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INTERACTIONS OF DRUGS ACTIVE AT OPIATE RECEPTORS AND DRUGS ACTIVE AT α_2-RECEPTORS ON VARIOUS TEST SYSTEMS

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1 The actions of the opiate receptor drugs, morphine, methionine-enkephalin (Met-enkephalin) and naloxone were compared with the actions of the α_2-receptor drugs, clonidine, xylazine and yohimbine on analgesic tests, *in vitro* bioassay (guinea-pig ileum and mouse vas deferens), and radioligand displacement studies on rat brain membrane preparations.

2 Both opiate and α_2-agonist drugs showed analgesic activity but whilst the α_2-agonist analgesic activity was antagonized by only α_2-antagonists, the analgesic activity of morphine was antagonized by both naloxone and yohimbine. In the *in vitro* tests, both groups of agonists inhibited electrically evoked activity; however in these experiments only antagonism of opiates by naloxone and α_2-agonists by yohimbine could be shown and it is concluded that in these systems the activity of the α_2-agonists is mediated via the presynaptic α_2-receptors only.

3 In the radioligand studies the drugs acting at α_2-receptors were active in the micromolar range at displacing labelled opioid ligands but opiates did not displace labelled α_2-ligands.

4 It is concluded that drugs which act on α_2-receptors interfere with the *in vivo* analgesic effects of opiates and weakly displace opioid radioligand binding, but opioids do not affect α_2 agonist analgesia and do not appear to displace α_2-agonist radioligand binding.

Introduction

Xylazine is a veterinary sedative drug, and clonidine, a hypotensive agent; both agents are strong α_2-adrenoceptor agonists at adrenergic neurones and can be antagonized at this site by yohimbine (Drew 1976). There are also reports of the existence of presynaptic α_2-adrenoceptors on cholinergic neurones (Drew 1978).

Xylazine has been shown to possess activity in the rat tail flick analgesic test and to be capable of displacing radiolabelled dihydromorphine in rat brain membrane preparations (Lawrence & Livingston, 1981a). Clonidine has been shown to reverse withdrawal symptoms in opiate addicts (Gold, Redmond & Kleber, 1978) and an overlap in the distribution of α_2- adrenoceptors and opiate receptors in the rat brain has been demonstrated autoradiographically (Young & Kuhar, 1979; 1980). The chronic administration of morphine seems to increase the number of α_2-binding sites in rat brain (Hamburg & Tallman, 1981) and the α_2-antagonist yohimbine is capable of reversing morphine-induced hypothermia in rats (Lawrence & Livingston (1981b).

The object of this study was to investigate the interactions between the drugs which react with α_2-receptors and those which exert their effects via opiate receptors. A preliminary report of part of this study has been given (Lawrence & Livingston, 1981c).

Methods

Analgesic testing: the rat tail flick method as described by Sewell & Spencer (1976) was used to assess the analgesic potency of the drugs. The test consisted of immersing the end of the rat's tail in water maintained at 55°C and measuring the response time to a maximum permitted at 15 s. The test was performed at 10 min intervals before and after injection of the appropriate drug. The analgesic effects of morphine, xylazine and clonidine and the antagonism of naloxone and yohimbine were measured.

Mouse vas deferens bioassay: the assay described by Hughes, Kosterlitz & Leslie (1975) using the vas deferens from an adult LACG mouse suspended in Tyrode solution was used. The vas was attached to an isotonic transducer and stimulated transmurally with a square wave pulses at 0.1 Hz, 1 ms duration and supramaximal voltage.

Guinea-pig ileum bioassay: portions of guinea-pig ileum were suspended in an organ bath in Krebs bicarbonate solution as described by Paton & Vizi (1969). The tissue was stimulated transmurally with supramaximal voltage at 0.1 Hz with pulses of 1 ms

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duration.

Opiate receptor binding: the method of measuring specific opiate receptor binding was based on that described by Pert & Snyder (1973) as described by Lawrence & Livingston (1981a) using a suspension of membranes from rat brain. Specific [^{3}H]-dihydromorphine binding (60%) was calculated by subtracting the binding in the presence, from that in the absence, of 10 μM morphine, and specific [^{3}H]-Ala^2-D-Leu^5-enkephalin binding (74%) was calculated from the binding in the presence and absence of 10 μM Met-enkephalin.

Clonidine receptor binding: the binding of [phenyl-4-^{3}H]-clonidine hydrochloride to rat brain membranes was studied by a method based on that described by Hamburg & Tallman (1981) which was similar to that described by U'Pritchard & Snyder (1980). Specific binding (72%) was calculated from the binding in the presence and absence of 10 μM clonidine.

Statistical analysis: Student's t test was used for testing the significance of difference between groups.

Drugs used: the drugs used were naloxone hydrochloride, kindly supplied by Endo Laboratories, clonidine hydrochloride, kindly supplied by Bohringer Ltd., morphine hydrochloride (McFarlan Smith Ltd.), Met-enkephalin (Peninsular Laboratories Ltd.), xylazine hydrochloride, kindly supplied by Bayer Ltd., yohimbine hydrochloride (Sigma Chemical Co.) and [^{3}H]-dihydromorphine, [^{3}H]-D-Ala^2-D-Leu^5 enkephalin and [phenyl-4-^{3}H]-clonidine hydrochloride (Radiochemical centre).

Results

Analgesic testing

Morphine (5 mg/kg i.p.) increased reaction time over a 50 min period. This effect was antagonized significantly by naloxone (5 mg/kg i.p.) given 5 min beforehand at each point measured. The analgesic activity of morphine also showed significant reduction at 10,20,30 and 40 min when the animals were pretreated 5 min beforehand with yohimbine (2 mg/kg i.p., Figure 1a).

Xylazine, at a dose rate of 30 mg/kg intraperitoneally, also showed analgesic activity, although the duration of this effect was shorter than that seen with morphine. These analgesic effects were significantly reduced at 10,20 and 30 min following pretreatment with yohimbine (2 mg/kg i.p.), but not with naloxone. There was a significant prolongation of the analgesic effects of the xylazine in the presence of naloxone at 40 and 50 min (Figure 1b).

Clonidine, (0.2 mg/kg i.p.) elicited analgesic activity similar to that seen with xylazine, although the drug was clearly more potent. Yohimbine (2 mg/kg i.p.) given 5 min beforehand, but not naloxone (5 mg/kg i.p.), significantly reduced the analgesic effects of clonidine at 30,40 and 50 min (Figure 1c).

Mouse vas deferens bioassay

The inhibitory effects of morphine, Met-enkephalin, xylazine and clonidine are shown in Figure 2a, with

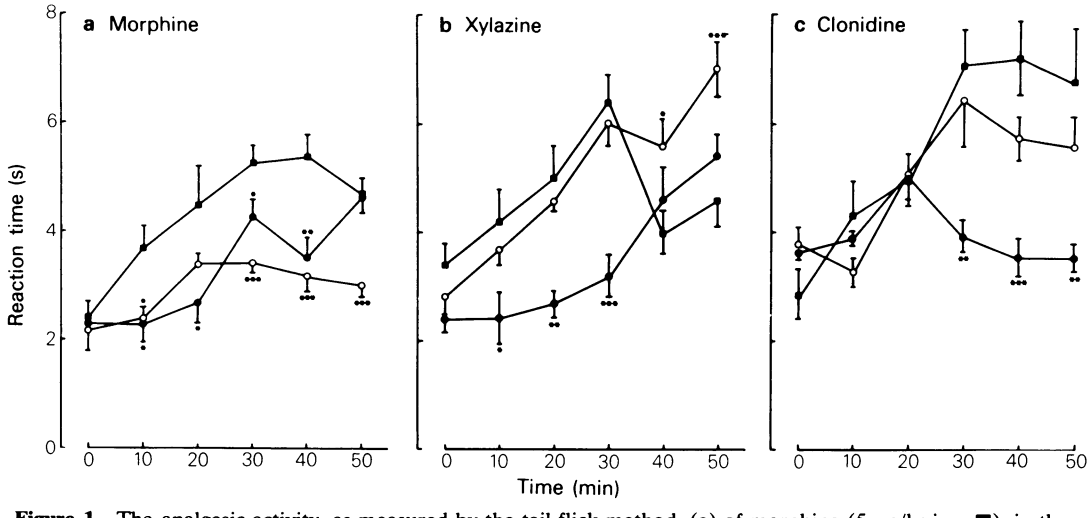

Figure 1 The analgesic activity, as measured by the tail flick method, (a) of morphine (5 mg/kg i.p., ■), in the presence of naloxone (5 mg/kg i.p., ○) and in the presence of yohimbine (2 mg/kg i.p., ●). (b) Analgesic activity of xylazine (30 mg/kg i.p., ■), in the presence of naloxone (5 mg/kg i.p., ○) and in the presence of yohimbine (2 mg/kg i.p., ●). (c) Analgesic activity of clonidine (0.2 mg/kg i.p., ■), in the presence of naloxone (5 mg/kg i.p., ○) and in the presence of yohimbine (2 mg/kg i.p., ●). All values are means of eight observations; vertical lines indicate s.e.mean; $^*P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

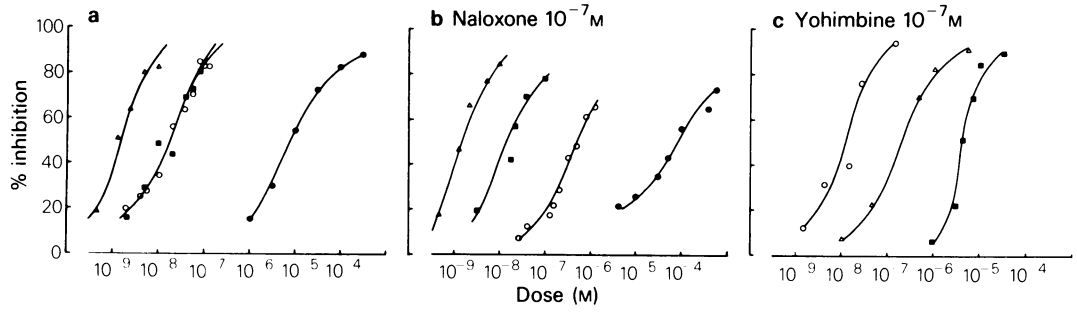

Figure 2 (a) Dose-response curves for the inhibition of electrically evoked contractions of the mouse vas deferens by morphine (●), Met-enkephalin (○), clonidine (△) and xylazine (■). (b) Dose-response curves for the inhibition of electrically evoked contractions of the mouse vas deferens in the presence of 10^{-7}M naloxone by morphine (●), Met-enkephalin (○), clonidine (△), and xylazine (■). (c) Dose-response curves for the inhibition of electrically evoked contractions of the mouse vas deferens in the presence of 10^{-7} M yohimbine by Met-enkephalin (○) clonidine (△) and xylazine (■). All values are the means of four experiments.

clonidine being the most potent (IC_{50} 2 nM), xylazine and Met-enkephalin being equipotent (IC_{50} 18 nM) and morphine the least potent (IC_{50} 8 μM). In the presence of 10^{-7}M naloxone (Figure 2b) there was an antagonism of the effects of Met-enkephalin (IC_{50} 0.5 μM) and morphine (IC_{50} 80 μM) but not of xylazine (IC_{50} 14 nM) or clonidine (IC_{50} 2 nM). In the presence of 10^{-7}M yohimbine (Figure 2c) the effects of xylazine (IC_{50} 4 μM) and clonidine (IC_{50} 0.2 μM) were antagonized but Met-enkephalin activity was unchanged (IC_{50} 13 nM).

Guinea-pig ileum bioassay

Dose-response curves for the inhibitory effects of morphine, Met-enkephalin, xylazine and clonidine are shown in Figure 3a. Morphine (IC_{50} 23 nM) and Met-enkephalin (IC_{50} 23 nM) were marginally less active than xylazine (IC_{50} 18 nM) and clonidine (IC_{50} 10 nM). In the presence of 5×10^{-7}M naloxone (Figure 3b) the dose-response curves for morphine (IC_{50} 18 μM) and Met-enkephalin (IC_{50} 0.6 μM) were shifted to the right but xylazine's action was unchanged (IC_{50} 18 nM). Figure 3c shows the effects of 10^{-7}M yohimbine; there was no significant reduction in the effects of Met-enkephalin (IC_{50} 40 nM), but a clear reduction in the activity of xylazine (IC_{50} 23 μM) and clonidine (IC_{50} 1 μM).

Opiate receptor binding

Specific [^{3}H]-dihydromorphine binding was displaced by all the agents tested (Figure 4a). The opiate drugs were more potent with IC_{50} values in the nanomolar range (morphine 65 nM, Met-enkephalin 25 nM, and naloxone 5 nM) whilst the α_2-

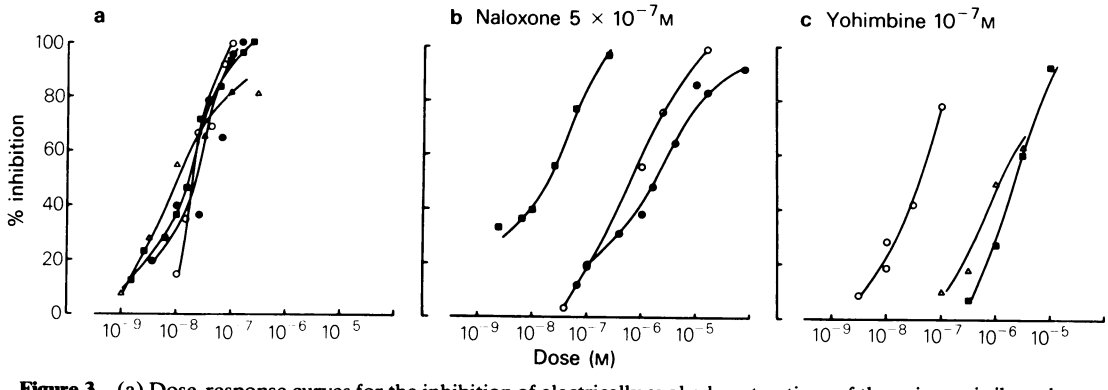

Figure 3 (a) Dose-response curves for the inhibition of electrically evoked contractions of the guinea-pig ileum by morphine (●), Met-enkephalin (○), clonidine (△) and xylazine (■). (b) Dose-response curves for the inhibition of electrically evoked contractions of the guinea-pig ileum in the presence of 5×10^{-7} M naloxone by morphine (●), Met-enkephalin (○) and xylazine (■). (c) Dose-response curves for the inhibition of electrically evoked contractions of the guinea-pig ileum in the presence of 10^{-7} M yohimbine by Met-enkephalin (○), clonidine (△) and xylazine (■). All values are the means of four experiments.

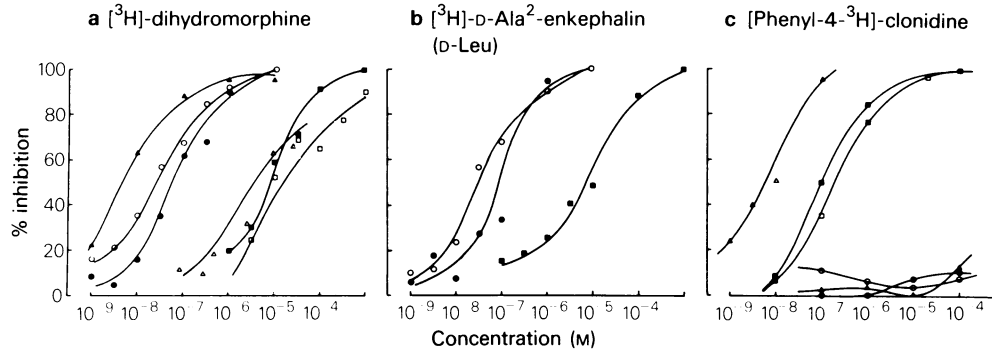

Figure 4 (a) The inhibition of specific [^{3}H]-dihydromorphine binding to rat brain membrane preparations by naloxone (▲), Met-enkephalin (○) morphine (●), clonidine (△), xylazine (■) and yohimbine (□). (b) The inhibition of specific [^{3}H]-D-Ala^2-enkephalin (D-Leu) binding to rat brain membrane preparations by Met-enkephalin (○), morphine (●) and xylazine (■). (c) The inhibition of specific [phenyl-4-^{3}H]-clonidine binding to rat brain membrane preparations by naloxone (▲), Met-enkephalin (○), morphine (●), clonidine (△), xylazine (■) and yohimbine (□). All values are the means of three determinations.

adrenoceptor ligands had activities in the micromolar range, (IC_{50} values: xylazine 8 μM, clonidine 5 μM and yohimbine 11 μM). Specific [^{3}H]-D-Ala^2-enkephalin (DADLE) binding was also displaced by both drug groups but again the opiates were more potent, (IC_{50} values: 40 nM, morphine 100 nM and xylazine 8 μM, (Figure 4b).

Clonidine receptor binding

The displacement of specific [phenyl-4-^{3}H]-clonidine binding showed a very different pattern from that seen with the opiate binding (Figure 4c). The α_2-adrenoceptor ligands were potent in displacing clonidine binding, (IC_{50} values: clonidine 6 nM, xylazine100 nM and yohimbine 200 nM), but morphine, Metenkephalin and naloxone were ineffective displacers of [^{3}H]-clonidine binding.

Discussion

The findings from the *in vivo* analgesic tests, that morphine analgesia could be antagonized by yohimbine as well as naloxone, whilst the analgesia produced by α-agonists was not sensitive to naloxone, suggest that whilst α_2-agonist analgesia did not involve opioids, the analgesia produced by morphine either involved a yohimbine-sensitive step or yohimbine possessed opiate receptor antagonist activity.

The receptor binding studies showed that the drugs active at α_2-receptors were weak displacers of opioid binding, but that opoids were not capable of displacing α_2 agonist binding even at high concentrations. The effects seen on opioid binding with the α_2-agonist xylazine were similar for both radioligands [^{3}H]-dihydromorphine and [^{3}H]-DADLE indicating that there was no difference in the ability of the α_2-agonist to displace opioids from the μ or δ receptor site.

The finding that opiate effects and binding can be affected by drugs active at α_2-receptors supports the studies of Gold *et al.* (1978) who demonstrated that treatment with the α_2-agonist, clonidine, could suppress withdrawal symptoms of opiate addiction in man. These findings may also be relevant in the interpretation of the studies of Hamburg & Tallman (1981) who demonstrated that the chronic administration of morphine in rats could apparently increase the number of clonidine binding sites in brain membrane preparations, if it is considered that their experimental protocol of chronic morphine treatment could give rise to an increase in morphine receptor sites, and that these sites could also bind clonidine and thus give rise to an apparent increase in clonidine binding sites.

In the study of the α_2-agonists on the opiate bioassay systems, the guinea-pig ileum and the mouse vas deferens, there was no indication of an interaction between the two groups of drugs. If a similar effect to that seen in the binding and analgesic testing studies were to operate one might have expected to see little effect when the α_2-agonist assays were performed in the presence of naloxone, but there would have been reduction of both the α_2-agonist and the opiate activity in the presence of yohimbine. This was not seen and there are several possible explanations for this failure to demonstrate any interaction. The first is that there may have been such a high concentration of presynaptic α_2-receptors that the effects seen at the adrenergic site (Langer, 1974; Drew, 1976) and the cholinergic site (Drew, 1978) on the vas deferens and the ileum respectively completely masked any effects that could have been mediated via opiate receptors.

The second possibility was that the interactions between opiate receptors and α_2-agonist drugs are restricted to those receptor sites within the central nervous system and finally the effects of the α_2 active drugs on the opiates in the CNS might have been indirectly mediated.

The results from the various groups of experiments on the drugs active at α_2-receptor sites and those active at opiate receptors suggest that the α_2-agonists may exert some of their central effects on opiate pathways or receptors, but that these interactions do not extend to the peripheral sites of action of the opiates.

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