlitz *et al.*, 1974). In the mouse vas deferens, cyclorphan is also a potent agonist (Table 2) but because of its shallow dose-response curve, the antagonist properties were demonstrable (Table 3).

When the ratios of the K_e values obtained in the mouse vas deferens and the guinea-pig ileum were calculated, it was found that the mouse vas deferens was 3-6 times less sensitive to the antagonist action than the guinea-pig ileum. For reasons already discussed, no ratio was obtained for cyclorphan which is a potent antagonist in the mouse vas deferens.

Antagonist effects of mixtures of normorphine and nalorphine

Gyang & Kosterlitz (1966) drew attention to the fact that it was not possible to demonstrate the antagonist action of nalorphine in the guinea-pig ileum by adding mixtures of morphine and nalorphine to the organ bath.

Table 3 Comparison of the dissociation constants as measure of the antagonist potencies in the mouse vas deferens (K_{em}) and the guinea-pig ileum (K_{eg}).

Drug	K_{em} *(nM)*	K_{eg} *(nM)*	K_{eg}/K_{em}
Naloxone	3.66 ± 0.29 (4)	1.22 ± 0.03 (6)	0.33
Nalorphine	28.7 ± 3.2 (8)	4.47 ± 0.59 (6)	0.16
Levallorphan	7.11 ± 0.48 (6)	1.12 ± 0.23 (6)	0.16
(−)-Cyclazocine	4.86 ± 0.92 (7)	0.75 ± 0.19 (6)	0.15
(−)-Cyclorphan	1.58 ± 0.23 (6)	–	–

The values are the means ± s.e. mean; the number of observations are given in parentheses. The values in the guinea-pig ileum are from Kosterlitz & Watt (1968); Kosterlitz *et al.* (1972).

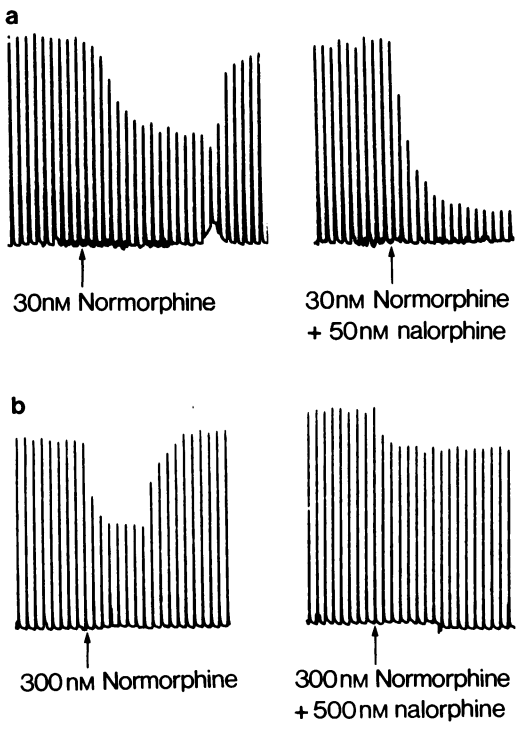

Figure 7 Comparison of the effects of mixtures of normorphine and nalorphine in (a) a segment of guinea-pig ileum and (b) in the mouse vas deferens. Stimulation was supramaximal at 0.1 Hz. The contractions of the ileum were recorded isometrically and those of the vas deferens isotonically.

When such mixtures were added to the bath fluids in which a mouse vas deferens or a segment of ileum were suspended, quite different results were obtained with the two preparations (Figure 7). In the guinea-pig ileum, the agonist action of normorphine was increased by nalorphine, whereas in the mouse vas deferens the agonist action of normorphine was antagonized by nalorphine. This difference in effect was independent of the ratios of nalorphine to normorphine which varied from 0.2-33.

Discussion

The first aim of this investigation was the confirmation and further analysis of the finding of Henderson *et al.* (1972) that narcotic analgesics depress evoked noradrenaline output and the associated longitudinal contraction of the mouse vas deferens, and that this effect is due to an interaction with a morphine receptor.

It has been shown that these effects are stereospecific since they are produced only by levorphanol and not by its (+)-isomer, dextrorphan, and that they are antagonized by the antagonists naloxone and naltrexone. Thus, the requirements for a specific action have been fulfilled.

The classical view holds that noradrenaline released from sympathetic nerves in the vas deferens causes contractions of the smooth muscle by action on α-adrenoceptors. Ambache, Dunk, Verney & Zar (1972) have advanced reasons for doubting an excitatory transmitter role for noradrenaline in the vas deferens, but a number of authors have produced results consistent with the classical role of noradrenaline (Jacobowitz & Koelle, 1965; Wadsworth, 1973; Furness, 1974) and refute the hypothesis of Ambache and his co-workers. The most cogent reason for accepting the neurotransmitter role of noradrenaline in the vas deferens is that drugs that block noradrenaline release such as bretylium and cocaine (Hughes, 1972), lysergic acid diethylamide (Hughes, 1973) and morphine (Henderson *et al.*, 1972; this paper) cause a corresponding block of the contractile response to nerve stimulation. In the present investigation it was found that there was a very close correlation between the concentrations of normorphine required to produce similar inhibitions of the twitch and of the output of labelled catecholamine.

The possibility cannot be excluded that morphine may not directly inhibit noradrenaline release but block some more central link in the transmission process. Inhibition of ganglionic transmission cannot be entirely eliminated but it is unlikely since the effects of morphine are not reduced by hexamethonium or hyoscine (Henderson *et al.*, 1972), or by stripping the outer coat of the mouse vas deferens (Henderson & Hughes, unpublished observations).

The second aim of this paper was to examine whether the mouse vas deferens is a suitable model for the prediction of the agonist and antagonist properties of narcotic analgesic drugs for use in analgesia in man. There is little doubt that this preparation predicts with considerable accuracy the agonist activities of compounds without antagonist properties, e.g. pethidine, heroin and levorphanol. The potency of codeine is lower than would be expected from its analgesic properties; a similar low activity has been found in the guinea-pig ileum (Kosterlitz & Watt, 1968) and in binding studies on brain homogenates (Pert & Snyder, 1973). The discrepancy found with codeine is probably due to the fact that codeine undergoes biotransformation to morphine in man (Adler, 1963) but not in the mouse vas deferens,

guinea-pig ileum or brain homogenate.

Complications arose with the assessment of agonist activities of compounds with dual agonist and antagonist properties since the dose-response curves of these compounds are very shallow. These difficulties were partly overcome by determining the lowest concentrations of these compounds which would depress the height of contraction by about 20% and relating this concentration to a concentration of normorphine causing the same depression. When this was done the correlation of the agonist activities with those found in the guinea-pig ileum was good. Since the results in the guinea-pig ileum predict satisfactorily the agonist effects in man (Kosterlitz *et al.*, 1974), the values obtained in the mouse vas deferens by the method of lowest active concentration also have good predictive properties.

The antagonist potencies of compounds with and without agonist properties are well correlated with those found in the guinea-pig ileum which in turn are in good agreement with those obtained in the morphine-dependent rhesus monkey (Kosterlitz *et al.*, 1974). The low sensitivity of the mouse vas deferens to the agonist action of higher concentrations of compounds with dual action makes this preparation particularly suitable for the estimation of the antagonist potency of these compounds.

It would appear therefore that the pharmacological properties of the morphine receptors in the mouse vas deferens, the myenteric plexus of the guinea-pig ileum, and the human central nervous system show considerable similarities, although the absolute sensitivity of the mouse vas deferens to agonists and antagonists is only 15-20% of that of the guinea-pig ileum. However, there are differences between the agonist activities of compounds with dual agonist and antagonist actions in the mouse vas deferens and guinea-pig ileum. This difference is mirrored by the variation in sensitivity of certain antinociceptive tests *in vivo*. Thus, compounds with dual agonist and antagonist actions have relatively little agonist activity in the mouse hot-plate test or the rat tail pressure test but show agonist activity correlated to human analgesia in the writhing test or the tail-shock test (Taber, 1974). Obviously, these differences are of importance for an understanding of the mechanisms underlying the action of narcotic analgesic drugs but on the evidence available at present any further discussion would be highly speculative.

A comparison of the usefulness of the two preparations, the mouse vas deferens and the guinea-pig ileum, for the prediction of the properties of new compounds indicates that the guinea-pig ileum is to be preferred because it is much more robust than the mouse vas deferens and gives constant twitch height over the long periods required for the assays. The agonist potencies of compounds with dual agonist and antagonist properties estimated by the guinea-pig ileum are more closely correlated to human values than those obtained by the mouse vas deferens unless special precautions are taken. On the other hand, the antagonist potencies of compounds with dual action are more readily assayed in the mouse vas deferens.

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