binding sites, particularly on a brain regional basis, and may confirm the existence of separate 5-HT receptors.

As proposed by others (Hruska & Silbergeld 1980), the present data also show that caution should be exercised when drugs dissolved in ethanol are evaluated in receptor binding assays.

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(—)-*m*-Chlorophenyl-piperazine, a central 5-hydroxytryptamine agonist, is a metabolite of trazodone

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Trazodone an antidepressant introduced recently in clinical practice, was found to inhibit 5-hydroxytryptamine (5-HT) 5-HT uptake in the rat brain and blood platelets (Garattini et al 1976). In this study, a possible metabolite of trazodone, (−)-*m*-chlorophenylpiperazine (mCPP) also inhibited 5-HT uptake, suggesting that it might contribute to the effect of the parent compound. Subsequent studies with mCPP showed that it behaves like a central 5-HT-agonist (Samanin et al 1979; Rokosz-Pelc et al 1980) and this agrees with its effectiveness in displacing [^{3}H] 5-HT binding to brain membranes (Samanin et al 1980).

A recent article (Maj et al 1979) reports that trazodone at low doses has anti-5-HT properties, while at higher doses it acts as a central 5-HT agonist. This latter effect was attribute to the formation of mCPP in trazodone-treated animals (Maj et al 1980) although this compound was not measured directly. mCPP has been recently identified as the *N*-glucuronide in rat urine (Melzacka et al 1979) confirming this route of trazodone's metabolism.

The aim of the present study was to prove that substantial amounts of mCPP are formed in the brain after oral administration of trazodone. To obtain preliminary information on the significance of mCPP formation for the effects of trazodone, brain levels of mCPP in rats treated with trazodone were compared with those found after pharmacologically and biochemically effective doses of mCPP (Samanin et al 1979).

Male CD-COS rats (Charles River, Italy), ≈250 g, were treated orally with trazodone hydrochloride (12·5, 25 and 50 mg kg^{-1}) or mCPP hydrochloride (1 and 5 mg kg^{-1}) and killed at various times after. mCPP was extracted from plasma (2 ml) and brain homogenates (0·1 M HCl, 6: v/w) with 5 ml of benzene, after addition of 1 M NaOH and 4-amino-1-(6-chloro-2-pyridil)-piperidine as an internal marker. To 1 ml of the benzene phase, 50 μl of heptafluorobutyric anhydride (a 25% v/v solution methyl acetate) was added and the samples left to stand for 30 min at 60 °C. After the reaction the samples were washed with water (1 ml) and 5% aqueous ammonia solution (0·5 ml) and 1–2 μl of the benzene phase were injected into the gas chromatographic column.

Samples were analysed on a C. Erba Fractovap Mod. 2150 equipped with an electron capture detector, using a 2 m by 3 mm internal diam. column containing 3% OV 17 on Supelcoport (Supelco, Inc.). The conditions were as follows: column temp. 205 °C, injector and detector temperature 250 °C. Carrier gas was nitrogen at a flow rate of 35 ml mm^{-1}. Standard curves were determined for each experiment by adding known amounts of mCPP and internal standard to brain homogenates and plasma samples and determining the ratio of the mCPP to internal standard peak areas. Specificity of the analyses was confirmed by gas chromatography combined with mass spectrometry (g.l.c.-m.s.). The sensitivity of the method was 10 ng ml^{-1} plasma or 50 ng g^{-1} brain. The recovery from plasma and brain was 90 ± 5% and 83 ± 7% respectively.

Table 1 shows that the biotransformation of trazodone in rats yields measurable amounts of mCPP in both plasma and brain. After oral trazodone (12·5, 25 and 50 mg kg^{-1}) the metabolite reached peak plasma concentrations between 1 and 2 h, declining thereafter in a monoexponential manner with an apparent half-life

* Correspondence.

Table 1. Plasma (P) and brain (B) concentrations (ng ml^{-1} or g) (with s.d.) of *m*-chlorophenyl-piperazine after oral administration of trazodone to rats.

Time after administration	12·5 mg kg^{-1} oral		25 mg kg^{-1} oral		50 mg kg^{-1} oral	
	P	B	P	B	P	B
15 min	10 (5)	120 (13)	25 (3)	515 (69)	49 (6)	984 (199)
30 min	14 (2)	325 (60)	32 (7)	1072 (103)	61 (9)	1670 (201)
1 h	15 (3)	424 (123)	35 (6)	1085 (283)	78 (18)	2388 (275)
2 h	15 (5)	315 (130)	44 (5)	1173 (192)	81 (9)	2474 (175)
4 h	10 (5)	84 (13)	15 (3)	404 (10)	31 (13)	774 (150)
6 h	<10	<50	10 (2)	161 (20)	20 (8)	402 (111)
AUC (ng ml^{-1} or g × h)	50+	1162	174	4333	348	9086
$Tt_{\frac{1}{2}}$ (h)	N.D.	1·24	1·87	1·40	1·98	1·52

Each value is the mean of 4 rats.
The kinetic parameters were calculated assuming a one-compartment open model.
* Calculated only up to 4 h.

($t_{\frac{1}{2}}$) of about 1·9 h. At the lowest dose tested (12·5 mg kg^{-1} orally) it was impossible to calculate any kinetic parameters because the mCPP concentrations reached a plateau close to the sensitivity limits of the method (10 ng ml^{-1}).

mCPP disappeared slightly faster from brain than from plasma; the brain $t_{\frac{1}{2}}$ of mCPP was about 1·2–1·5 h, which is comparable with the half-life of trazodone in the rat (1·7 h) (Yamato et al 1974).

Unlike trazodone (Yamato et al 1974), mCPP accumulated in the brain, reaching concentrations several times those in plasma. These findings were reflected in the area under the curve (AUC), the kinetic parameter that indicates the total exposure in relation to the time. At all the oral doses tested the brain AUCs of the metabolite were about 25 times the plasma AUCs.

Both plasma and brain AUCs were influenced by a factor greater that the dose increase. When the dose of trazodone was raised 2 or 4 times the AUCs of the metabolites rose about 3·5 and 8 times.

These differences in relation to the dose were also evident after oral administration of mCPP to rats (Table 2), suggesting that the kinetics of this compound may be dose-dependent.

Table 2. Plasma (P) and brain (B) concentrations (ng ml^{-1} or g (with s.d.) of *m*-chlorophenylpiperazine after oral administration to rats.

Time after administration	1 mg kg^{-1} oral		5 mg kg^{-1} oral	
	P	B	P	B
15 min	16 (4)	355 (21)	86 (16)	1660 (540)
30 min	25 (5)	705 (35)	99 (10)	3170 (170)
1 h	15 (1)	362 (28)	106 (12)	3230 (430)
2 h	12 (2)	225 (26)	55 (8)	1530 (250)
4 h	<10	87 (37)	36 (12)	880 (188)
6 h	<10	<50	12 (2)	200 (40)
AUC (ng ml^{-1} or g × h)	55	1160	323	8547
$t_{\frac{1}{2}}$ (h)	1·53	1·24	1·71	1·33

Each value is the mean of 4 rats.
The kinetic parameters were calculated assuming a one-compartment open model.

In these experimental conditions mCPP reached a peak between 0·5 and 1 h with plasma and brain concentrations slightly higher than after oral administration of trazodone. However, that plasma and brain AUCs of mCPP after oral administration of 1 and 5 mg kg^{-1} were comparable to those after oral administration of 12·5 and 50 mg kg^{-1} of trazodone.

These results confirm the suggestion (Maj et al 1980) that mCPP may play an important role in the 5-HT-agonistic effects of relatively high doses of trazodone in the rat. Whether this is also true for the anti-5-HT effects found after low doses of trazodone remains to be clarified.

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