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ON THE ROLE OF 5-HYDROXYTRYPTAMINE IN DRUG-INDUCED ANTINOCICEPTION

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1 The effects of four specific inhibitors of 5-hydroxytryptamine (5-HT) uptake on morphine-, methadone- or pethidine-induced antinociception was studied in rats. Antinociception was assessed by means of hot plate (55°C) reaction times. The effect of the compounds on the uptake of [^{3}H]-5-HT into rat whole brain synaptosomes was also investigated.

2 Pretreatment with Org 6582, citalopram, zimelidine or femoxetine at doses devoid of antinociceptive activity potentiated morphine- but not methadone- or pethidine-induced antinociception.

3 A temporal correlation existed between the ability of Org 6582 to potentiate morphine-induced antinociception and to block synaptosomal [^{3}H]-5-HT uptake.

4 5-HT plays a critical role in the antinociceptive effect of morphine but not of methadone or pethidine.

Introduction

There is considerable evidence to suggest that the antinociceptive effect of morphine is dependent upon intact central 5-hydroxytryptaminergic pathways (Messing & Lytle, 1977; Sewell & Spencer, 1977). The elevation of brain content of 5-hydroxytryptamine (5-HT) either by the intracerebroventricular (i.c.v.) injection of 5-HT (Sparkes & Spencer, 1971) or by administering the 5-HT precursor 5-hydroxytryptophan (Contreras & Tamayo, 1967) potentiates morphine-induced antinociception in the rat. An alternative procedure for increasing 5-HT availability at the receptor is by inhibiting the uptake of the monoamine and it has been observed that pretreating rats with fluoxetine, a specific inhibitor of 5-HT uptake (Fuller, Perry & Molloy, 1975), results in a potentiated antinociceptive response to morphine (Messing, Phebus, Fisher & Lytle, 1975; Sugrue & McIndewar, 1976; Larson & Takemori, 1977). In contrast to morphine, methadone- or pethidine-induced antinociception was unaltered by fluoxetine pretreatment (Sugrue & McIndewar, 1976). The enhanced response to morphine following fluoxetine was attributed to an increased availability of 5-HT in the synaptic cleft as a consequence of 5-HT uptake inhibition. However, the possibility that the augmented response is due to factors other than blockade of 5-HT uptake warrants consideration. For example, the metabolism of morphine may be altered and it is of interest to note that fluoxetine potentiates barbiturate sedation in rats by retarding hepatic metabolism (Fuller, Rathbun & Parli, 1976). One of the objectives of this study was to determine if the ability to potentiate morphine-, but not methadone- or pethidine-induced antinociception is a property common to other specific inhibitors of 5-HT uptake. The other objective was to determine whether morphine potentiation could be correlated with inhibition of [^{3}H]-5-HT uptake into a synaptosome-rich homogenate obtained from rat whole brain. The specific inhibitors of 5-HT uptake studied were Org 6582 (±)-8-chloro-11-anti-amino-benzo-(b)-bicyclo-[3.3.1]-nona-3,6a (10a)-diene hydrochloride) (Sugrue, Goodlet & Mireylees, 1976; Mireylees, Goodlet & Sugrue, 1978) citalopram (Lu 10-171) (Hyttel, 1977), femoxetine (FG 4963) (Buus Lassen, Squires, Christensen & Molander, 1975) and zimelidine (H 102/09) (Ross, Ögren & Renyi, 1976).

Methods

In all experiments male Wistar rats weighing 180 to 250 g were used. Antinociceptive activity was assessed by means of the hot plate (55°C) test. Rats were individually placed on the hot plate 30 min after the subcutaneous injection of analgesic or saline (0.9% w/v NaCl solution). Reaction time was the time between placement on the hot plate and the licking or flicking

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of the hind paws. Reaction times were timed to the nearest second and each result was the mean of 6 to 8 observations. Statistical significance was determined by means of Student's t test (two tailed).

The uptake of [^{3}H]-5-HT into a synaptosome-rich homogenate of whole brain was determined as described in detail elsewhere (Mireylees *et al.*, 1978). Briefly, rats were killed and the brain quickly removed. The weighed brain was homogenized in 9 volumes of 0.32 M sucrose to yield a uniform suspension. Following centrifugation at 1000 g for 10 min at 4°C, the resultant supernatant was decanted and stirred to give a uniform suspension. A 0.1 ml aliquot of the suspension was preincubated for 10 min in 0.95 ml of a modified Krebs-bicarbonate buffer at 37°C under an atmosphere of 95% O_2 and 5% CO_2 in a Dubnoff metabolic shaker. [G-^{3}H]-5-HT creatinine sulphate (14.0 Ci/mmol) was purchased from the Radiochemical Centre, Amersham. Fifty µl of [^{3}H]-5-HT (final concentration of 2.6×10^{-8} M) was added and incubated for 5 min. Incubation was terminated by addition of 5 ml ice cold saline and by standing the beakers in ice for 10 min. The homogenate was separated from the medium by filtration under vacuum and after a further rinse with 5 ml ice cold saline the filter disc was placed in a counting vial to which 15 ml of Bray's scintillant was added and the samples counted. The concentration of [^{3}H]-5-HT taken up was calculated by dividing d min^{-1} g^{-1} of original tissue by d min^{-1} ml^{-1} of medium and was corrected for diffusion by subtracting the amount taken up at 0°C. Results were expressed either as % of control values or as the ED_{50} value which is defined as the dose of drug (mg/kg) required to inhibit uptake by 50% and was obtained from % uptake inhibition/log dose-response curves constructed by the method of least squares. Each log dose-response curve had at least three points and each point was the mean of at least four observations.

The following drugs were dissolved in saline: citalopram hydrobromide, femoxetine hydrochloride, methadone hydrochloride, morphine hydrochloride, pethidine hydrochloride and zimelidine dihydrochloride. Org 6582 was dissolved in distilled water. All doses refer to the free base.

Table 1 Effects of Org 6582, citalopram, zimelidine and femoxetine on hot plate reaction times

Agent	*Dose (mg/kg)*	*Reaction times (s)* *Control*	*Treated*
Org 6582	10	6.5 ± 0.6	7.2 ± 0.6
Citalopram	10	7.2 ± 0.6	6.5 ± 0.6
Zimelidine	10	7.0 ± 0.6	6.3 ± 0.5
Femoxetine	20	6.2 ± 0.5	7.3 ± 0.7

Reaction times (s) are the mean ± s.e. mean of 6 to 8 observations. Agents were injected intraperitoneally 1 h before rats were placed on hot plate (55°C).

Results

Antinociceptive studies

Hot plate reaction times were unaltered 1 h after the intraperitoneal injection of Org 6582 (10 mg/kg), citalopram (10 mg/kg), zimelidine (10 mg/kg) or femoxetine (20 mg/kg) (Table 1).

The dose of analgesic selected was one which approx. doubled the reaction time of saline-treated rats. Morphine (2 mg/kg), methadone (1 mg/kg) and pethidine (10 mg/kg) all significantly increased hot plate reaction times 30 min after subcutaneous injection (Table 2). The reaction time of morphine-treated rats was significantly increased following 30 min pretreatment with Org 6582, citalopram, zimelidine or femoxetine. In contrast, the prior administration of the 5-HT uptake inhibitors did not alter the reaction times of rats receiving methadone or pethidine

Table 2 Effects of 5-hydroxytryptamine uptake inhibitors on drug-induced antinociception

Pretreatment	*Dose (mg/kg)*	*Reaction times (s)* *Morphine*	*Methadone*	*Pethidine*
None		13.9 ± 1.4**	12.5 ± 1.1*	11.6 ± 0.5**
Org 6582	10	27.0 ± 1.2†	13.3 ± 1.2	13.1 ± 1.1
Citalopram	10	25.4 ± 1.7†	14.4 ± 1.4	12.0 ± 1.3
Zimelidine	10	24.6 ± 2.7†	13.3 ± 1.0	11.6 ± 1.2
Femoxetine	20	23.6 ± 2.5†	14.3 ± 1.5	12.7 ± 1.6

Uptake inhibitors were injected intraperitoneally 30 min before the analgesic. Morphine (2 mg/kg) methadone (1 mg/kg) or pethidine (10 mg/kg) was injected subcutaneously 30 min before rats were placed on hot plate. Each result is mean ± s.e. mean of 6 to 8 observations. Control reaction times for analgesic groups were: morphine 6.7 ± 0.4, methadone 7.7 ± 0.3 and pethidine 6.5 ± 0.6 s.
*Differs from control *$P < 0.01$; **$P < 0.001$; †differs from morphine †$P < 0.001$.

(Table 2). The effect of different doses of the 5-HT uptake inhibitors on morphine-induced antinociception is shown in Table 3. The minimum doses required for a significant increase in reaction times were Org 6582 and zimelidine 5 mg/kg, citalopram 10 mg/kg and femoxetine 20 mg/kg. Following 24 h pretreatment only Org 6582 (10 mg/kg, i.p.) significantly increased reaction times to morphine. Org 6582 (10 mg/kg, i.p.) was ineffective following 48 h pretreatment (Table 4).

Uptake studies

The effect of 1 h pretreatment with Org 6582, citalopram, femoxetine or zimelidine on the uptake of [^{3}H]-5-HT into synaptosomes obtained from rat whole brain was investigated and results are summarized in Table 5. ED_{50} values ranged from 3.2 to 6.5 mg/kg, with Org 6582 being the most potent and zimelidine the least potent. Following 24 h pretreatment only Org 6582 (10 mg/kg, i.p.) markedly decreased the uptake of [^{3}H]-5-HT by rat brain synaptosomes. Org 6582 (10 mg/kg, i.p.) was devoid of effect 48 h after injection (Table 6).

Discussion

There is evidence to suggest that morphine-induced antinociception is dependent upon intact central

Table 3 Effect of different doses of 5-hydroxytryptamine uptake inhibitors on morphine-induced antinociception

Pretreatment	*Dose (mg/kg)*	*Pretreatment plus morphine Reaction times (s)*
None		10.8 ± 1.2†
Org 6582	2.5	10.7 ± 0.7
Org 6582	5.0	21.3 ± 1.6**
Zimelidine	2.5	11.7 ± 1.0
Zimelidine	5.0	15.1 ± 1.1*
Citalopram	5.0	13.3 ± 1.2
Citalopram	10.0	20.0 ± 1.2**
Femoxetine	10.0	11.3 ± 1.2
Femoxetine	20.0	19.4 ± 1.3**

Dose of morphine was 2 mg/kg s.c. and control reaction time was 6.5 ± 0.5 s. Remainder of legend as for Table 2.
Differs from control †$P < 0.01$; *differs from morphine *$P < 0.05$; **$P < 0.001$.

Table 5 Blockade of [^{3}H]-5-hydroxytryptamine uptake into synaptosomes obtained from rat whole brain

Compound	*ED_{50} (mg/kg)*	*95% confidence limits (mg/kg)*
Org 6582	3.2	2.8 – 3.5
Citalopram	4.6	3.9 – 5.3
Femoxetine	5.6	4.9 – 6.2
Zimelidine	6.5	5.4 – 7.5

Compounds were injected intraperitoneally 1 h before death. ED_{50} values were obtained from % uptake inhibition/log dose-response curves constructed by the method of least squares. Each log dose-response curve had at least 3 points and each point was the mean of at least 4 determinations.

Table 4 Effect of 5-hydroxytryptamine uptake inhibitors at different pretreatment times on morphine-induced antinociception

Pretreatment	*Time (h)*	*Dose (mg/kg)*	*Pretreatment plus morphine Reaction times (s)*
None			14.1 ± 1.4†
Org 6582	24	10	20.9 ± 2.7*
Org 6582	48	10	13.3 ± 0.9
Citalopram	24	10	13.3 ± 0.9
Zimelidine	24	10	15.9 ± 1.3
Femoxetine	24	20	15.3 ± 2.5

Uptake inhibitors were injected intraperitoneally 24 or 48 h before morphine (2 mg/kg, s.c.) and control reaction times were 6.2 ± 0.5 s. Remainder of legend as for Table 2.
Differs from control †$P < 0.001$; differs from morphine *$P < 0.05$.

Table 6 Blockade of rat brain synaptosomal [^{3}H]-5-hydroxytryptamine uptake at different times after injection

Compound	*Pretreatment time (h)*	*Dose (mg/kg)*	*% of control*
Org 6582	24	10	43.3 ± 1.0
Org 6582	48	10	99.0 ± 2.0
Citalopram	24	10	102.2 ± 2.8
Zimelidine	24	10	100.9 ± 1.3
Femoxetine	24	10	82.8 ± 1.3
Femoxetine	24	20	80.3 ± 1.3

Uptake inhibitors were injected intraperitoneally at times stated before death. Results are expressed as % of control and are the mean ± s.e. mean of 4 determinations.

ascending and descending 5-hydroxytryptaminergic nerve tracts (Yaksh, Plant & Ruddy, 1977; Deakin & Dostrovsky, 1978). The results of this study confirm the importance of 5-HT in the antinociceptive action of morphine. In contrast, 5-HT may not play such a critical role in methadone- or pethidine-induced antinociception.

Org 6582, citalopram, femoxetine and zimelidine are all potent inhibitors of 5-HT uptake, as assessed by the effect of 1 h pretreatment on the uptake of [^{3}H]-5-HT into synaptosomes obtained from rat brain. The intraperitoneal doses required for a 50% inhibition of uptake ranged from 3.2 to 6.5 mg/kg.

Hot plate reaction times were unaltered following 1 h pretreatment with Org 6582, citalopram, zimelidine (all 10 mg/kg, i.p.) or femoxetine (20 mg/kg, i.p.). Following 30 min pretreatment with these doses, all four 5-HT uptake inhibitors potentiated the antinociceptive effect of morphine. One of the objectives of this study was to determine if morphine potentiation could be correlated with inhibition of 5-HT uptake. A precise correlation does not exist between the two phenomena. For example, femoxetine and zimelidine are essentially equipotent at blocking [^{3}H]-5-HT uptake (ED_{50} values of 5.6 and 6.5 mg/kg respectively) yet zimelidine at a dose of 10 mg/kg potentiates the ability of morphine to increase hot plate reaction times whereas the same dose of femoxetine does not. This lack of total correlation may be due to the possibility that 5-HT uptake inhibitors vary quantitatively in their efficacy in blocking 5-HT uptake in different brain regions and that it is blockade of 5-HT uptake in a critical brain region which accounts for the observed potentiation of morphine-induced antinociception. An excellent temporal correlation exists between morphine potentiation and [^{3}H]-5-HT uptake inhibition as indicated by the finding that of the four agents studied, only Org 6582 markedly blocked [^{3}H]-5-HT uptake and potentiated antinociception after 24 h pretreatment. Following 48 h pretreatment Org 6582 was devoid of effect on both paradigms.

There is evidence to suggest that 5-HT may not be critically implicated in the antinociceptive effect of methadone or pethidine. For example, lesions of the nucleus raphé medianus attenuate the antinociceptive effect of morphine, but not that of methadone or pethidine (Samanin, Ghezzi, Mauron & Valzelli, 1973; Chance, Krynock & Rosecrans, 1978). A further difference between morphine and pethidine is the finding that the antinociceptive effect of the former is attenuated whereas that of the latter is increased in rats depleted of brain 5-HT content after feeding on a tryptophan deficient diet (Phebus, 1978). Moreover, of morphine, methadone and pethidine, only morphine increases rat brain 5-HT turnover following acute administration and this effect of morphine is blocked by pretreatment with the specific narcotic antagonist, naloxone (Goodlet & Sugrue, 1974; Sawa & Oka, 1976). The inability of Org 6582, citalopram, femoxetine or zimelidine, at doses potentiating morphine, to alter the antinociceptive response to methadone or pethidine is in agreement with previous data for fluoxetine, another specific inhibitor of 5-HT uptake (Sugrue & McIndewar, 1976). In contrast to morphine, *in vitro* studies have revealed that methadone is a potent and pethidine a moderate inhibitor of 5-HT uptake into rabbit brain synaptosomes (Ciofalo, 1974) and human platelets (Ahtee & Saarnivaara, 1973) respectively. Sewell & Spencer (1977) have speculated that the inability of 5-HT uptake inhibitors to potentiate the antinociceptive effect of methadone or pethidine may be due to the fact that 5-HT uptake is partially inhibited by the two analgesics. However, *in vivo* findings argue against this hypothesis. For example, *in vivo* 5-HT uptake inhibition is often studied by determining the effect of drug pretreatment on the ability of *p*-chloroamphetamine to lower rat brain 5-HT levels. Org 6582, citalopram, femoxetine and zimelidine are all more potent than chlorimipramine at blocking the *p*-chloroamphetamine-induced fall in rat brain 5-HT content (Sugrue, Charlton, Mireylees & McIndewar, 1978; unpublished observations). In contrast, the ability of *p*-chloroamphetamine to lower the concentration of 5-HT in rat brain was unaltered by methadone pretreatment (Fuller & Perry, 1976). Other studies have also revealed that a very large dose of methadone (30 mg/kg) was needed for an approx. 50% inhibition of [^{3}H]-5-HT uptake into rat hypothalamic synaptosomes 1 h after injection (Moffat & Jhamandas, 1976). Finally, Carlsson & Lindqvist (1969) have shown that pethidine is a very weak inhibitor of the 4-methyl-α-ethyl-*m*-tyramine (H 75/12)-induced fall in the level of 5-HT in mouse brain.

In summary, four specific inhibitors of 5-HT uptake, Org 6582, citalopram, femoxetine and zimelidine, potentiate the antinociceptive effect of morphine, but not that of methadone or pethidine, in rats. There is an excellent temporal correlation between the ability of Org 6582 to potentiate morphine-induced antinociception and to block 5-HT uptake by rat whole brain. The findings of this study emphasize the importance of 5-HT in morphine-, but not methadone- or pethidine-induced antinociception.

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