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Studies on the antinociceptive action of α-agonist drugs and their interactions with opioid mechanisms

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1 A modified abdominal constriction test, whereby the drugs used are injected intraperitoneally when the writhing response is maximal, has been used to study the antinociceptive activity of various sympathomimetic drugs. Of those tested, clonidine was the most potent, with an ID_{50} value in the nanomolar range. (−)-Isoprenaline, (−)-adrenaline and (−)-noradrenaline were only a little less potent. Phenylephrine, the least potent, had only about one-sixtieth of the activity of clonidine.

2 The antinociceptive action appears to occur within the peritoneum, since it was apparent almost immediately after the drugs were injected and was produced by doses far smaller than were effective by the subcutaneous route.

3 α-Adrenoceptors appear to be involved in the reaction, since noradrenaline showed stereospecificity, and the α-adrenoceptor antagonists phentolamine and piperoxan both shifted the dose-response curves of the α-adrenoceptor agonist drugs to the right, usually parallel to the control curves.

4 The high antinociceptive potency of clonidine and oxymetazoline, indicate the importance of α_2-adrenoceptors and this was supported by the finding that piperoxan was a more effective antagonist than phentolamine. The moderate potency of phenylephrine suggests that α_1-adrenoceptors may also be involved, although the selective α_1-antagonist, prazosin, did not antagonize noradrenaline and had antinociceptive activity of its own.

5 β-Adrenoceptors also appear to be involved in the antinociceptive response, since propranalol antagonized the effect of isoprenaline, but not that of clonidine.

6 Piperoxan was a very effective antagonist of morphine, while phentolamine had a weaker action. Naloxone had little action against the α-adrenoceptor agonists.

7 Mice pretreated with clonidine or oxymetazoline but not noradrenaline showed a very great cross-tolerance to morphine. Morphine pretreatment caused marked desensitization of itself, but little cross-tolerance to clonidine or oxymetazoline.

8 It is suggested that sensory nerves in the mouse peritoneum have α_2- and β-adrenoceptors on their terminals, and possibly α_1-receptors also. It is possible that when activated by the appropriate agonists they depress the generation of pain impulses. There is an interaction between the α-adrenoceptors and opioid receptors in the mouse peritoneum.

Introduction

In 1981 Bentley, Newton & Starr reported that morphine and several other opioid drugs, as well as Met- and Leu-enkephalin, when given by intraperitoneal injection to mice had antinociceptive activity when tested by modification of the abdominal constriction technique. Because several of these drugs were effective at nanomolar levels, and because the action was maximal within 1 or 2 min of administration, it was concluded that the antinociceptive effect occurred within the peritoneum, and it was also suggested that several different types of opioid receptors, resembling μ, δ, and κ-receptors, were involved. It was postulated that these receptors were situated on sensory nerve endings in the mouse peritoneum, and that, when they combined with opioid agonists, they depressed the generation of pain impulses. These findings support those of Ferreira & Nakamura (1979) who have shown that morphine administered locally in very low doses, can reverse the hyperalgesia caused by an injection of prostaglandin E_1 (PGE_1) into the rat paw.

In an earlier paper, Bentley, Copeland & Starr

(1977), using the conventional abdominal constriction test, observed that a number of α-adrenoceptor agonists also had antinociceptive activity. Some of these substances appeared to produce their effects by an action within the peritoneum. We now describe a more detailed study of the antinociceptive effects of various α-adrenoceptor agonists, using the modified abdominal constriction test. Our results indicate that all the agonists tested can act within the peritoneum, and, in addition, a strong cross-tolerance between morphine and oxymetazoline and clonidine, but not noradrenaline, can occur.

Methods

Male Balb-C mice, from the Monash University Central Animal House were used. They weighed between 20 and 25 g. For measuring antinociceptive activity, a modification of the abdominal constriction test was used, as described by Bentley *et al.* (1981). Briefly, this modification consists of injecting mice intraperitoneally with 0.6% acetic acid, 1.0 ml/100 g body weight. It was found that this concentration produced the maximum number of writhes, and higher concentrations were no more effective. Eight min after this injection, the rate of writhing reached a plateau, and unless some further treatment was given, remained at this level for at least 20 min. Eight min after the acetic acid injection, the antinociceptive agent was injected, also by the intraperitoneal route, and the writhes were then counted immediately, usually for two periods of 2 min, but in some cases, for four 1 min periods.

In a few experiments, intraperitoneal acetylcholine, 3.2 mg/kg (Collier, Dineen, Johnson & Schneider, 1968) was used as the noxious stimulus. This produced immediate writhing, but by 6 min the effect had ceased. For this reason, the antinociceptive agents were mixed with the acetylcholine, and given simultaneously with it. The writhes were then counted immediately for two periods of 2 min.

Each substance tested for antinociceptive activity was administered intraperitoneally in three ascending doses to 12 mice at each dose level. A fourth group of 12 mice acted as a control, and received 0.9% w/v NaCl solution (saline) intraperitoneally. All treatments were given in random order. The doses used were in logarithmic ratio (2×) and were chosen so that, at the highest level, the writhing response was completely suppressed in only a few mice.

When antagonist drugs were used, these were given subcutaneously 15 min before the acetic acid injection, and thus, 23 min before the antinociceptive drugs were administered. In order to determine the time at which the maximum effect of piperoxan and phentolamine occurred, experiments were performed where the ID_{50} value of noradrenaline was determined in mice predosed with the antagonist drugs, which were given 23 and 38 min before the noradrenaline injection.

For constructing dose-response curves, the mean number of writhes of the 12 mice in the group (those receiving either saline, or the antagonist drugs) was calculated. The writhing score for each individual mouse in the three groups receiving agonist drugs was substracted from this mean value, and, using a computer programme, a line of best fit and the ID_{50} value with its 95% confidence value, were calculated. Where an antagonist drug was used, the resulting dose-response curve was compared with the appropriate control curve, and again using a computer programme, an ANOVA was performed (Colquhoun, 1971) to calculate the dose-ratio and its 95% confidence limits.

Drugs

The following drugs (sources in parentheses) were used: (−)-adrenaline bitartrate (Sterling Winthrop), clonidine hydrochloride (Boehringer Ingelheim), (−)-noradrenaline bitartrate (Sigma), (+)-noradrenaline bitartrate (Sterling Winthrop), (−)-isoprenaline bitartrate (Sigma), (±)-isoprenaline hydrochloride (Sigma). lofexidine base (Merrill), morphine hydrochloride (T & H Smith), naloxone hydrochloride (Endo), oxymetazoline hydrochloride (Glaxo), phentolamine hydrochloride (Ciba Geigy), phenylephrine hydrochloride (I.C.I.), piperoxan hydrochloride (May & Baker), prazosin hydrochloride (Pfizer), propranolol hydrochloride (I.C.I.) and St 91 (2-(2, 6-diethylphenylimino)-2-imidazolidine hydrochloride (Boehringer-Ingelheim).

Results

Adrenoceptor agonists

Several of the α-adrenoceptor agonists showed extremely high antinociceptive potency in the modified writhing test, and they all produced their effect very rapidly (Table 1). Clonidine was the most potent substance tested (ID_{50}, 5.06×10^{-9} mol/kg), followed closely by (−)-adrenaline (1.13×10^{-8} mol/kg) and (−)-noradrenaline ($1.24 - 10^{-8}$ mol/kg). Phenylephrine was the least active drug tested (2.79×10^{-7} mol/kg). Adrenaline, noradrenaline, dopamine and phenylephrine were all tested at 1 min intervals following their injection, and, with few exceptions none of the observed reductions in writhing differed significantly from each other. Similarly, except for clonidine, the 2 min val-

Table 1 The antinociceptive potencies of several α-adrenoceptor agonist drugs and isoprenaline, measured at successive one-or two-min intervals

Drug	*ID_{50} value with 95% confidence limits* (mol/kg)			
	1st min	2nd min	3rd min	4th min
Acetic acid as the noxious stimulus				
Clonidine		5.06 $(4.76-5.50)\times10^{-9}$		5.56 $(5.43-5.91)\times10^{-9}$
(−)-Adrenaline	1.13 $(1.04-1.22)\times10^{-8}$	1.15 $(1.06-1.24)\times10^{-8}$	1.06 $(0.98-1.14)\times10^{-8}$	2.11 $(1.90-2.33)\times10^{-8}$
(−)-Noradrenaline	1.24 $(1.15-1.32)\times10^{-8}$	1.19 $(1.12-1.28)\times10^{-8}$	1.19 $(1.12-1.28)\times10^{-8}$	0.99 $(0.91-1.05)\times10^{-8}$
(+)-Noradrenaline		1.25 $(0.92-1.58)\times10^{-7}$		1.33 $(0.92-1.80)\times10^{-7}$
Oxymetazoline		1.37 $(1.30-1.45)\times10^{-8}$		1.35 $(1.29-1.40)\times10^{-8}$
St 91		4.71 $(4.45-5.00)\times10^{-8}$		4.84 $(4.52-5.21)\times10^{-8}$
Lofexidine		8.80 $(8.60-9.11)\times10^{-8}$		
Dopamine	1.08 $(1.00-1.16)\times10^{-7}$	0.93 $(0.87-0.98)\times10^{-7}$	1.00 $(0.94-1.06)\times10^{-7}$	0.95 $(0.89-1.00)\times10^{-7}$
Phenylephrine	2.79 $(2.48-3.19)\times10^{-7}$	2.92 $(2.65-3.24)\times10^{-7}$	3.10 $(2.77-3.56)\times10^{-7}$	2.66 $(2.35-3.05)\times10^{-7}$
(−)-Isoprenaline		7.15 $(6.77-7.53)\times10^{-9}$		7.01 $(6.63-7.43)\times10^{-9}$
(±)-Isoprenaline		2.02 $(1.92-2.13)\times10^{-8}$		2.00 $(1.90-2.11)\times10^{-8}$
Acetylcholine as the noxious stimulus				
(−)-Noradrenaline		1.31 $(1.22-1.40)\times10^{-8}$		
Clonidine		1.00 $(0.94-1.07)\times10^{-8}$		
Phenylephrine		2.97 $(2.72-3.25)\times10^{-7}$		

ues for the other drugs did not differ significantly from each other. Thus the α-adrenoceptor agonists all produced a maximal depression of writhing within 1 to 2 min of their administration. To test for stereospecificity, (+)-noradrenaline was also used. It had an ID_{50} value of 1.25×10^{-7} mol/kg, and was thus about 10 times less potent than the (−)-isomer.

Noradrenaline, phenylephrine and clonidine were also tested with acetylcholine as the noxious stimulus. The ID_{50} values for noradrenaline and phenylephrine were not significantly different from those obtained when acetic acid was used, although that for clonidine was almost twice as large.

Only one β-adrenoceptor agonist was used, isoprenaline. The (−)-isomer was the most potent of all the catecholamines tested, and had an ID_{50} value of 7.15×10^{-9} mol/kg, while the racemic mixture had less than half this activity with an ID_{50} value of 2.02×10^{-8} mol/kg (Table 1).

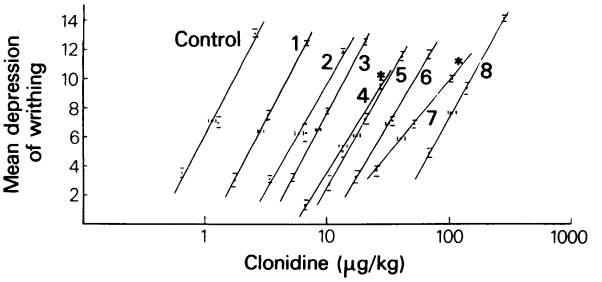

Figure 1 Dose-response curves to clonidine alone and in the presence of piperoxan, phentolamine, naloxone and propranalol. Curve (1) propranalol 6.76×10^{-6}; (2) piperoxan 5.0×10^{-6}; (3) naloxone, 1.5×10^{-6}; (4) piperoxan 1.0×10^{-5}; (5) naloxone 3.0×10^{-5}; (6) piperoxan 1.5×10^{-5}; (7) phentolamine 3.0×10^{-5} and (8) piperoxan 3.0×10^{-5} mol/kg. * denotes significant deviation from parallelism with control curves.

Interactions with antagonist drugs

α-Adrenoceptor antagonists The two α-adrenoceptor antagonists, piperoxan and phentolamine, were tested against several agonist drugs, and, in addition, prazosin was tested against noradrenaline only.

Phentolamine at doses of 1.5 and 3.0×10^{-5} mol/kg s.c. given 15 min before acetic acid, had no effect on the writhing score. Piperoxan at the same dose levels usually caused a small increase in writhing. On the other hand, prazosin given subcutaneously was quite potent at depressing the writhing, with an ID_{50} value of 1.93×10^{-6} mol/kg. However, at a level of 2.38×10^{-6} mol/kg it had negligible effect.

Both piperoxan and phentolamine shifted the dose-response curves of the α-adrenoceptor agonists to the right. For both these drugs, the antagonism was completely surmountable, so that, by increasing the dose of the agonist drugs, it was still possible to block the writhing response completely. The shifts in the dose-response curves were usually parallel with the

Table 2 Interactions of piperoxan and phentolamine with various agonist drugs

Agonist	*Dose piperoxan* (mol/kg, s.c.)	*Dose-ratio and 95% confidence limits*
Piperoxan		
Clonidine	5.0×10^{-6}	4.89 (4.48 – 5.36)
	1.0×10^{-5}	14.72 (13.56 – 16.03)*
	1.5×10^{-5}	28.68 (26.46 – 31.09)
	3.0×10^{-5}	83.88 (77.91 – 90.12)
(−)-Noradrenaline	5.0×10^{-6}	1.98 (1.73 – 2.27)
	1.5×10^{-5}	16.50 (15.03 – 18.07)*
	3.0×10^{-5}	97.83 (91.85 – 104.07)
(−)-Adrenaline	1.5×10^{-5}	9.20 (8.55 – 9.91)
	3.0×10^{-5}	110.64 (103.33 – 118.35)
Phenylephrine	3.0×10^{-5}	6.84 (6.31 – 7.40)*
Isoprenaline	3.0×10^{-5}	3.48 (3.26 – 3.71)
Morphine	1.5×10^{-5}	11.67 (10.76 – 12.63)
	3.0×10^{-5}	66.37 (60.82 – 72.16)
Phentolamine		
Clonidine	3.0×10^{-5}	46.54 (42.72 – 50.79)*
(−)-Noradrenaline	3.0×10^{-5}	12.84 (11.64 – 14.13)
(−)-Adrenaline	3.0×10^{-5}	52.77 (49.33 – 56.49)*
Phenylephrine	3.0×10^{-5}	5.31 (4.83 – 5.81)
Morphine	3.0×10^{-5}	18.30 (17.00 – 19.65)

*Indicates significant deviation from parallelism with control curve.

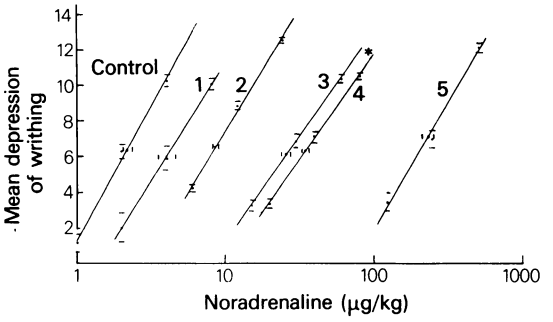

Figure 2 Dose-response curves to noradrenaline, alone, and in the presence of piperoxan, phentolamine, and naloxone. Curve (1) piperoxan 5×10^{-6}; (2) naloxone 1.5×10^{-5}; (3) phentolamine 3.0×10^{-5}; (4) piperoxan 1.5×10^{-5} and (5) piperoxan 3.0×10^{-5} mol/kg. *denotes significant deviation from parallelism with control curve. The curve for propranalol, 6.76×10^{-6} mol/kg almost completely co-incides with that for phentolamine 3.0×10^{-5} mol/kg, and therefore has not been included.

control curves, but with both antagonists, there were occasional small but statistically significant deviations. The dose-ratios generated by these antagonist drugs varied considerably according to the agonist used. For example, with piperoxan 3.0×10^{-5} mol/kg the dose-ratios varied from 6.8 for phenylephrine to 110.6 for adrenaline. Phentolamine, which was always less effective an antagonist than piperoxan, also produced dose-ratios varying from 5.3 for phenylephrine to 52.8 for adrenaline. However, piperoxan was a very ineffective antagonist of isoprenaline and at a concentration of 3.0×10^{-5} mol/kg, it produced a dose-ratio of only 3.5. In contrast, it was a remarkably effective antagonist of morphine, and at this same dose, produced a dose-ratio of 66.4. Phentolamine was less effective as an antagonist of morphine, and at 3.0×10^{-5} mol/kg it produced a dose-ratio of 18.3 (Table 2, Figures 1, 2 and 3).

Prazosin, in contrast to the other α-adrenoceptor antagonists, did not antagonize noradrenaline but actually slightly increased its antinociceptive potency, so that the ID_{50} value was decreased to 1.38×10^{-8} mol/kg.

A Schild plot (Arunlakshan & Schild, 1959) for noradrenaline plus piperoxan was constructed, despite the fact that only three concentrations of piperoxan were used, and, for one of these (1.5×10^{-5} mol/kg) there was significant deviation from parallelism. The slope of this Schild plot was −3.31 and the intercept was 5.15. Hence competitive antagonism seems unlikely and the intercept indicates an apparent pA_2 value different from that for piperoxan on peripheral tissues (Edvinsson & Owman, 1974; Borowski, Starke, Ehrl & Endo, 1977). A Schild plot was also constructed for piperoxan vs. clonidine, for which four concentrations of the antagonist were available. Again, the calculated slope of −1.55 differed from the theoretical value of −1 and the intercept was 5.76.

When piperoxan 3.0×10^{-5} mol/kg, was given 30 min before the acetic acid, and thus, 38 min before noradrenaline, it was found that the ID_{50} value for noradrenaline was 1.47 (1.38–5.57) $\times 10^{-7}$ mol/kg. This is only slightly larger than the ID_{50} value at the 23 min period, 1.37 (1.26–1.48) $\times 10^{-7}$ mol/kg. In a similar experiment using phentolamine 3.0×10^{-5} mol/kg, given 38 min before noradrenaline, the ID_{50} value was 1.64 (1.55–1.72) $\times 10^{-7}$ mol/kg, as compared with the value of 1.53 (1.42–1.63) $\times 10^{-7}$ mol/kg at the 23 min period. Thus for both these antagonists the values at the two different times are only just significantly different, and therefore one may conclude that the effect of both antagonists are very close to maximum at the 23 min period.

The antinociceptive effect of prazosin was also studied briefly. It was found that when it was given subcutaneously, together with piperoxan 1.0×10^{-5} mol/kg, the ID_{50}, measured 15 min later, was 4.5×10^{-6} mol/kg. This is only about 2.3 times greater than the value for prazosin given alone, subcutaneously.

β-Adrenoceptor antagonist. Propranolol, 3.0×10^{-5} mol/kg caused a marked depression of writhing, which persisted even when tested 36 min after subcutaneous injection. However, at the lower dose of 6.76×10^{-6} mol/kg, it showed no antinociceptive activity, but antagonized both isoprenaline and noradrenaline, giving dose-ratios of 17.4 and 12.4 respectively. However, when tested against clonidine, it had little antagonist action, and produced a dose-ratio of only 2.72 (Table 3).

Naloxone At doses of 1.5 and 3.0×10^{-5} mol/kg, naloxone produced dose-ratios of 20.5 and 172.2 respectively against morphine (Bentley *et al.*, 1981). It also had moderate antagonist activity against several α-adrenoceptor agonists, and at 1.5×10^{-5} mol/kg it produced dose-ratios ranging from 2.8 for phenylephrine to 7.3 for clonidine. At a dose of 3.0×10^{-5} mol/kg, naloxone produced a dose-ratio of 17.5 against clonidine (Figure 3 and Table 3).

Cross-tolerance experiments. Because of the interactions between the α-antagonists and morphine described above, experiments were conducted to determine whether any cross-tolerance could be demonstrated between morphine on the one hand, and the α-adrenoceptor agonists oxymetazoline, clonidine, or noradrenaline, on the other. Mice were pre-dosed subcutaneously with morphine, 5×10^{-9} and 5×10^{-6} mol/kg, and, 3 h later, dose-response curves

Table 3 Interactions of naloxone, and propranalol with various agonist drugs

Agonist	*Dose antagonist* (mol/kg)	*Dose-ratio with 95% Confidence limits*
Naloxone		
Clonidine	1.5×10^{-5}	7.30 (6.90 – 7.72)
	3.0×10^{-5}	7.53 (16.22 – 18.98)
Noradrenaline	1.5×10^{-5}	4.60 (4.27 – 4.95)
Phenylephrine	1.5×10^{-5}	2.78 (2.57 – 3.01)*
Morphine	1.5×10^{-5}	20.45 (19.32 – 21.62)
	3.0×10^{-5}	171.21 (162.74 – 182.18)
Propranalol		
Noradrenaline	6.8×10^{-6}	12.42 (11.30 – 13.60)
Isoprenaline	6.8×10^{-6}	17.36 (16.13 – 18.63)
Clonidine	6.8×10^{-6}	2.72 (2.52 – 2.93)

*Indicates significant deviation from parallelism with control curve.

were constructed to morphine, oxymetazoline, and clonidine. In other experiments mice were pretreated with oxymetazoline, 5×10^{-9}, clonidine 5×10^{-8}, and noradrenaline 5×10^{-9} mol/kg subcutaneously, and, 3 h later dose-response curves to morphine were constructed. In addition, control experiments were conducted to determine whether pretreatment with any of the above drugs caused any residual antinociceptive effect 3 h after their administration.

It was found that pretreatment with oxymetazoline 5×10^{-9} mol/kg (s.c.) caused no residual antinociceptive effect when tested 3 h later. Mice so treated showed only a small degree of tolerance to a second dose of oxymetazoline, and the ID_{50} of this substance increased by a factor of only 4.6. However, oxymetazoline pretreatment increased the ID_{50} of morphine 258 times, as compared with saline pretreated mice. Bentley *et al.* (1981) had shown that pretreatment with morphine, 5×10^{-9} mol/kg, caused a 23.1 fold reduction in the potency of morphine, when tested 3 h later. Thus, at equimolar doses, oxymetazoline has almost 10 times the ability to desensitize morphine as compared with morphine itself. However, when mice were pretreated with morphine, 5×10^{-9} mol/kg there was a negligible change in the ID_{50} of oxymetazoline, when measured 3 h later.

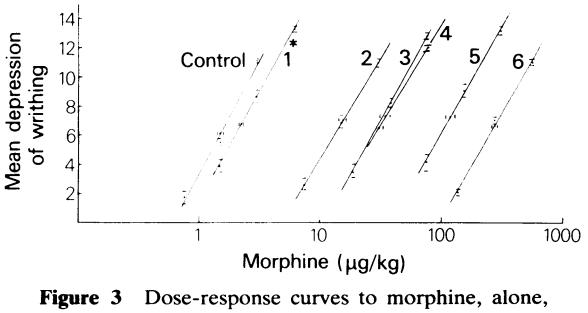

Figure 3 Dose-response curves to morphine, alone, and in the presence of piperoxan, phentolamine, and naloxone. Curve (1) piperoxan 7.5×10^{-6}; (2) piperoxan 1.5×10^{-5}; (3) phentolamine 3.0×10^{-5}; (4) naloxone 1.5×10^{-5}; (5) piperoxan 3.0×10^{-5} and (6) naloxone 3.0×10^{-5} mol/kg. *denotes significant deviation from parallelism with control curve.

Clonidine, 5×10^{-8} mol/kg, s.c. given 3 h before morphine, also caused cross-tolerance, so that the ID_{50} of morphine increased by a factor of 400. Even when the clonidine pretreatment was given 17 h before morphine, a 145.5 fold cross-tolerance was still present. As with oxymetazoline, treatment with clonidine either 3 or 17 h before testing caused no residual antinociceptive effect. In contrast to the experiments described above, it was found that pretreatment with noradrenaline, 5×10^{-9} mol/kg s.c., 3 h beforehand, caused a sensitization to the antinociceptive effect of morphine. The ID_{50} value was reduced from the control value of 5.5×10^{-9} mol/kg to 2.26×10^{-9} mol/kg. Pretreatment with noradrenaline did not cause any residual antinociceptive action when tested 3 h later (Table 4).

An experiment was also undertaken to test the antinociceptive effect of morphine and clonidine given simultaneously. The two drugs were mixed in the same ratio as their ID_{50} values, given singly, and a dose-response curve was constructed to this mixture. The ID_{50} value was calculated as morphine 1.68 (1.57–1.78) $\times 10^{-9}$ mol/kg plus clonidine 1.92 (1.80–2.04) $\times 10^{-9}$ mol/kg. Thus giving these two drugs together has potentiated their antinociceptive action. This is in agreement with the finding of Spaulding, Fielding, Venaftro & Lal (1979) who used the tail-flick assay in mice.

Discussion

This study has shown that, when the modified ab-

Table 4 The effect of pretreating mice with morphine and various α-adrenoceptor agonists on the antinociceptive potency of morphine, and of morphine pretreatment on the antinociceptive potencies of clonidine and oxymetazoline

Pretreatment (mol/kg)	*Antinociceptive agonist* and ID_{50} *mol/kg)*		*Desensitization factor*
Saline	Morphine	5.5×10^{-9}	
Morphine 5×10^{-9}	Morphine	1.27×10^{-7}	23.1
Morphine 5×10^{-6}	Morphine	5.67×10^{-6}	1030.9
Saline	Oxymetazoline	1.37×10^{-8}	
Oxymetazoline 5×10^{-9}	Oxymetazoline	6.43×10^{-8}	4.69
Oxymetazoline 5×10^{-9}	Morphine	1.42×10^{-6}	258.2
Morphine 5×10^{-9}	Oxymetazoline	1.71×10^{-8}	1.25
Saline	Clonidine	5.06×10^{-9}	
Morphine 5×10^{-9}	Clonidine	1.57×10^{-8}	3.10
Clonidine 5×10^{-8}	Morphine	2.20×10^{-6}	400
Clonidine 5×10^{-8} (17 h previously)	Morphine	8.0×10^{-7}	145.5
Noradrenaline	Morphine	2.26×10^{-9}	0.41

dominal constriction test is used in mice, a number of α-adrenoceptor agonist drugs have antinociceptive activity in nanomolar doses. Of those drugs tested, clonidine was the most potent, with a very similar activity to morphine (Bentley *et al.*, 1981). Adrenaline and noradrenaline were only a little less potent than clonidine. The β-agonist isoprenaline was the most potent of the four catecholamines tested, while phenylephrine was the least active drug used in this study. This antinociceptive effect, as with the opioid agonists (Bentley *et al.*, 1981) appears to occur within the peritoneum, since the effective doses are many times lower than when the drugs are given subcutaneously (Paalzow, 1974; Schmitt, Le Douarec & Petillot, 1974; Bentley *et al.*, 1977), and the antinociceptive effect occurs almost immediately after the drugs are injected. This suggestion conflicts with the statement by Major & Pleuvry (1971) that α-agonist drugs do not have a peripheral antinociceptive action. However, these authors used the hot-plate test, which is considerably less sensitive than even the conventional writhing test, and certainly would not give information about actions within the peritoneum.

Noradrenaline, phenylephrine and clonidine were also tested using acetylcholine as the noxious stimulus. For the first two drugs the ID_{50} values were very similar with both the noxious stimuli. Collier *et al.* (1968) have suggested that acetic acid triggers the production of irritant substances within the peritoneum, which cause the writhing response, while acetylcholine acts directly on the sensory nerve endings. The fact that noradrenaline and phenylephrine were equally effective against both noxious stimuli indicates that these drugs do not depress the production of these endogenous irritants, but presumably act directly on sensory nerve endings. Clonidine on the other hand, had only about one-half the activity against acetylcholine that it had against acetic acid, and thus its mode of action is less clearly defined.

The suggestion that α-adrenoceptors are involved in the antinociceptive action of the α-adrenoceptor agonists is supported by two pieces of evidence. These are finding that noradrenaline shows stereospecificity, so that the (+)-isomer has only about one-tenth of the potency of (−)-noradrenaline, and also the ability of both piperoxan and phentolamine to antagonize all the α-adrenoceptor agonists tested. These two α-antagonists drugs, given subcutaneously either 23 or 38 min before acetic acid, had no significant effect on the writhing score.

In order to determine the time at which the maximum effect of piperoxan and phentolamine occurred, these drugs were given 23 and 38 min before an injection of noradrenaline. It was found that the ID50 value for this α-agonist was almost identical at the two times, indicating that, at the 23 min interval, both antagonist drugs were producing an effect that was only just significantly less than maximal. Since it was more convenient to use the shorter time interval, this procedure was adopted for all experiments.

Dose-response curves to noradrenaline, clonidine and adrenaline were constructed in the presence of several doses of piperoxan, and to phenylephrine, isoprenaline and morphine in the presence of only one concentration (3.0×10^{-5} mol/kg) of piperoxan. In addition, all the above α-adrenoceptor agonists and morphine were also tested in the presence of phentolamine, 3.0×10^{-5} mol/kg. It was found that both the α-antagonist drugs shifted the dose-response curves of all the α-adrenoceptor agonists to the right, but the size of the dose-ratios so produced varied greatly according to the agonist drug used. With piperoxan, there was a 16.3 fold difference between the dose-ratio for phenylephrine (6.8) and

for adrenaline (110.6), while with phentolamine, which was always a less effective antagonist, the dose-ratios for these two agonists varied by 9.9 times. In all cases, the antagonism was completely surmountable by increasing the concentration of the agonist drugs, and, in most cases, the curves in the presence of the antagonists were parallel to the control curves, although occasionally there were small but statistically-significant deviations. These variations in dose-ratio between different agonists may be due to the fact that several different receptor types appear to be involved in the antinociceptive response. Although phenylephrine was the weakest α_1-adrenoceptor agonist tested, its antinociceptive potency was nevertheless quite respectable. Phenylephrine is stated to act principally on α-adrenoceptors (Starke, Montel & Endo, 1975) and hence the α_1-adrenoceptor presumably can initiate analgesia. Clonidine and oxymetazoline, which are primarily α_2-agonists, both showed high antinociceptive potency, indicating that α_2-adrenoceptors are important in causing analgesia. β-Adrenoceptors also appear to be involved, since isoprenaline, which, like noradrenaline, showed stereospecificity, was second only to clonidine in antinociceptive potency. Propranalol was a very effective antagonist of this action. In addition, as will be discussed below, there is evidence that clonidine and oxymetazoline but not noradrenaline, can also act on opioid receptors. Thus it is not surprizing that piperoxan and phentolamine, which have different ratios (Borowski *et al.*, 1977) of α_1- and α_2-antagonism should produce different dose-ratios with the various α-adrenoceptor agonists tested.

The diversity of adrenoceptors involved in the antinociceptive response could also explain the steep slopes of the Schild plots constructed for clonidine and noradrenaline with piperoxan. In both cases, these differed greatly from -1, suggesting non-competitive antagonism. However, since propranalol was such an effective antagonist of noradrenaline (though not of clonidine) it is likely that at least part of the antinociceptive action of this agonist would be mediated by β-adrenoceptors. Hence one should not expect the Schild plot with piperoxan to be -1, and the intercept would not correspond with the affinity constant measured in tissues having only α-adrenoceptors. The slope of the Schild plot for clonidine against piperoxan was also steeper than -1, though not as steep as that for noradrenaline. While clonidine is unlikely to have actions on β-adrenceptors, it is likely that this apparent non-competitive antagonism is due to the involvement of both α_1- and α_2-adrenoceptors and perhaps even opioid receptors.

Although phenylephrine had considerable antinociceptive activity, it was surprizing to find that the selective α_1-antagonist, prazosin (Doxey & Roach, 1980), not only did not antagonize noradrenaline, but had considerable antinociceptive action of its own. Since this was only weakly antagonized by piperoxan, it is likely that the effect does not involve a receptor interaction at all, but may be due to some other cause such as local anaesthetic action. Propranalol is known to have high local anaesthetic activity (see Bowman & Rand, 1980) and it showed antinociceptive action in this study. There are other reports in the literature suggesting that α_1-adrenoceptor activity is not important in the antinociceptive action of clonidine. Paalzow & Paalzow (1976) showed the fairly selective α_2-adrenoceptor antagonist, yohimbine (Borowski *et al.*, 1977) is effective in depressing the antinociceptive actions of clonidine in rats whereas both phenoxybenzamine and chlorpromazine potentiated its action.

Studies on the interactions of morphine and α-adrenoceptor agonists and antagonists produced some puzzling results. There are indications that the α-agonists and antagonists can act in some way on opioid receptors, although the effects of the opioid drugs on α-adrenoceptor mechanisms are less apparent. For example, piperoxan had between one third and one-quarter of the potency of naloxone in antagonizing morphine, athough phentolamine was considerably weaker. In contrast, naloxone had little ability to antagonize the α-adrenoceptor agonists. This is consistent with the report by Paalzow & Paalzow (1976) that naloxone was completely inactive in antagonizing the antinociceptive action of clonidine in rats. Other indications of a 'one-way' interaction between opioid and α-adrenoceptor mechanisms comes from the studies where mice were pretreated with either morphine or α-adrenoceptor agonists, and, 3 h later, antinociceptive tests were undertaken. None of the drugs used for pretreatment (morphine, clonidine, oxymetazoline or noradrenaline) altered the writhing score estimated 3 h later. Hence there was no sign of residual agonist action at this time. Pretreatment of mice with a single dose of either clonidine, morphine or oxymetazoline caused a marked reduction in the potency of a subsequent dose of morphine. In fact, the desensitizing effect of oxymetazoline against morphine was greater than that of morphine itself. In addition, the desensitizing effect of clonidine was still marked when measured 17 h after predosing. In contrast, noradrenaline pretreatment caused a significant sensitization to morphine at the 3 h period. Morphine pretreatment had only a slight desensitizing action against oxymetazoline given 3 h later. It seems improbable that the α-adrenoceptor agonists are acting as persistent antagonists at the opioid receptor, since when clonidine was given simultaneously with morphine, the antinociceptive effects were potentiated. These

findings are consistent with those of Paalzow (1978), who also showed that clonidine could cause a cross-tolerance to morphine. However, Paalzow used much higher doses of clonidine (up to 3000 μg/kg), and dosed his animals (rats) over seven days. Further studies are planned to explore these drug interactions in more detail.

There are several previous reports in the literature suggesting the interaction of opioid- and α-adrenoceptor agonists at a receptor level. Cicero, Wilcox & Meyer (1974) found that naloxone could inhibit the binding of both phenoxybenzamine and phentolamine to brain homogenates, while Spiehler, Fairhurst & Randall (1978) showed that phenoxybenzamine could cause a dose-dependent displacement of stereospecifically-bound naloxone from mouse brain homogenates, and that this effect was sodium-dependent. These authors calculated that the 'sodium ratio' of phenoxybenzamine was similar to that of mixed agonist-antagonist opiates. There is also the report by Glossmann & Hornung (1980) who showed that the binding of clonidine to brain homogenates was, like opiate drugs, dependent on the sodium levels in the bathing medium. On the other hand, Golombiowska-Nikitkin, Pilc & Ventulani (1980), who used rat brain homogenates, were unable to show any displacement of clonidine by morphine or other opiates. Finally, Atlas & Sabol (1980) have shown that the substance N-(4-hydroxyphenylacetyl)-4-amino clonidine, which is a potent α-adrenoceptor ligand, also has properties in common with the opioids. It causes a naloxone-reversible inhibition of adenylcyclase in neuroblastoma-× glioma hybrid cells, and, in addition, inhibits the binding of [^{3}H]-D-Ala^2-Met^5 enkephalinamide and [^{3}H]-dihydromorphine to rat brain opiate receptors. These authors state that the structure of this clonidine derivative has 'common elements in the ligand binding sites of α- and opiate receptors'.

The findings of the present study further emphasize the link between opioid and α-adrenoceptors. One possible explanation for the present findings is that the opioid receptor is linked to its effector mechanism through an α-adrenoceptor, and that opioid agonists in some way activate the α-adrenoceptor. This theory would accommodate the findings that opioid effects can be markedly attenuated both by α-adrenoceptor antagonists, and by pretreatment with some α-adrenoceptor agonists. It is possible that these drugs could cause some allosteric modification of the α-adrenoceptor, thus disrupting its effective coupling with the opioid receptor and so reducing the effectiveness of morphine.

It is planned to study further the effects of various ions and adenine and guanine nucleotides on the antinociceptive effects of opioid and α-adrenoceptor agonists.

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