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Blockade by trazodone of naloxone-precipitated jumping in morphine-dependent rats: correlation with brain levels of *m*-chlorophenylpiperazine

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Recent studies have shown that *m*-chlorophenylpiperazine (mCPP) displaces [^{3}H]-5-hydroxytryptamine (5-HT) binding to rat brain membranes (Samanin et al 1980a) and produces pharmacological and biochemical effects indicative of a stimulatory action on postsynaptic 5-HT receptors in the brain (Samanin et al 1979; Garattini 1979).

Since mCPP has been found in rat urine and brain after treatment with trazodone (Melzacka et al 1979; Caccia et al 1981a), a recently introduced antidepressant (Fabre et al 1979), the possibility exists that trazodone exerts 5-HT-like effects through the formation of mCPP. This hypothesis was recently proposed on the basis of pharmacological studies with trazodone in rats (Maj et al 1979, 1980), although these experiments provided no direct evidence that substantial amounts of mCPP were indeed present in the brain of trazodone-treated rats.

In an attempt to obtain more direct information on trazodone's ability to form sufficient mCPP to cause 5-HT-like effects, we measured brain mCPP concentrations in rats treated with doses of trazodone and mCPP producing comparable effects in a test useful to reveal 5 HT-activities. Naloxone-precipitated jumping in morphine-dependent rats was selected since recent findings have shown that this sign is selectively blocked by drugs increasing 5-HT transmission in the brain (Samanin et al 1980b).

Materials and methods

Male CD-COBS rats (Charles River, Italy), about 200 g at the beginning of the experiments, received two intraperitoneal injections of 10 mg kg^{-1} of morphine hydrochloride at 10 a.m. and 6 p.m. on the first day of treatment. The dose of morphine was doubled every other day to reach a total daily dose of 160 mg kg^{-1} on the 7th day. The largest dose was given for 3 more days. On the 11th day the animals received the last injection of morphine at 10 a.m., and 4 h later the abstinence syndrome was precipitated by an intraperitoneal injection of naloxone HCl (1 mg kg^{-1}). Trazodone (12·5 and 25 mg kg^{-1}) and mCPP (0·25, 0·5 and 1 mg kg^{-1}) were administered orally 1 h before the narcotic antagonist. Withdrawal symptoms within 30 min were recorded by observers unaware of the treatments, according to Samanin et al (1980b). The data are expressed as the numbers of positive animals in the various experimental groups, and differences were analysed statistically by the χ^2 test. At the end of the experiments (30 min after naloxone injection), the animals were killed and their brains removed for the determination of trazodone and mCPP by the gas liquid chromatographic method described by Caccia et al (1981b).

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Results and discussion

In agreement with previous findings (Samanin et al 1980b), jumping, together with ptosis and diarrhoea, was mostly observed in the morphine-dependent animals when injected with naloxone. Other signs described in the literature such as wet-dog shakes, flat posture, teeth chattering, salivation, vocalization on touch and dyspnoea were observed, but less frequently. As shown in Table 1, doses of 12·5 and 25 mg kg^{-1} trazodone blocked jumping to the same extent as 0·25 and 0·5 mg kg^{-1} of mCPP. Since comparable brain mCPP concentrations were found after treatment with these doses of trazodone and mCPP, the results suggest that trazodone blocks jumping in morphine-dependent rats through the formation of mCPP.

Table 1. Withdrawal signs and brain mCPP concentrations in trazodone- and mCPP-treated rats. Drugs were given 1 h before naloxone and the animals were killed 30 min after naloxone injection for the determination of trazodone and mCPP brain concentration. See methods for details of morphine dependence induction and assessment. * $P < 0{\cdot}01$ compared with saline (χ^2 test).

Treatment mg kg^{-1} orally	Withdrawal signs jumping	Brain concns (μg g^{-1}) Trazodone	mCPP
0·9% NaCl	16/20	—	—
mCPP 0·25	8/20*	—	0·20 ± 0·06
mCPP 0·5	1/20*	—	0·50 ± 0·07
mCPP 1·0	0/20*	—	1·23 ± 0·25
0·9% NaCl	11/20	—	—
Trazodone 12·5	4/20*	0·44 ± 0·10	0·29 ± 0·06
Trazodone 25·0	2/20*	1·32 ± 0·11	0·65 ± 0·10

The present findings support the recent suggestion that trazodone causes 5HT-like activities through the formation of mCPP (Maj et al 1979; Caccia et al 1981a). These results raise the possibility that mCPP may be formed in depressed patients treated with trazodone, contributing to its antidepressant activity. It may be relevant to mention that at doses higher than 1 mg kg^{-1} mCPP increases noradrenaline metabolism in the rat brain (Invernizzi et al 1981), an effect which may also contribute to the antidepressant activity. The present data further suggest that trazodone may inhibit some withdrawal syndrome signs in subjects from whom narcotics had been withdrawn.

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Observations on urinary excretion of codeine in illicit heroin addicts

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The metabolism of morphine and heroin in man is still under discussion. Baselt (1978) and Gorrod & Beckett (1978) reported codeine as a minor morphine metabolite via *O*-methylation in man, Boerner & Abbott (1973) during opiate screening of urines of 75 heroin addicts, found codeine and morphine in 85% of the samples, the codeine in amounts between 12 and 15% of the morphine present. To prove that the codeine formed occurred via *O*-methylation of morphine the authors examined 5 subjects (4 non-tolerant and 1 tolerant) given morphine sulphate in various daily doses. Codeine was present in all urines with a significant larger percentage in the tolerant subject (0·7–6% relative to morphine).

Yeh (1974) in a study on urine specimens collected from post-addict volunteers given morphine sulphate (240 mg daily) detected codeine in minute amounts (0·015% relative to morphine), but attributed its presence to it being an impurity (0·04%) in the morphine injected and not to a biotransformation product. The large amount of codeine found in urine samples by Boerner & Abbott (1973) might be the result of either on-column acetylation of the urine extract, or contamination of heroin of illicit source with acetylcodeine. Boerner & Roe (1975) and Yeh (1975) continued their controversy.

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Yong & Lik (1977) report a study of the urinary excretion patterns in opiates addicts: where illicit morphine and heroin had been injected, codeine was also detected in the urines, because codeine was an impurity in illicit morphine and acetylcodeine an impurity in illicit heroin. In cases of licit morphine and heroin intake, codeine was not detected. In 1979, Yeh et al reported new metabolites of morphine in several mammalian species, but did not detect codeine. We have carried out a study on 220 urine samples with previously positive results for opiates by EMIT (DAU—Syva Corp., Palo Alto, California) assay.

The urine specimens were divided in two groups:
Group I: 70 urine samples of subjects known to be receiving morphine only, in doses of 60–120 mg daily (18 in chronic pain treatment and 52 opiates addicts on a controlled deconditioning program on morphine only).
Group II: 150 urine samples of known heroin addicts.

Methods

Each of the urine samples was divided in two aliquots, the first for morphine detection and the second for codeine detection. Morphine extraction and its quantitative determination were carried out according to Felby et al (1974). The codeine was extracted with ether at pH = 14 (NaOH 20%) on acidic hydrolysed urines. The detection was carried out by t.l.c. on silica gel precoated plates with ethanol–benzene–dioxane–ammonia (50:40:5:5) as solvent; iodoplatinate and Marquis as reagents (Clarke 1975).