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Brain-to-blood partition and *in vivo* inhibition of 5-hydroxytryptamine reuptake and quipazine-mediated behaviour of nefazodone and its main active metabolites in rodents

[1]Angelo Nacca, [1]Giovanna Guiso, [1]Claudia Fracasso, [1]Luigi Cervo & [1,2]Silvio Caccia

[1]Istituto di Ricerche Farmacologiche "Mario Negri" Via Eritrea 62, 20157 Milan, Italy

1 The brain/plasma partition of nefazodone, hydroxynefazodone (OHNFZ) and m-chlorophenyl-piperazine (mCPP) and their antagonism of p-chloroamphetamine (PCA)-induced 5-hydroxytryptamine (5-HT) depletion and quipazine-induced head twitches were compared in rodents.

2 Nefazodone (30 mg kg^{-1}, i.p.) rapidly entered the brain but concentrations were exceeded by mCPP, the metabolic ratio being 47 and 10 in the mouse and rat respectively. OHNFZ was detectable in plasma but never in brain.

3 Brain concentrations of OHNFZ in the mouse (30 mg kg^{-1}, i.p.) were less than 10% of those in plasma, confirming a poor blood-brain barrier penetration. Concentrations of its metabolite mCPP were similar to those after 5 mg kg^{-1}(i.p.) mCPP.

4 In the mouse, nefazodone (30 mg kg^{-1}) antagonized the 5-HT depleting effect of PCA 2 h after dosing, when it had disappeared from brain but when mCPP concentrations were similar to those after 5 mg kg^{-1} (i.p.) mCPP. However, mCPP antagonized PCA less than nefazodone.

5 In the rat, nefazodone pretreatment (30 mg kg^{-1}, 15 min) prevented (97% of inhibition) quipazine-induced head twitches. The effect was weaker (65% of inhibition) but significant when only mCPP was detected in brain. Analysis of brain concentrations of the two compounds after their ED_{50} against quipazine indicated that both contributed to the effect, although nefazodone was more active than mCPP in terms of concentrations required to obtain a comparable reduction of twitches.

6 These findings show that mCPP concentrates in the brain following injection of nefazodone and may play a role in preventing quipazine-induced behaviour and PCA-induced 5-HT depletion. In contrast OHNFZ poorly enters the brain and its *in vivo* activity is mostly due to its biotransformation to mCPP.

Keywords: Nefazodone; active metabolites; pharmacokinetics; inhibition of 5-HT-reuptake and quipazine-induced headshakes; rodents

Abbreviations: 5-HT, 5-hydroxytryptamine; AUC, area under the curve; C_{max}, maximum concentration; EM, extensive metabolizers; mCPP, m-chlorophenyl-piperazine; OHNFZ, hydroxynefazodone; PCA, p-chloroamphetamine; PM, poor metabolizers; $t_{1/2}$, half-life; t_{max}, time to maximum concentration

Introduction

Nefazodone is one of the newer antidepressant agents. While it shares some structural similarities with trazodone, which also has a chlorophenyl-piperazine side-chain, its mechanism of action differs from all current antidepressants. *In vitro* and *in vivo* studies in rodents indicate that nefazodone has potent antagonistic effects on $5\text{-}HT_{2A}$ postsynaptic receptors and moderate 5-hydroxytryptamine (5-HT) transporter inhibiting properties which together may modulate serotoninergic neurotransmission through postsynaptic receptors, particularly $5\text{-}HT_{1A}$ receptors (Taylor *et al*., 1995; Davis *et al*., 1997).

Nefazodone is extensively biotransformed in all species before excretion (Shukla *et al*., 1993; Mayol *et al*., 1994; Greene & Barbhaiya, 1997). Of the known metabolites two retain pharmacological activity *in vitro* and *in vivo*, namely hydroxynefazodone (OHNFZ) and m-chlorophenyl-piperazine (mCPP). OHNFZ shows affinity *in vitro* for $5\text{-}HT_{2A}$ receptors and the 5-HT uptake site and has pharmacological actions in rodents similar to nefazodone (Taylor *et al*., 1986; 1995). mCPP displays a potency similar to the parent drug for 5-HT reuptake site and has a lower affinity for $5HT_{2A}$ receptors (Taylor *et al*., 1995). However its pharmacological properties also include agonist (or partial agonist) effects at $5\text{-}HT_1$ subtypes and $5\text{-}HT_{2C}$ receptors and it may be an antagonist at $5\text{-}HT_3$ receptors (Murphy *et al*., 1991; Kennett 1993; Kennett & Curzon, 1991; Fiorella *et al*., 1995).

The metabolites OHNFZ and mCPP are both detectable in human plasma at the usual therapeutic doses of nefazodone. Systemic exposure to OHNFZ, in terms of plasma maximum concentrations (C_{max}) and area under the curve (AUC), is about 30–40% of that of nefazodone, supporting pharmacological findings that this metabolite presumably contributes to the final outcome (Greene & Barbhaiya, 1997; Davis *et al*., 1997). Exposure to mCPP is generally low, except in poor metabolizers (PM) of dextromethorphan CYP2D6-mediated O-demethylation in whom exposure to the metabolite is about half that of nefazodone after repeated dosing. This has led to the suggestion that in most populations it plays a minor role in the drug's overall effect (Greene & Barbhaiya, 1997; Davis *et al*., 1997).

Information is scarce on the pharmacokinetics of nefazodone and its active metabolites in rodents. The brain-to-blood partition of metabolites compared to that of the parent drug is also unavailable despite the fact that total blood concentrations do not always reflect the metabolite-to-parent drug ratio

[2]Author for correspondence.

in the central nervous system (CNS) (Caccia & Garattini, 1990; Lin & Lu, 1997; Caccia, 1998). Therefore the relative contribution of the parent drug and its two main metabolites to the overall pharmacological activity is still not clearly established, complicating the extrapolation of pharmacological findings in rodents to the clinical situation.

The present study assessed the plasma and brain kinetics of nefazodone, OHNFZ and mCPP after single i.p. doses of nefazodone in the mouse and rat. The activity of the metabolites was compared with that of the parent drug in *in vivo* tests believed to reflect 5-HT_{2A} receptor blockade (inhibition of quipazine-induced head twitches in rats) and 5-HT reuptake inhibition (PCA-induced depletion of brain 5-HT). Brain concentrations of OHNFZ and mCPP were also compared with those obtained after administration of neuropharmacologically active doses of the metabolites to gain further information on their role in the parent drug's action.

Methods

Drug and sources

Nefazodone hydrochloride and OHNFZ hydrochloride were kindly supplied by the Bristol-Myers Squibb Company (U.S.A.). mCPP dihydrochloride, quipazine maleate and PCA hydrochloride were provided by Sigma Chemical Co. (U.S.A.).

Animals and drug treatment

Male CD1 albino mice, 20–25 g, and male CD-COBS rats, 150–175 g (Charles River, Italy) were used. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. n.116, G.U., suppl. 40, 18 Febbraio 1992, Circolare No. 8, G.U., 14 Luglio 1994) and international laws and policies (EEC Council Directive 86/609, OJ L 358,1, Dec. 12, 1987; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996).

Nefazodone was dissolved in 5% Tween 80 in water and its metabolites in distilled water. All compounds were injected i.p. at the constant volume of 2 ml kg^{-1} body weight.

Pharmacokinetic studies

Animals were injected with 30 mg kg^{-1} nefazodone hydrochloride and killed by decapitation at various times thereafter for parallel determination of the parent compound and its metabolites in plasma and whole brain. Mixed arteriovenous trunk blood was collected in heparinized tubes, centrifuged at $3000 \times g$ for 10 min and the plasma was stored at $-20°C$. Brain was removed immediately and brain areas were dissected, blotted with paper to remove excess surface blood and quickly frozen in dry ice.

Nefazodone and its metabolites were extracted from plasma and brain homogenate by a solid-liquid extraction procedure and quantified by high-performance liquid chromatography (HPLC) with UV detection (254 nm). Briefly, after adding the internal standard (trazodone) the plasma samples were deproteinized with CH_3OH (1 : 2 v/v) and centrifuged at $3000 \times g$ for 10 min (at 4°C). One ml of 0.1 N phosphate buffer, pH 5, was added to the supernatant and the samples were added to LC 18 SPE cartridges (Supelco, U.S.A.) pre-wetted with 2 ml of CH_3OH, 2 ml of H_2O and 2 ml of 0.1 N phosphate buffer, pH 5. Cartridges were washed with 4 ml 0.1 N phosphate buffer, 6 ml of CH_3CN-H_2O (60 : 40 v/v) and 0.3 ml of 1 M NH_4OH in CH_3OH. The compounds were removed by eluting the cartridges with 3 ml of 1 M NH_4OH in CH_3OH, which was evaporated to dryness. The residue was dissolved in the mobile phase – CH_3OH:CH_3CN:0.01 M $(NH_4)_2HPO_4$ (5 : 32 : 63, v/v) containing 0.01 M tetramethyl-ammonium hydroxide – and injected into the HPLC column (Phenyl μBondapack, 300 mm × 3.9 mm I.D).

Brain tissue was homogenized (10 ml g^{-1}) in 0.1 M KH_2PO_4-CH_3CN, pH 5, (60 : 30 v/v) and 2.5 ml were centrifuged at $3000 \times g$ for 15 min (at 4°C). The supernatant was processed essentially as described for plasma (the wash with 0.3 ml of 1 M NH_4OH was unnecessary).

The lower limits for quantification of nefazodone and its metabolite in plasma were about 0.05 nmol ml^{-1} in the rat and 0.2 nmol ml^{-1} in the mouse, using respectively 1 ml and 0.3 ml of plasma; in brain the limit was about 0.3 nmol g^{-1} in both species, using 2 ml of brain homogenate or approximately 200 mg of brain tissue. At these concentrations the coefficients of variation (C.V.) for the precision and reproducibility of the assay were between 10–15%. Higher concentrations gave C.V. less than 10%.

Antagonism of PCA-induced depletion of 5-HT

Mice were injected with nefazodone (30 mg kg^{-1}), OHNFZ (30 mg kg^{-1}) and mCPP (5 mg kg^{-1}) 15 min before PCA (21 mg kg^{-1}) (Hemrick-Luecke *et al*., 1994) and animals were killed 120 min after the test compound. Brains were removed, the cortex was rapidly dissected and tissue frozen at $-80°C$ until analysis. Half the cortex was used for monoamine analysis and the remainder for drug analysis. Cortical 5-HT contents were determined using HPLC with electrochemical detection (Caccia *et al*., 1993).

Antagonism of quipazine-induced head-twitching

Using a similar method to Eison *et al*. (1990) rats were injected with quipazine maleate (5 mg kg^{-1}, s.c.) 15 min (determination of the ED_{50}) and 150 min (time-course studies, with nefazodone only) after the test compound. Head movements were counted within 30 min of quipazine dosing. In some studies the animals were killed by decapitation at the specified times and brain tissue was analysed for parent drug and metabolite contents. Because in the previous HPLC conditions quipazine overlapped the mCPP peak it was necessary to modify the chromatographic conditions to quantify mCPP; samples were therefore analysed on a gradient system where solvent A was CH_3CN:0.01 M $(NH_4)H_2PO_4$, containing 0.01 M tetramethylammonium hydroxide, buffered to pH 4 (20 : 80, v/v); solvent B was CH_3CN:0.01 M $(NH_4)H_2PO_4$, pH 4 (50 : 50, v/v). A linear gradient from 0–100% solvent B was applied over 45 min for elution.

Calculations

The area under the plasma concentration-time curve from zero to the last measurable plasma concentration (AUC_t) was determined by the trapezoidal rule and extrapolated to infinity (AUC) by a conventional method. The terminal slope was determined by nonlinear least-squares regression, using the data points of the terminal log-linear region of the plasma concentration-time curves. When possible the elimination half-life ($t_{1/2}$) was determined from the terminal slope by the usual equation. The plasma and brain C_{max} and the time when they

were reached (t_{max}) were taken directly from the analytical data.

The frequencies of head twitching and cortical 5-HT concentrations were analysed by analysis of variance, with *post hoc* comparisons of individual means by Duncan's multiple range test. To compare the compounds' relative potencies for inhibiting quipazine-induced head twitching the results for each treatment group were converted to percentages of the mean control value. The ED_{50} was calculated from four dose levels, with five animals per group at each dose, according to the method of De Lean *et al*. (1978). In all cases probabilities (P) less than 0.05 were considered statistically significant.

Results

Plasma and brain pharmacokinetics

In the mouse, nefazodone (30 mg kg^{-1}, i.p.) reached mean plasma and brain C_{max} at 5 min, rapidly declining thereafter to below the limits of quantification from 60 min after dosing (Figure 1). OHNFZ had a similar kinetic profile, however it was never detectable in brain tissue. The elimination $t_{1/2}$ of nefazodone and OHNFZ from plasma and brain could not be adequately estimated because of the small number of experimental points.

The metabolite mCPP reached plasma and brain C_{max} values within 15–30 min and then declined, with mean elimination $t_{1/2}$ of about 1.7 h in both tissues. The plasma AUC of mCPP was about 2.5 times that of the parent drug in plasma but approximately 50 times more in brain because of differences in brain uptake (brain/plasma AUC ratio) (Table 1).

In the rat nefazodone achieved mean C_{max} comparable to (brain) or slightly lower (plasma) than those seen in the mouse (Figure 2). However the drug concentrations in both tissues declined at a slower rate (mean elimination $t_{1/2}$ about 40 min) than in the mouse. Again, OHNFZ peaked in plasma at approximately the same time as the parent drug, disappearing thereafter with a comparable elimination $t_{1/2}$, whereas it was undetectable in brain.

mCPP peaked slightly later and disappeared more slowly from plasma and brain ($t_{1/2}$, 1–1.2 h) than nefazodone and OHNFZ. Its plasma and brain C_{max} and AUC were lower than in the mouse. The mCPP/nefazodone AUC ratio averaged 0.6 in plasma but about 10 in brain because the metabolite's brain uptake was even higher than in the mouse (Table 1).

To clarify the kinetics of OHNFZ it was injected i.p. at 30 mg kg^{-1} in the mouse. The compound appeared rapidly in plasma declining to levels close to the limits of analytical sensitivity within 30 min (AUC 1.7 nmol ml^{-1} h). Mean brain concentrations (Figure 3) apparently paralleled those in plasma but were approximately nine times lower in terms of AUC_t (0.2 nmol g^{-1} h) within the first 15 min. These brain concentrations were largely exceeded by those of mCPP, which reached mean AUC (65 nmol g^{-1} h) close to that obtained after 5 mg kg^{-1} mCPP (71 nmol g^{-1} h).

Table 1 Area under the plasma and brain concentration-time curves of nefazodone and its active metabolites

Tissue	*NFZ*	*OHNFZ* (nmol ml^{-1} *or* g^{-1} h^{-1})	*mCPP*
Mouse			
Plasma	1.7	0.6 (0.3)	4.3 (2.5)
Brain	2.2	a	104.0 (47)
Brain/plasma	1.3		24
Rat			
Plasma	2.1	0.6 (0.3)	1.3 (0.6)
Brain	3.8	a	39.7 (10)
Brain/plasma	1.8		31

The dose of nefazodone (NFZ) was 30 mg kg^{-1}, i.p. [a]Hydroxynefazodone (OHNFZ) was undetectable in brain with the analytical procedure used. The metabolite-to-parent drug ratio is shown in parentheses.

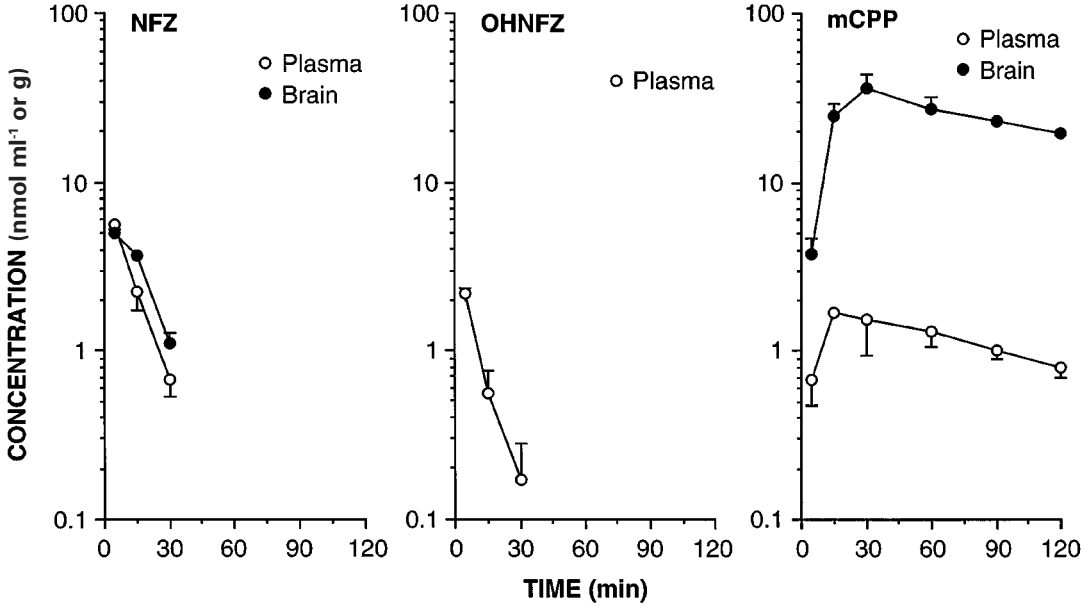

Figure 1 Mean plasma and brain concentration-time curves of nefazodone (NFZ), hydoxynefazodone (OHNFZ) and m-chlorophenylpiperazine (mCPP) after intraperitoneal injection of 30 mg kg^{-1} nefazodone hydrochloride in the mouse. Brain concentrations of OHNFZ were below the limit of sensitivity of the analytical procedure. Each point is the mean ± s.d. of three animals.

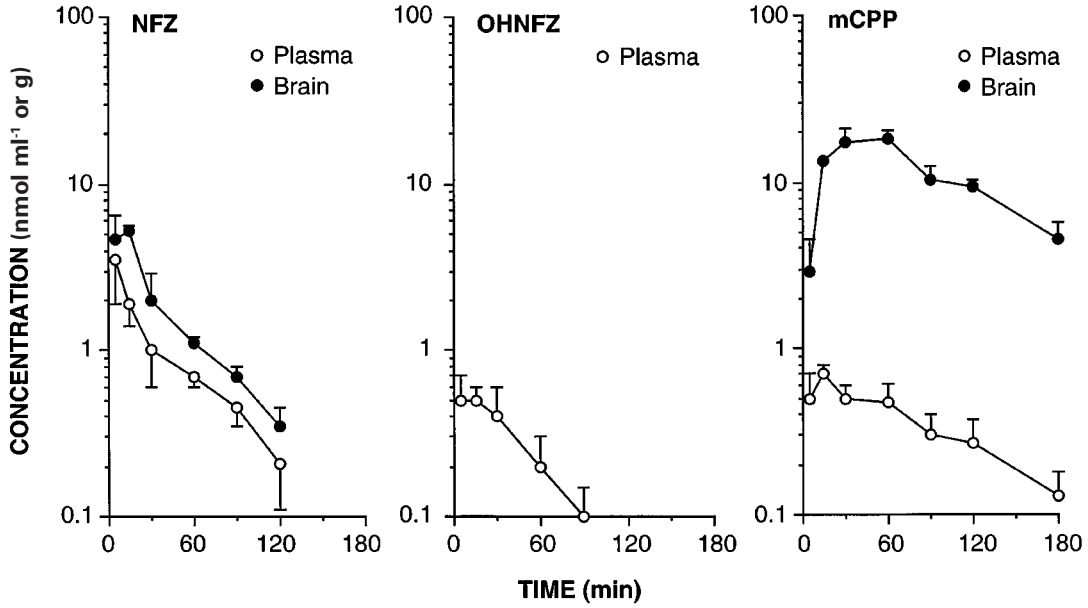

Figure 2 Mean plasma and brain concentration-time curves of nefazodone (NFZ), hydoxynefazodone (OHNFZ) and m-chlorophenyl-piperazine (mCPP) after intraperitoneal injection of 30 mg kg^{-1} nefazodone hydrochloride in the rat. Brain concentrations of OHNFZ were below the limit of sensitivity of the analytical procedure. Each point is the mean ± s.d. of three animals.

Table 2 Antagonism by nefazodone, OHNFZ and mCPP of PCA-induced 5-HT depletion in mouse cortex and drug concentrations

Compound (mg kg^{-1})	*5-HT content* (nmol g^{-1})	*Brain concentrations* *NFZ*	*ONHFZ* (nmol g^{-1})†	*mCPP*
Vehicle	1.0 ± 0.2	–	–	–
NFZ (30)	1.7 ± 0.1**	< 0.3 (2.2)	< 0.3 (ND)	19.1 ± 2.5 (50)
OHNFZ (30)	1.3 ± 0.1*+	–	< 0.3 (0.2)	14.4 ± 1.0 (36)
mCPP (5)	1.3 ± 0.2*+	–	–	16.0 ± 1.8 (52)

Mice were given p-chloroamphetamine (PCA) 15 min after the test compound and were killed 105 min later (i.e. 120 min after the test compound). At this time PCA (20.7 mg kg^{-1}, i.p.) caused 35% of 5-hydroxytryptamine (5-HT) depletion compared to vehicle-treated mice (1.4 ± 0.2 nmol g^{-1}). Each value is the mean ± s.d. of five mice for nefazodone (NFZ), hydroxynefazodone (OHNFZ) and m-chlorophenyl-piperazine (mCPP). †The area under the curve ($AUC_{0-2\,h}$ for mCPP) is shown in parentheses. N.D. = Not determinable. $^{**}P < 0.01$; $^{*}P < 0.05$ vs PCA alone (vehicle). $^{+}P < 0.01$ vs NFZ.

Antagonism of PCA-induced depletion of cortical 5-HT

In the mouse nefazodone at 30 mg kg^{-1}, i.p., antagonized PCA-induced depletion of cortical 5-HT 2 h after administration (Table 2). It was not possible to assess nefazodone 5-HT uptake sites inhibiting activity at shorter times because in the mouse PCA did not significantly deplete cortical 5-HT soon after dosing.

In the same experimental conditions OHNFZ (30 mg kg^{-1}, i.p.) antagonized the indole-depleting effect of PCA less than nefazodone. Its effect was comparable to that of mCPP (5 mg/kg^{-1}) which achieved 2 h post dosing cortical concentrations and $AUC_{2\,h}$ (i.e. the interval from PCA injection until the animals were killed) close to those of the metabolite after OHNFZ. The brain concentrations of mCPP were also very similar to those of the metabolite after 30 mg kg^{-1} nefazodone. However mCPP caused a weaker neurochemical response than nefazodone (Table 2).

Antagonism of quipazine-induced headshakes

In the rat nefazodone, 30 mg kg^{-1} i.p., fully antagonized quipazine-induced headshakes, when this was given 15 min

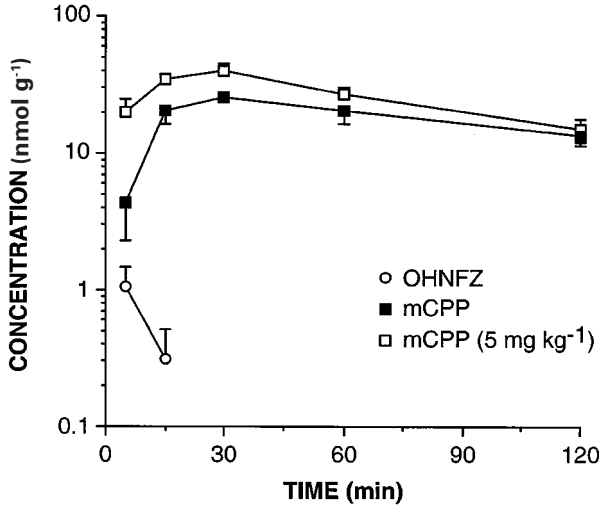

Figure 3 Mean brain concentration-time curves of hydroxynefazodone (OHNFZ) and its metabolite m-chlorophenyl-piperazine (mCPP) after intraperitoneal injection of 30 mg kg^{-1} OHNFZ hydrochloride or 5 mg kg^{-1}, mCPP dihydrochloride in the mouse. Each point is the mean ± s.d. of 3-4 animals.

Table 3 Antagonism of nefazodone, OHNFZ and mCPP of quipazine-induced head twitches in rat and drug brain concentrations

Compound (mg kg^{-1})	*Time*† (min)	*Quipazine antagonism* (% inhibition)	*Brain concentrations* *NFZ*	*OHNFZ* (nmol g^{-1})	*mCPP*
NFZ (30)	45	97±5**	1.3±0.6	<0.3	11.5±5.9
	180	65±20**	<0.3	<0.3	4.2±1.8
OHNFZ (30)	45	98±4**	–	<0.3	15.3±2.1
mCPP (5)	45	84±17**	–	–	13.4±6.6

†Time after nefazodone or its metabolites; quipazine maleate (5 mg kg^{-1}, s.c.) was injected 15 and 150 min (NFZ only) after the test compound and head twitches were counted the next 30 min. Each value is the mean±s.d. of six rats for nefazodone (NFZ), hydroxynefazodone (OHNFZ) and m-chlorophenyl-piperazine (mCPP). **$P<0.01$ compared to quipazine alone.

later (i.e. approximately at the t_{max} of nefazodone). At the end of the observation period (30 min later, i.e. 45 min after nefazodone) the brain concentration of nefazodone was about 1 nmol g^{-1}, whereas mCPP was higher and close to that obtained after 5 mg kg^{-1} mCPP, which caused a comparable reduction of head twitches (Table 3). mCPP was the only compound that could be detected in rat brain (about 4 nmol g^{-1}) 180 min after nefazodone, when the inhibition of the head movements caused by quipazine still amounted to about 65%. However, there was a weak correlation between brain concentrations of mCPP and the pharmacological response for individual animals, considering all treatment groups ($r^2=0.35$; $n=24$).

The ED_{50} for antagonism of quipazine-induced head twiching of nefazodone and mCPP was calculated from four doses levels and (±s.e.mean) was 6.2±1.5 mg kg^{-1} for nefazodone and 2.5±0.4 mg kg^{-1} for mCPP (11.3 and 6.7 μmol kg^{-1} respectively, in terms of free bases; $P=$NS). Analysis of the brain concentrations of the two compounds after these (equiactive) doses indicated that mCPP concentrations around 8 nmol g^{-1} must be reached in the rat in order to inhibit head twitching in 50% of the animals. After nefazodone the metabolite brain concentration was only about 4 nmol g^{-1} (Table 4). This suggested that mCPP and nefazodone may have contributed equally to the pharmacological response early after dosing, although the parent drug was more active in terms of brain concentrations required to obtain a comparable reduction of head twitching. The fact that the active brain concentrations of mCPP were about twice those of the metabolite 180 min after 30 mg kg^{-1} nefazodone further suggested that other metabolite(s) may also contribute to the parent drug's effect at longer times, besides residual brain concentrations of nefazodone. However, it is unlikely that OHNFZ has a significant role. Although it fully antagonized head twitching shortly after dosing (30 mg kg^{-1}), this was probably mostly due to its biotransformation to mCPP because this achieved brain concentrations comparable to those after mCPP as such (5 mg kg^{-1}) (Table 3).

Table 4 ED_{50} of nefazodone and m-chlorophenyl-piperazine for antagonism of quipazine-induced head twitches in the rat

Compound	*ED_{50}* (mg kg^{-1}, ±s.d.)	*Brain concentrations* *NFZ*	*OHNFZ* (nmol g^{-1})†	*mCPP*
NFZ	6.2±1.5	0.3±0.1	<0.3	4.0±0.4
mCPP	2.5±0.4	–	–	8.1±4.2

Quipazine maleate (5 mg kg^{-1}) was injected 15 min after the test compound and head twitches were counted the next 30 min). †Each value is the mean±s.d. of five rats for nefazodone (NFZ), hydroxynefazodone (OHNFZ) and m-chlorophenyl-piperazine (mCPP).

Discussion

At a dose (30 mg kg^{-1}, i.p.) within the range of those effective in rodent models of depression (Taylor *et al*., 1995; Davis *et al*., 1997) nefazodone rapidly reached the systemic circulation and entered the brain. From the peak, disappearance from plasma and brain was almost parallel and rapid, particularly in the mouse. The results in the rat are at variance with Taylor *et al*. (1995) who reported an elimination $t_{1/2}$ for NFZ in this species of 2–3 h. Since these studies did not give details it is not possible to know whether they used radiolabelling techniques, which do not distinguish between the parent drug and its metabolites. Moreover the pharmacokinetics of nefazodone is nonlinear in some species (Shukla *et al*., 1993; Greene & Barbhaiya, 1997). Thus the longer $t_{1/2}$ reported for NFZ in the rat may be due to its reduced clearance at doses higher than the pharmacological one used in the present study.

OHNFZ and mCPP were present in mouse and rat plasma as main metabolites. The metabolite-to-parent drug ratio for OHNFZ averaged 0.3 which is similar to that in humans after single and repeated oral doses of nefazodone. For mCPP the metabolic ratio was higher in the mouse than the rat, being similar on a molar basis in rats to that in extensive metabolizers (EM) of dextromethorphan after single dosing. In poor metabolizers (PM), who do not metabolize mCPP efficiently, the metabolic ratio is higher than in the mouse, decreasing after repeated dosing because in man mCPP formation is a saturable process (Greene & Barbhaiya, 1997; Davis *et al*., 1997; Caccia, 1998).

In mouse and rat brain only mCPP could be detected with our analytical procedure, besides the unchanged compound. Brain concentrations of OHNFZ were always below the detection limits. After i.p. injection of the hydroxylated compound brain concentrations hardly reached 10% of those in plasma, confirming that this metabolite poorly penetrates the blood-brain barrier. Very likely therefore it does not substantially contribute to the central effects of nefazodone in rodents.

The mouse showed consistently higher mCPP brain concentrations than the rat, in spite of the fact that the brain uptake for the metabolite was slightly lower in the mouse. Already at 30 min after nefazodone (30 mg kg^{-1}) the mouse brain contained about 30 times as much mCPP as nefazodone. The parent drug was no longer measurable at later times. However it antagonized the 5-HT-depleting effect of PCA 2 h after dosing. Antagonism of PCA-induced depletion of brain

5-HT requires inhibition of the 5-HT transporter throughout the test period (Hemrick-Luecke *et al*., 1994), so the effect did not depend exclusively on the brain concentration of the unchanged drug. mCPP may therefore have contributed to the parent drug's effect, although other metabolites may also play a part. Accordingly, the brain $AUC_{2\,h}$ of the metabolite after 30 mg kg^{-1} nefazodone were similar to that after 5 mg kg^{-1} mCPP, but the neurochemical effect was stronger in nefazodone- than in mCPP-treated animals.

In the rat relatively large doses of mCPP (25 mg kg^{-1}, i.p.) only slightly attenuated PCA-induced 5-HT depletion (Fuller *et al*., 1981). This is consistent with its relatively low affinity for the 5-HT uptake site *in vitro* in rat cortex, although this is comparable to nefazodone (Taylor *et al*., 1995). Together with previous pharmacokinetic data on oral mCPP (Caccia *et al*., 1981), this suggests that in this species the metabolite contributes even less to 5-HT uptake inhibition by the parent drug. In the rat antagonism of PCA-induced depletion is directly correlated with serum nefazodone concentrations 3 h after dosing, although 5-HT uptake inhibition was significant at doses well above those active in models of antidepressant activity (Owens *et al*., 1995).

Nefazodone displays particularly affinity for $5\text{-}HT_{2A}$ receptors *in vitro* in rat cortex. *In vivo* in the rat it dose-dependently antagonizes the head twitches induced by quipazine, suggesting that it acts as a $5\text{-}HT_{2A}$ antagonist; this agrees with its ability *in vitro* to inhibit the $5\text{-}HT_{2A}$-mediated turnover of phosphatidylinositol in rat cortex (Taylor *et al*., 1995).

mCPP too dose-dependently blocks the head twitches induced by quipazine in the rat, behaving as a full antagonist of 5-HT-induced phosphatidylinositol hydrolysis *in vitro* in cortex (Conn & Sander-Bush, 1987; Simansky & Schechter, 1988). The present study found that mCPP was less potent than nefazodone in terms of the brain concentrations required to achieve an equal antagonism of quipazine-induced twitching, as would be expected from the comparative potency of the two compounds in inhibiting $5\text{-}HT_{2A}$ binding *in vitro* (Taylor *et al*., 1995). Comparing the brain concentrations of mCPP after equiactive doses (ED_{50}) of the metabolite and its parent compounds it appears however that this active metabolite makes a major contribution to the inhibition of head twitching induced by i.p. nefazodone in rats.

Whether other metabolites of nefazodone also play a role remains to be clarified. At least one triazoledione derivative of nefazodone was found to retain some affinity for $5\text{-}HT_{2A}$ receptors *in vitro* (Davis *et al*., 1997). After oral administration of nefazodone in man and dogs exposure to this metabolite is greater than that of nefazodone (Mayol *et al*., 1994; Davis *et al*., 1997) but the brain uptake and concentrations reached at the site of action compared with the parent drug are still not known.

In conclusion, mCPP concentrates in the brain more than nefazodone, possibly contributing largely to its antagonism of quipazine-induced head twitching and, to a lesser extent, PCA-induced depletion of 5-HT. By contrast OHNFZ poorly enters the brain and its *in vivo* activity is probably mainly due to its rapid biotransformation to mCPP. Poor blood-brain barrier penetration compared to the parent drug has been observed for metabolites of other centrally acting drugs, illustrating how total blood concentrations may be a poor indication of the metabolite-to-parent drug ratio at the site of action in the CNS (Caccia & Garattini, 1990; Lin & Lu, 1997).

Despite probable species differences in drug brain uptake, brain/plasma ratios of these compounds in man can be reasonably assumed to be proportionally similar to those in rodents. Possibly therefore mCPP concentrates more than nefazodone in human brain and the mCPP/nefazodone ratios at the site of action may be higher than in plasma. This may have clinical implications because mCPP has a large spectrum of recognized pharmacological actions some of which have been attributed to actions mediated by post-synaptic $5HT_{2C}$ receptors (Kennett & Curzon, 1988;1991; Murphy *et al*., 1991; Kennett 1993; Mazzola-Pomietto *et al*., 1996). In healthy subjects and psychiatric patients it induces neuroendocrine, behavioural, temperature and cardiovascular responses after single doses (Murphy *et al*., 1991; Bagdy & Arato, 1998), although as in animals (Mazzola-Pomietto *et al*., 1996; Fone *et al*., 1998) tolerance develops to some of these effects with repeated administration (Benjamin *et al*., 1996). Some of these effects (dose-related increases in plasma prolactin and raises in oral temperature) have been observed after oral nefazodone in healthy subjects (Walsh *et al*., 1993). The role of mCPP in the overall effects of nefazodone therefore needs furher investigation.

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References

BAGDY, G. & ARATO, M. (1998). Gender-dependent dissociation between oxytocin but not ACTH, corticol or TSH responses to m-chlorophenylpiperazine in healthy subjects. *Psychopharmacology,* **136,** 342–348.

BENJAMIN, J., GREENBERG, B.D. & MURPHY, D.L. (1996). Daily administration of m-chlorophenylpiperazine to healthy human volunteers rapidly attenuates many of its behavioural, hormonal, cardiovascular and temperature effects. *Psychopharmacology,* **127,** 140–149.

CACCIA, S. (1998). Metabolism of the newer antidepressants. An overview of the pharmacological and pharmacokinetic implications. *Clin. Pharmacokinet.,* **34,** 281–302.

CACCIA, S., ANELLI, M., CODEGONI, A.M., FRACASSO, C. & GARATTINI, S. (1993). The effects of single and repeated anorectic doses of 5-hydroxytryptamine uptake inhibitors on indole levels in rat brain. *Br. J. Pharmacol.,* **110,** 355–359.

CACCIA, S., BALLABIO, M., SAMANIN, R., ZANINI, M.G. & GARATTINI, S. (1981). (−)-m-Chlorophenyl-piperazine, a central 5-hydroxytryptamine agonist, is a metabolite of trazodone. *J. Pharm. Pharmacol.,* **33,** 477–478.

CACCIA, S. & GARATTINI, S. (1990). Formation of active metabolites of psychotropic drugs: an updated review of their significance. *Clin. Pharmacokinet.,* **18,** 434–459.

CONN, P.J. & SANDERS-BUSH, E. (1987). Relative efficacies of piperazines at the phosphoinositide hydrolysis-linked serotonergic (5-HT-2 and 5-HT-1c) receptors. *J. Pharmacol. Exp. Ther.,* **242,** 552–557

DAVIS, R., WHITTINGTON, R. & BRYSON, H.M. (1997). Nefazodone. A review of its pharmacology and clinical efficacy in the mamagement of major depression. *Drugs,* **53,** 608–636.

DE LEAN, A., MUNSON, P.J., RODBARD, D. (1978). Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assays, and physiological dose-response curves. *Am. J. Physiol.,* **235,** E97 – E102.

EISON, A.S., EISON, M.S., TORRENTE, J.R., WRIGHT, R.N. & YOCCA, F.D. (1990). Nefazodone: preclinical pharmacology of a new antidepressant. *Psychopharmacol. Bull.,* **26,** 311 – 315.

FIORELLA, D., RABIN, R.A. & WINTER, J.C. (1995) The role of the 5-HT_{2A} and 5-HT_{2C} receptors in the stimuls effects of m-Chlorophenylpiperazine. *Psychopharmacology,* **119,** 222 – 230.

FONE, K.C.F., AUSTIN, R.H., TOPHAM, I.A., KENNETT, G.A. & PUNHANI, T. (1998). Effect of chronic m-CPP on locomotion, hypophagia, plasma corticosterone and 5-HT_{2C} receptor levels in the rat. *Br. J. Pharmacol.,* **123,** 1707 – 1715.

FULLER, R.W., SNODDY, H.D., MASON, N.R. & OWEN, J.E. (1981). Disposition and pharmacological effects of m-chloro-phenylpiperazine in rats. *Neuropharmacology,* **20,** 155 – 162.

GREENE, D.S. & BARBHAIYA, R.H. (1997). Clinical pharmacokinetics of nefazodone. *Clin. Pharmacokinet.,* **33,** 260 – 275.

HEMRICK-LUECKE, S.K., SNODDY, H.D. & FULLER, R.W. (1994). Evaluation of nefazodone as a serotonin uptake inhibitor and a serotonin antagonist in-vivo. *Life Sci.,* **55,** 479 – 483.

KENNETT, GA. (1993). 5-HT_{1C} receptors and their therapeutic relevance. *Curr. Opin. Invest. Drugs,* **2,** 317 – 362.

KENNETT, G.A. & CURZON, G. (1988). Evidence that mCPP may have behavioural effects mediated by central 5-HT_{1C} receptors. *Br. J. Pharmacol.,* **94,** 137 – 147.

KENNETT, G.A. & CURZON, G. (1991). Potencies of antagonists indicate that 5-HT_{1C} receptors mediate 1 – 3(chlorophenyl) piperazine-induced hypophagia. *Br. J. Pharmacol.,* **103,** 2016 – 2020.

LIN, J. H. & LU, A.Y.H. (1997). Role of pharmacokinetics and metabolism in drug discovery and development. *Pharmacol. Rev.,* **49,** 403 – 449.

MAYOL, R.F., COLE, C.A., LUKE, G.M., COLSON, K.L. & KERNS, E.H. (1994). Characterization of the metabolites of the antidepressant drug nefazodone in human urine and plasma. *Drug Metab. Dispos.,* **21,** 304 – 311.

MAZZOLA-POMIETTO, P., AULAKH, C.S., WOZNIAK, K.M. & MURPHY, D.L. (1996). Evidence that m-chlorophenylpiperazine-induced hyperthermia in rats is mediated by stimulation of 5-HT_{2C} receptors. *Psychopharmacology,* **123,** 333 – 339.

MURPHY, D.L., LESCH, K.P., AULAKH, C.S. & PIGOTT, T.A. (1991). III. Serotonin-selective arylpiperazines with neuroendocrine, behavioral, temperature, and cardiovascular effects in humans. *Pharmacol. Rev.,* **43,** 527 – 552.

OWENS, M.J., IENI, J.R., KNIGHT, D.L., WINDERS, K. & Nemeroff, C.B. (1995). The serotoninergic antidepressant nefazodone inhibits the serotonin transporter in vivo and ex vivo studies. *Life Sci.,* **24,** 373 – 380.

SHUKLA, U.A., MARATHE, P.H., PITTMAN, K.A. & BARBHAIYA, R.H. (1993). Pharmacokinetics, absolute bioavailability, and disposition of [^{14}C] nefazodone in the dog. *Drug Metab. Dispos.,* **21,** 502 – 507.

SIMANSKY, K.J. & SCHECHTER, L.E. (1988). Properties of some 1-arylpiperazines as antagonists of stereotyped behaviors mediated by central serotoninergic receptors in rodents. *J. Pharmacol. Exp. ther.,* **247,** 1073 – 1081.

TAYLOR, D.P., CARTER, R.B., EISON, A.S., MULLINS, U.L., SMITH, H.L., TORRENTE, J.R., WRIGHT, R.N. & YOCCA, F.D. (1995). Pharmacology and neurochemistry of nefazodone, a novel antidepressant drug. *J. Clin. Psychiatry,* **56, Suppl. 6,** 3 – 11.

TAYLOR, D.P., EISON, A.S., MULLINS, U.L., HYSLOP, D.K., SMITH, D.W. & YOCCA, F.D. (1986). Pharmacologic studies of the α-hydroxy metabolite of the potential antidepressant nefazodone. *Soc. Neurosci. Abstr.,* **12,** 474.

WALSH, A.E.S., HOCKNEY, R.A., CAMPLING, G. & COWEN, P.J. (1993). Neuroendocrine and temperature effects of nefazodone in healthy volunteers. *Biol. Psychiatry,* **33,** 115 – 119.

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