Opiates and specific receptor binding of [^{3}H]clonidine

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Clonidine is a hypotensive drug that inhibits the signs of precipitated morphine withdrawal in rats (Tseng et al 1975; Vetulani & Bednarczyk 1977). It also abolishes the signs of methadone abstinence in man, thereby holding out promise in the treatment of opiate addicts (Gold et al 1978). Moreover, it is a potent analgesic (Paalzow 1974) and, although naloxone does not block its analgesic effect (Paalzow & Paalzow 1976), there is cross-tolerance to analgesic effects between clonidine and morphine (Paalzow 1978).

The study of Aghajanian (1978) strongly suggests that clonidine and morphine produce a similar action in the locus coeruleus (a depression of firing of the neurons) by acting on different receptors. However, it remains to be proved if clonidine and opiates interact with the same receptor.

To investigate this possibility we tested to see if specifically bound radioactive clonidine is displaced by morphine and the morphine-related drugs naloxone, azidomorphine and *N*-cyclopropylmethylnorazidomorphine (CAM), and by an enkephalin analogue (D-Met^2, Pro^5)-enkephalinamide.

The assays were carried out using the P_2 fraction from rat cortex (Whittaker & Barker 1971). The pellets were prepared from fresh tissue and stored at −20 °C. For incubation they were resuspended in 50 mM Tris buffer (pH 7·6) to obtain a concentration of 50 mg of original tissue cm^{-3}, and 400 mm^3 of the suspension (approx. 700 μg of protein) were mixed with 100 mm^3 of [^{3}H]clonidine (Boehringer Sohn; spec. act. 26·7 Ci mmol^{-1}) to make a final concentration of 2·125 nM, and 50 mm^3 of drug solution in 50 mM Tris buffer.

The displacing drugs were clonidine HCl (Boehringer Sohn), naloxone HCl (Endo Labs), morphine HCl (Polfa), (D-Met^2, Pro^5)-enkephalinamide acetate hydrate (synthesized in the Research Institute for Pharmaceutical Chemistry, Budapest, Hungary), azidomorphine and CAM (synthesized in the Department of Organic Chemistry, Kossuth Lajos University, Debrecen, Hungary). CAM was initially dissolved in few drops of 0·01 M HCl, and the solution was dissolved in Tris buffer.

Incubation was carried out at 25 °C for 30 min, and was terminated by filtration of the incubation mixture through GF/B glass fibre filters. The filters were placed in scintillation vials containing 10 ml of Bray's scintillation fluid. The radioactivity was measured in an Intertechnique liquid scintillation counter, at 31% yield.

Specific binding was defined as the excess of radioactivity over blanks incubated with 10 μM clonidine.

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The results are presented in Fig. 1. [^{3}H]Clonidine was specifically displaced by non-radioactive clonidine (IC50 = 10 nM), but was not displaced by enkephalinamide, a very potent opiate-like analgesic (Szekely et al 1977), morphine and naloxone (up to 1 mM). Azidomorphine, a semisynthetic morphine derivative of high analgesic activity but producing no appreciable opiate dependence (Knoll 1973), was also an extremely weak displacing agent (IC50 = 523 μM). CAM, a compound which in some respects acts as a potent morphine agonist, but is a potent antagonist of morphine analgesia (Knoll et al 1977), was more effective than azidomorphine (IC50 = 129 μM), but practically its effect was also negligible. CAM is a potent inhibitor of 'wet shakes' during the morphine withdrawal syndrome (Vetulani et al 1978), and was proposed to act as an antagonist of opiate receptors involved in the presynaptic control of noradrenaline release (Knoll et al 1977). However, it does not seem that it interferes directly with the noradrenergic receptor.

The present results suggest strongly that the interaction between opiates and the noradrenergic system, and particularly the interaction between clonidine and morphine or endogenous opiate-like substances does not result from an action at a common receptor.

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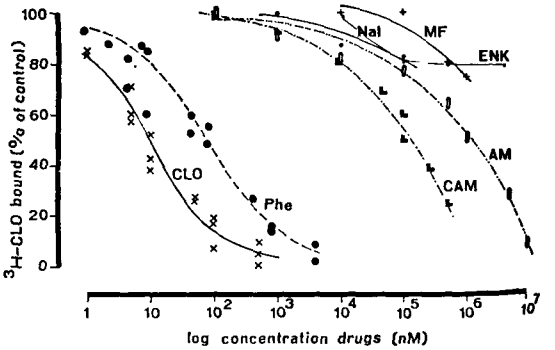

FIG. 1. The specific binding of [^{3}H]clondine (displaceable by clonidine) after increasing concentrations of drugs. The specific binding was approximately 32–45 fmol mg^{-1} protein, corresponding to about 700–800 specific counts min^{-1} per assay tube. CLO: clonidine; Phe: phentolamine; NAL: naloxone; MF: morphine; ENK: (D-Met^2, Pro^5) enkephalinamide; AM: azidomorphine; CAM: *N*-cyclopropylnorazidomophine. Ordinate: % inhibition of specific [^{3}H]clonidine binding Abscissa: drug concentration (nM).

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Effect of quipazine and fluoxetine on analgesic-induced catalepsy and antinociception in the rat

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Recent evidence indicates that brain 5-hydroxytryptamine (5-HT) may be implicated in morphine activity in the rat. For example, depletion of 5-HT with *p*-chlorophenylalanine (Goerlitz & Frey 1972; Tenen 1968), or 5,6-dihydroxytryptamine (Genovese et al 1973; Vogt 1974) reduces morphine analgesia. Destruction of either the midbrain ascending 5-HT system (Samanin et al 1970) or the descending medullary 5-HT system (Proudfit & Anderson 1975; Chance et al 1978) also blocks the analgesic effect of systemically administered morphine. Increasing 5-HT concentrations by injection of 5-hydroxytryptophan (Tulunay et al 1976) potentiates morphine-induced antinociception.

Recently, Sugrue & McIndewar (1976) reported that fluoxetine a potent inhibitor of rat brain 5-HT re-uptake (Fuller et al 1975), potentiated the antinociceptive effect of morphine but not that of methadone and pethidine.

Quipazine, a new type of antidepressant (Rodriguez & Pardo 1971) can increase central 5-HT-ergic activity by a direct action on central 5-HT receptors (Rodriguez et al 1973).

We describe the influence of quipazine and fluoxetine on two of the most characteristic effects of morphine in rats: analgesia and catalepsy. We have also used the analgesics codeine, fentanyl and pentazocine which is devoid of cataleptogenic activity (Malec et al 1977).

Male albino Wistar rats, 160–220 g, were used. Catalepsy was measured in six tests according to Simon et al (1969) these were: 3 cm block, 9 cm block, parallel bars, four corks, Buddha test, limb crossing. In the first four tests the catalepsy was considered as present if the rat remained immobile for at least 10 s. In the other tests the time was at least 2 s. The number of positive responses was counted in all tests for each animal at 15, 30, 45, 60, 90 and 120 min after i.p. injecton of analgesics.

The antinociceptive effect of analgesics was tested by the hot plate method (56 °C) using as reaction time the time between the rat being placed on the hot plate and it licking its paws or jumping off. Animals failing to reach the end point after 30 s, were removed and scored 30. Each rat was exposed to the hot plate once before treatment and again at 30 min after injection of morphine, codeine, pentazocine, and 15 min after fentanyl. The time between exposures was 60 min. Fluoxetine (10 mg kg^{-1}) and quipazine (10 and 15 mg kg^{-1}) were injected i.p. 30 min before morphine, codeine, pentazocine and 45 min before fentanyl (the action of fentanyl is short lasting). In other experiments, quipazine (15 mg kg^{-1}) was injected 10 min after morphine, codeine, pentazocine, and simultaneously with fentanyl. All doses refer to the salts.

Fluoxetine (10 mg kg^{-1}) significantly increased and prolonged catalepsy induced by morphine, codeine and fentanyl, although after 15 min of observation a reduction in morphine catalepsy was observed (Table 1). Similarly, quipazine (10 mg kg^{-1}), when injected before an analgesic, increased catalepsy (Table 1). This dose of quipazine did not alter the rat behavioural activity (Green et al 1976), but after a higher dose of quipazine alone (15 mg kg^{-1}), we observed, 5–60 min after injection, an increase in locomotor activity, stereotyped sniffing and the other effects, which suggest (Grabowska et al 1974a; Green et al 1976) the involvement of brain dopamine, as well as 5-HT. Grabowska et al (1974b) reported that quipazine (2·5, 10 mg kg^{-1}) reduced turn-

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