Br. J. Pharmac (1987), **92**, 243–255

Neuropharmacological and neurochemical properties of N-(2-cyanoethyl)-2-phenylethylamine, a prodrug of 2-phenylethylamine

[1]Glen B. Baker, Ronald T. Coutts & [2]Tadimeti S. Rao

PMHAC Research Unit, Department of Psychiatry and Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada

1 N-(2-cyanoethyl)-2-phenylethylamine (CEPEA) was examined as a possible prodrug of 2-phenylethylamine (PEA).

2 Pharmacokinetics of PEA and CEPEA were investigated in rat brain, blood and liver by gas chromatography with electron-capture detection (GC-ECD). Interactions of PEA and CEPEA with putative neurotransmitter amines were investigated by use of high performance liquid chromatography with electrochemical detection (h.p.l.c.-e.c.).

3 Administration of PEA caused transient increases in PEA concentrations which decreased rapidly in brain and blood and at a slower rate in liver. Administration of CEPEA caused sustained elevations of PEA concentrations and elimination of PEA was markedly decreased in these tissues relative to the situation after administration of PEA itself.

4 Administration of CEPEA caused more prolonged decreases in brain noradrenaline, dopamine and 5-hydroxytryptamine concentrations than those observed after PEA administration, although values increased to control levels eventually.

Introduction

2-Phenylethylamine (PEA) is an endogenous 'trace amine' (Usdin & Sandler, 1976) which is thought to act as a neuromodulator in the central nervous system of mammals (Boulton & Juorio, 1982; Wolf & Mosnaim, 1985). Several groups of researchers have suggested that a functional deficiency of PEA may be involved in the aetiology of affective disorders (Dewhurst, 1968; Boulton & Milward, 1971; Sabelli & Mosnaim, 1974; Sandler *et al.*, 1979).

The administration of monoamine oxidase (MAO)-inhibiting antidepressant drugs to experimental animals results in marked increases in the concentrations of PEA relative to the increases seen with the classical neurotransmitters noradrenaline (NA), dopamine, and 5-hydroxytryptamine (5-HT) (Philips & Boulton, 1979). The enormous increase in concentrations of trace amines such as PEA and tryptamine may contribute in part to the therapeutic effectiveness of these drugs (Dewhurst, 1984). In order to learn more about the function of PEA in the central nervous system and/or about its role in depression and in the action of antidepressant drugs, it is important to be able to cause a selective increase in brain concentrations of this amine. Excellent substrate characteristics with regard to MAO and a very short biological half-life (Yang & Neff, 1973; Cohen *et al.*, 1974; Wu & Boulton, 1975; Shannon *et al.*, 1982; Coutts *et al.*, 1985) preclude the use of PEA itself to elevate selectively the PEA concentrations. MAO inhibitors, while leading to marked increases in concentrations of PEA, also cause an elevation of several other amines, making interpretation of experimental data difficult. A more logical approach would seem to be the use of 'prodrugs' to elevate selectively brain concentrations of PEA. This entails the use of analogues of PEA that are metabolized in the body to produce elevated, sustained concentrations of PEA. Studies in our laboratories have indicated that the N-2-cyanoethyl analogue of amphetamine is effectively N-dealkylated to amphetamine in the rat (Coutts *et al.*, 1986), thus acting as a prodrug. We have therefore conducted in the rat detailed pharmacokinetic and neurochemical studies on N-(2-cyanoethyl)-2-phenylethylamine (CEPEA) as a possible prodrug of PEA. These studies have included investigations on concentrations of PEA and CEPEA in brain, liver and blood and on

[1] Author for correspondence at Dept. of Psychiatry, University of Alberta.

[2] Present address: Neuroscience Research, Ciba-Geigy Ltd, Summit, New Jersey 07901, U.S.A.

effects of administration of CEPEA on brain concentrations of putative neurotransmitter amines. The results of this research are described here.

Methods

Animals

Male Sprague-Dawley rats (175–230 g) were obtained from Bio-Science Animal Services, Ellerslie, Alberta, Canada. The animals were housed in plastic cages on cedar chip bedding in a temperature controlled room (21°C), with alternate 12 h light and dark schedule. Food and water were provided *ad libitum*. Animals were fed with Lab-Blox Feed (from Wayne Food Division, Continental Grain Co., Chicago, U.S.A.), composed of 4.0% crude fat (minimum), 4.5% crude fibre (maximum), and 24% crude protein (minimum).

Gas chromatography

A Hewlett Packard (HP) 5880 gas chromatograph fitted with 15 mCi ^{63}Ni linear electron-capture detector and fused silica capillary column (25 m, 0.31 mm i.d., 0.5 μm film of 5% phenylmethylsilicone as stationary phase, Hewlett Packard Co. Palo Alto, U.S.A.) was used. The carrier gas was helium at a flow rate of 2 ml min^{-1} and the makeup gas was 5% methane in argon at a flow rate of 35 ml min^{-1} at the detector. The injection port and detector temperatures were 200°C and 300°C respectively. The oven temperature was programmed to increase from an initial temperature of 105°C (held for 0.5 min) at a rate of 25°C per min to 280°C (held for 10 min). An HP 5880A integrator was used to measure peak heights.

Experimental procedure

Groups of rats were dosed intraperitoneally with either saline or PEA or CEPEA (0.1 mmol kg^{-1}) as solutions in isotonic saline. At 5, 15, 30, 60, 90, 120 and 180 min after administration of the drugs, groups of rats were killed by cervical dislocation. In acute studies, groups of rats were injected with CEPEA 0.2 or 0.4 mmol kg^{-1} and were killed 90 min after dosing. Brains and livers were dissected out as quickly as possible and frozen solid in isopentane on solid carbon dioxide. Blood samples were collected into vials containing 100 μl of saturated disodium edetate solution and were frozen solid by immersing in isopentane on solid carbon dioxide. Samples were stored at −60°C until the time of analysis, which was carried out using a modification of the procedure of Nazarali *et al.* (1987b). Partially thawed tissues were weighed and homogenized in 5 volumes of ice cold 0.4 N perchloric acid in a homogenizer (Tri-R Stir-R, model S63C with a Teflon pestle and glass mortar (with 0.1–0.15 mm clearance). The homogenates were centrifuged at 10,000 *g* for 15 min at 4°C (IEC, B20 Centrifuge). Aliquots (3 ml) of homogenates were transferred to a set of test tubes and 2000 ng of the internal standard (IS) 2,6-dichlorophenoxypropylamine was added to all tubes. In the case of blood, the samples were allowed to thaw out completely and 1 g portions were weighed into polyethylene tubes to which 2 ml of 0.4 N perchloric acid was added; the tubes were sonicated for 5 min, centrifuged at 1000 *g* for 3 min, and the entire clear supernatants were used. Simultaneously, a set of standards of various known amounts of PEA and CEPEA and a fixed amount (2000 ng) of the IS was also included in the assay to be carried through the entire procedure. Excess acid was neutralized by the addition of solid $KHCO_3$ and the precipitate of potassium perchlorate was removed by centrifugation (1000 *g*, 5 min). The supernatants were adjusted to pH 7.8 and extracted with 3 volumes of DEHPA (2.5% v/v in chloroform) by shaking vigorously for 3 min. Following a brief centrifugation, the aqueous layers were aspirated off and the organic layers were transferred to clean tubes and extracted for 2 min with equal volumes of 0.5 N HCl. After a brief centrifugation, the aqueous layers were transferred to another set of clean tubes. The amines, PEA, CEPEA and the IS were converted to their N-pentafluorobenzoyl (PFB) derivatives by basifying the aqueous layer with solid K_2CO_3 and by shaking vigorously for 15 min with 3 ml of a mixture of toluene:acetonitrile:PFBC (9:1:0.01). After centrifugation the organic layers were transferred to another set of tubes and taken to dryness under nitrogen. The residues were dissolved in 300 μl of toluene and briefly washed with 500 μl of 1.0 N NH_4OH. After a brief centrifugation, the toluene layers were transferred to microfuge tubes and a 1 μl aliquot from each tube was injected into the gas chromatograph. The retention times of derivatized PEA, CEPEA, and IS were 8.3, 10.1 and 13.7 min respectively. The standard curves were linear in the concentration range of 1–2000 ng per 3 ml with a correlation coefficient $r > 0.99$. The PFB derivatives were stable up to a month at −20°C. Although gas chromatography with electron-capture detection was employed routinely for the analysis of PEA and CEPEA as their PFB derivatives, their structures were first confirmed by employing combined gas chromatography-mass spectrometry (for details see Nazarali *et al.*, 1987b).

High-performance liquid chromatography with electrochemical detection (h.p.l.c.-e.c.) was used to determine rat brain levels of NA, dopamine, DOPAC, HVA, 5-HIAA and 5-HT. The brain samples were dissected in half after partial thawing, and homogenized in 5 volumes of homogenizing solution containing 0.1 mol

perchloric acid, 0.05 mmol ascorbic acid and DHBA (50 ng ml^{-1}) as an IS and centrifuged at 12000 *g* for 10 min at 4°C. A portion (300 μl) of the resultant supernatant in each case was diluted further to 600 μl with homogenizing solution; this solution was put in the Waters Intelligent Sample Processor (WISP) system and a 20 μl aliquot was used for h.p.l.c.-e.c. analysis. The h.p.l.c. system was composed of a Waters model 510 pump, a WISP (Waters Associates, Milford, MA, U.S.A.), a LC-4B amperometric detector (Bioanalytical Systems (BAS), West Lafayette, IN, U.S.A.) and an HP 3392A integrator. The glassy carbon electrode (Model TL-5A, BAS) was set at 0.75 V against a Ag/AgCl reference electrode (model RE-1, BAS). The mobile phase was composed of 10 mmol Na_2HPO_4, 0.5 mmol disodium EDTA, 5 mmol SOS, 10% acetonitrile and 5% methanol; the solution was adjusted to a pH value of 2.5 and was pumped at a flow rate of 1 ml min^{-1} through a Econosphere-C_{18} (dimensions 4.6 mm × 250 mm, 5 μm particle size, Applied Science Labs, Avondale, PA, U.S.A.) column. Each day, a set of standards containing various concentrations of each compound (NA, HVA, DOPAC, dopamine, 5-HIAA and 5-HT) was prepared in the homogenizing solution containing 50 ng ml^{-1} of DHBA. The quantities of amines and metabolites in the brain samples were determined from the peak height ratios of each compound to the internal standard and comparing them with the standard curve. The standard curves were linear, with correlation coefficients $r > 0.99$ being obtained routinely.

Inhibition of monoamine oxidase (MAO) activity was determined *ex vivo* and *in vitro* by a modification of the method of Wurtman & Axelrod (1963). [^{14}C]-5-HT and [^{14}C]-PEA diluted with unlabelled compounds (to give final concentrations of 35 μmol) were employed as specific substrates for MAO-A and MAO-B isoenzymes, respectively.

In studies on brain levels of the drug and PEA, semilogarithamic plots of CEPEA or PEA concentrations against time were used to determine distribution and elimination half-lives (α and β respectively) employing the feathering technique (Gibaldi & Perrier, 1982). Concentration-time curves were employed to determine the area under the curve (AUC) employing the trapezoidal rule (Gibaldi & Perrier, 1982). Data were analysed with analysis of variance followed by independent *t*-tests in the case of single pair comparisons (*P* values as indicated in text) or Newman-Keuls tests in the case of multiple pairwise comparison ($\alpha = 0.05$). All probabilities are 2-tailed.

Drugs and chemicals

PEA.HCl, di-(2-ethylhexylphosphate) (DEHPA), (−)-noradrenaline (NA) HCl, 5-hydroxytryptamine (5-HT) creatinine sulphate, dopamine HCl, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindole-3-acetic acid (5-HIAA), and 3,4-dihydroxybenzyl-amine (DHBA) HCl were obtained from Sigma Chemical Co. (St Louis, MO, U.S.A.). Chloroform (ACS) was obtained from Anachemia (Toronto, Canada). Octane sulphonic acid sodium salt (SOS) and pentafluorobenzoyl chloride (PFBC) were purchased from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). β-[Ethyl-1-^{14}C]-phenylethylamine hydrochloride (50.2 mCi mmol^{-1}) and 5-[2-^{14}C]-hydroxytryptamine binoxalate (55.0 mCi mmol^{-1}) were purchased from New England Nuclear (NEN, Boston, MA, U.S.A.). All other chemicals and solvents were of highest purity commercially available. Water was twice distilled in an all-glass distillation apparatus.

Preparation of N-(2-cyanoethyl)-2-phenylethylamine (CEPEA)

A solution of PEA (6.0 g, 49.6 mmol) and acrylonitrile (6.54 g, 123.4 mmol) in toluene (20 ml) was heated under reflux for 24 h. Toluene and excess acrylonitrile were removed by vacuum distillation to give a pale yellow syrup. Kugelrohr distillation at 95–100°C (air bath temp.) and 0.025 mmHg gave a colourless mobile oil (5.55 g, yield 64%).

The hydrochloride salt was prepared and recrystallized from ethanol-ether to give white crystals. The product was characterized by melting point (MP), infrared (IR) spectrometric, nuclear magnetic resonance (n.m.r.) spectroscopic and electron-impact mass spectrometric (EI-MS) measurements and elemental analysis: MP 177–178°C; IR (HCl salt): 2686, 2448 (NH^+), 2252 (CN), 759 (Ph); ^{1}H-n.m.r. (free base): 1.20 (br s, 1H N*H*), 2.47 (t, 2H, J = 6.5 Hz, NcH$_2$ CH_2CN), 2.86 (br, s, 4H, PhCH_2CH_2N), 2.93 (t, 2H, J = 6.5 Hz, NCH_2CH_2CN), 7.12–7.30 (m, 5H, Ph); ^{13}C-n.m.r. (free base): 139.7, 128.7, 128.5, 126.3, 118.6, 50.4, 45.1, 36.4, 18.7, MS [mass-to-charge ratio (relative abundance)]: 174 (molecular ion, 1.5), 105 (3.7), 92 (1.5), 91 (4.7), 83 (100), 77 (2.4), 65 (2.3), 54 (9.5). Elemental analysis for $C_{11}H_{15}ClN_2$ (HCl salt) calculated: C, 62.70; H, 7.18; N, 13.29; found: C, 62.62; H, 6.90; N, 13.33. Electron-impact mass-spectrometric analysis of the N-pentafluorobenzoyl derivative of CEPEA revealed ion fragments characteristic of its structure [ion fragments, mass to-charge ratio (relative abundance)]; $C_6H_5CH_2CH_2\dot{N}(CH_2CH_2CN)COC_6F_5$, 368 (molecular ion, 1.5%), $C_6H_5CH_2\overset{+}{N}H_2(=CH_2)COC_6F_5$, 328 (0.2%) and $CH_2=\overset{+}{N}(CH_2CH_2CN)COC_6F_5$, 277 (5.1%).

Results

After administration of PEA, concentrations of PEA

increased dramatically in brain, blood and liver. In brain, concentrations approximately 1000 times control values were attained at 5 min; by 15 min these values decreased to less than 83 pmol g^{-1}, and by 180 min the concentrations had decreased to control values (Figure 1, approximately 16 pmol g^{-1}) [$F(7,40) = 53.34$, $P < 0.001$]. The half-lives of distribution (α) and elimination (β) were 1.1 and 342 min respectively. Concentrations of PEA in blood at 5 min were nearly 600 times control values, and decreased to nearly 20 times control values by 30 min and to control values by 180 min [$F(7,38) = 16.95$, $P < 0.01$] (Figure 2). The half-lives α and β were 3.0 and 69.0 min respectively. Concentrations of PEA at 5 min in brain and blood were significantly higher than those at other times ($P < 0.05$). In liver, the concentration-time profile of PEA was quite different (Figure 3). Peak concentrations of PEA at 5 min (the shortest time interval at which measurements were made) were only 120 times higher than control values and these decreased monoexponentially with a half-life (β) of 27 min [$F(7,37) = 52.39$, $P < 0.001$]. Concentrations of PEA in liver were significantly increased at 5, 15 and 30 min relative to other times ($P < 0.05$). Peak concentrations of PEA in brain and blood were not different ($P > 0.2$), but those in brain and liver and in blood and liver were significantly different from each other ($P < 0.001$ and $P < 0.02$ respectively). The availability of PEA as measured by AUC values was not different between any two tissues ($P > 0.1$, Table 1).

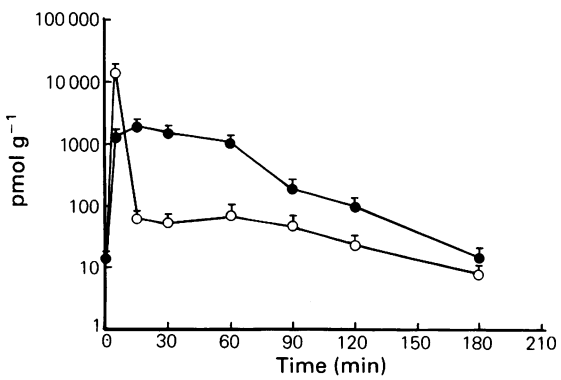

Figure 1 Rat brain 2-phenylethylamine (PEA) concentrations (pmol g^{-1}, mean of $n = 5$ or 6; s.e.mean shown by vertical lines) after PEA (O) and N-(2-cyanoethyl)-2-phenylethylamine (CEPEA, ●) administration (each 0.1 mmol kg^{-1}, i.p.). Concentrations of PEA in untreated rats were 17.4 ± 2.3 pmol g^{-1} (mean ± s.e.mean $n = 6$). Analysis of PEA concentrations in brain after administration of an equimolar dose of PEA and CEPEA indicated a significant difference between effects of drug treatments (i.e., PEA and CEPEA) [$F(1,75) = 18.64$, $P < 0.001$)] and of time [$F(7,75) = 56.30$, $P < 0.001$] and a significant interaction between drug treatment and time (indicative of differences in PEA concentration-time profiles) [$F(7,75) = 50.18$, $P < 0.001$]. Concentrations of PEA after administration of PEA and CEPEA at 5, 15, 30, 60 ($P < 0.001$), 90 and 120 min ($P < 0.05$) were significantly different from each other (t test).

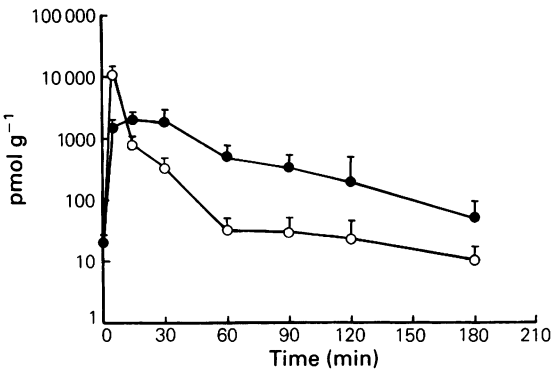

Figure 2 Rat blood 2-phenylethylamine (PEA) concentrations (pmol g^{-1}, mean of $n = 5$ or 6; s.e.mean shown by vertical lines) after PEA (O) and N-(2-cyanoethyl)-2-phenylethylamine (CEPEA, ●) administration (each 0.1 mmol kg^{-1}, i.p.). Concentrations in untreated rats were 20.7 ± 1.9 pmol g^{-1} (mean ± s.e.mean, $n = 6$). Analysis of PEA concentrations after PEA and CEPEA treatments indicated a significant difference in effects of drug treatments [$F(1,80) = 5.33$, $P < 0.025$] and of time [$F(7,80) = 21.54$, $P < 0.001$] and a significant interaction between drug treatment and time [$F(7,80) = 14.83$, $P < 0.001$]. Concentrations of PEA after administration of PEA and CEPEA at 5 ($P < 0.01$), 15 ($P < 0.02$), 30, 60 ($P < 0.001$) and 90 min ($P < 0.05$) were significantly different from each other (t test).

Concentrations of PEA increased slowly in brain after the administration of CEPEA (Figure 1). At 15 min, peak concentrations of nearly 140 times the control values were obtained; these decreased monoexponentially with a half-life (β) of 22.5 min [$F(7,40) = 34.01$, $P < 0.001$]. Brain PEA concentrations at 5, 15, 30 and 60 min were higher than those at other times ($P < 0.05$). In blood, peak concentrations that were 130 times control values were attained at 15 min and decreased monoexponentially with a half-life (β) of 27 min [$F(7,39) = 23.24$, $P < 0.001$]. Concentrations of PEA at 5, 15 and 30 min were significantly higher than those at other times ($P < 0.05$) (Figure 2). In liver (Figure 3), peak concentrations of PEA that were nearly 600 times control values were attained at 15 min and decreased monoexponentially with a half-life (β) of 30 min [$F(7,38) = 53.13$, $P < 0.001$]. At 5, 15, 30 and 60 min PEA concentrations were significantly higher than those measured at other times ($P < 0.05$). Peak con-

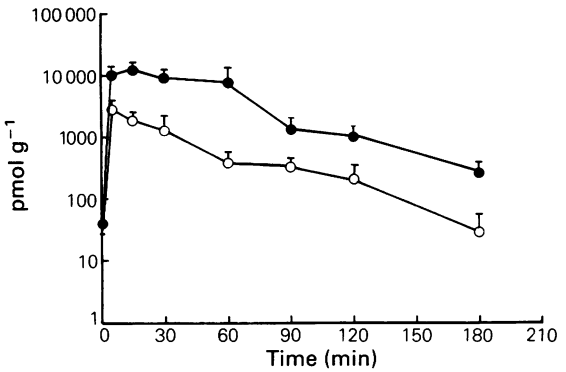

Figure 3 Rat liver 2-phenylethylamine (PEA) concentrations (pmol g^{-1}, mean of $n = 5$ or 6; vertical lines show s.e.mean) after PEA (○) and N-(2-cyanoethyl)-2-phenylethylamine (CEPEA, ●) administration (each 0.1 mmol kg^{-1}, i.p.). Liver concentrations in untreated rats were 28.9 ± 1.3 pmol g^{-1} (mean ± s.e.mean, $n = 6$). Analysis of PEA concentrations after PEA and CEPEA treatments indicated a significant difference between effects of drug treatments [$F(1,80) = 338.51$, $P < 0.001$] and of time [$F(7,80) = 83.05$, $P < 0.001$] and a significant interaction between drug treatment and time [$F(7,80) = 42.66$, $P < 0.001$]. Concentrations of PEA after administration of PEA and CEPEA at 5, 15, 30, 60, 90, 120 and 180 min were significantly different from each other ($P < 0.001$) (t test).

centrations of PEA after CEPEA administration in liver were significantly different from those in blood and brain ($P < 0.001$). The availability of PEA after CEPEA treatment as measured by AUC was significantly different between liver and brain and liver and blood ($P > 0.001$), but not between brain and blood ($P > 0.1$, Table 1). A comparison of the availability of PEA in brain, blood and liver after administration of an equimolar dose of PEA and CEPEA revealed that CEPEA treatment caused significantly more PEA available in liver ($P < 0.001$), but not in brain or blood ($P > 0.1$). However, as illustrated in the figures the pattern of the increase in PEA concentrations was very much different after CEPEA administration, with relatively sustained elevations being attained.

Peak CEPEA concentrations were attained in blood at 5 min, and these decreased biexponentially with half-lives α and β of 8 and 126 min respectively (Figure 4). Peak concentrations of CEPEA in brain were attained at 15 min and decreased biexponentially with half-lives α and β of 4.2 and 30 min respectively. The disposition of CEPEA in liver was similar to that in brain, with peak concentrations attained at 15 min that decreased with half lives α and β of 4.5 and 35 min respectively. The peak concentrations of CEPEA in brain and liver were significantly higher than those in blood ($P < 0.001$), but there was no difference between those in brain and liver ($P > 0.1$). The

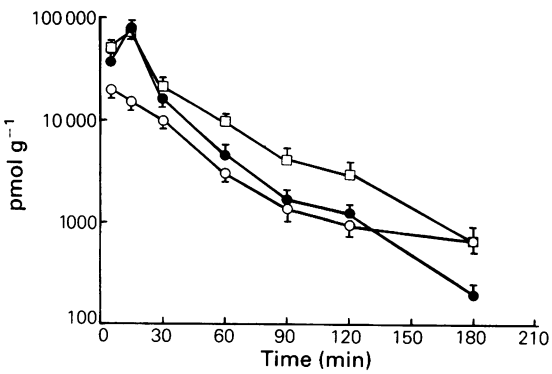

Figure 4 Rat blood (○), brain (●) and liver (□) N-(2-cyanoethyl)-2-phenylethylamine (CEPEA) concentrations (pmol g^{-1}, mean of $n = 5$–6; vertical lines show s.e.mean) after CEPEA administration.

Table 1 Pharmacokinetic parameters of 2-phenylethylamine (PEA) and N-(2-cyanoethyl)-2-phenylethylamine (CEPEA) in rat tissues

	Tissue	*AUC* (nmol min^{-1} g^{-1})	*Half-life of distribution* (α) (min)	*Half-life of elimination* (β) min	C_{max} (nmol g^{-1})
	Blood	102.1 ± 10.2	1.1	342.0	11.6 ± 2.7
PEA	Brain	123.0 ± 12.6	3.0	69.0	14.7 ± 2.0
	Liver	108.8 ± 16.1	—	27.0	3.0 ± 0.2
PEA	Blood	105.5 ± 8.7	—	27.0	2.2 ± 0.4
after	Brain	116.4 ± 7.6	—	22.5	2.1 ± 0.3
CEPEA	Liver	768.4 ± 48.3	—	30.0	13.5 ± 1.8
	Blood	741.4 ± 55.8	8.0	126.0	20.7 ± 2.3
CEPEA	Brain	1954 ± 148.8	4.2	30.0	82.8 ± 8.0
	Liver	2329 ± 51.7	4.5	35.0	70.1 ± 7.5

Values of AUC and C_{max} are mean ± s.e.mean

availability of CEPEA as measured by AUC was significantly different between brain and blood ($P<0.001$), brain and liver ($P<0.05$), and between blood and liver ($P<0.001$, Table 1). The AUC of CEPEA in liver was 1.2 times greater than that in brain and 3.1 times greater than that in blood.

Ex vivo inhibition of brain MAO at 5, 15, 30, 60 and 120 min after the administration of CEPEA, 0.1 mmol kg^{-1}, was determined, and the data are presented in Table 2. CEPEA caused weak inhibition of both MAO-A and MAO-B and by 120 min the enzyme activities were near normal. In a preliminary *in vitro* study, the inhibition of brain MAO-A and MAO-B enzyme activity (percentage, mean ± s.e.mean, $n = 4-6$) was 8.4 ± 1.6 and 7.9 ± 1.5 respectively at a concentration of CEPEA of 10 μM.

Table 3 shows the levels of rat brain, blood and liver PEA and CEPEA 90 min after 0.1, 0.2 and 0.4 mmol kg^{-1} doses of CEPEA.

Rat brain NA, dopamine, DOPAC, HVA, 5-HT and 5-HIAA concentrations after administration of PEA and CEPEA as percentages of respective saline control values are shown in Figures 5–10 respectively. After PEA administration, NA concentrations decreased significantly below control values at 30 and 60 min [$F(6,35) = 4.19$, $P<0.005$] whereas the administration of an equimolar dose of CEPEA resulted in significant decreases in NA concentrations at 30, 60, 90 and 120 min [$F(6,44) = 2.56$, $P<0.05$] (Figure 5). The concentrations of dopamine remained unchanged after PEA administration [$F(6,42) = 1.82$; $P>0.1$] whereas CEPEA administration resulted in significant decreases in dopamine concentrations at 30, 60, 90 and 120 min [$F(6,39) = 6.96$, $P<0.01$] (Figure 6). After PEA administration, DOPAC concentrations decreased below control values only at 15 min [$F(6, 35) = 4.35$, $P<0.005$] while after administration of CEPEA there were no changes in DOPAC concentrations [$F(6,53) = 1.76$, $P>0.1$] (Figure 7). Concentrations of HVA remained unchanged from control values after PEA administration [$F(6,34) = 2.37$, $P>0.05$] but increased above control values at 5, 15 and 30 min [$F(6,51) = 3.56$, $P<0.01$] following injection of CEPEA (Figure 8). There were no significant time-dependent changes in 5-HT concentrations after PEA administration [$F(6,39) = 1.77$, $P>0.1$] but they were significantly below control values at 30, 60, 90 and 120 min after CEPEA treatment [$F(6,51) = 3.53$, $P<0.01$] (Figure 9). There were significant decreases in 5-HIAA concentrations below control values at 5 and 15 min after PEA administration [$F(6,40) = 6.1$, $P<0.001$], whereas concentrations of 5-HIAA increased above control values at 15 and 180 min after CEPEA treatment [$F(6,46) = 2.38$, $P<0.05$] (Figure 10).

Table 2 Inhibition of rat brain monoamine oxidose (MAO) after administration of N-(2-cyanoethyl)-2-phenylethylamine (CEPEA, 0.1 mmol kg^{-1}, i.p.)

Time (min)	*MAO-A*	*MAO-B*
5	9.9 ± 4.8	8.9 ± 1.7
15	5.3 ± 4.5	22.0 ± 2.2
30	11.5 ± 6.6	19.7 ± 4.7
60	0.50 ± 0.3	7.7 ± 2.6
120	3.8 ± 3.7	1.1 ± 0.5

% inhibition, mean ± s.e.mean ($n = 5$ or 6) is shown.

Discussion

Soon after intraperitoneal administration of PEA, concentrations of PEA in brain, blood and liver were increased many fold above the control values. Significantly higher peak concentrations of PEA in brain than in liver are consistent with the known lipophilicity and ability of PEA to cross the blood-brain barrier (Cohen *et al.*, 1974; Wu & Boulton, 1975;

Table 3 Rat brain, blood and liver 2-phenylethylamine (PEA) and N-(2-cyanoethyl)-2-phenylethylamine (CEPEA) concentrations 90 min after CEPEA administration

CEPEA dose	*PEA (pmol g^{-1})*			*CEPEA (pmol g^{-1})*		
	Brain	*Blood*	*Liver*	*Brain*	*Blood*	*Liver*
Saline ($n = 6$)	17 ± 2	21 ± 2	29 ± 1	—	—	—
0.1 mmol kg^{-1} ($n = 5$ or 6)	206 ± 37	259 ± 45	1417 ± 218	1724 ± 345	862 ± 115	4195 ± 517
0.2 mmol kg^{-1} ($n = 5$ or 6)	508 ± 80	463 ± 54	3007 ± 226	4943 ± 345	1782 ± 172	8046 ± 747
0.4 mmol kg^{-1} ($n = 5$ or 6)	668 ± 89	1244 ± 181	5861 ± 825	9195 ± 1782	4885 ± 632	13973 ± 3103

Values are mean ± s.e.mean.

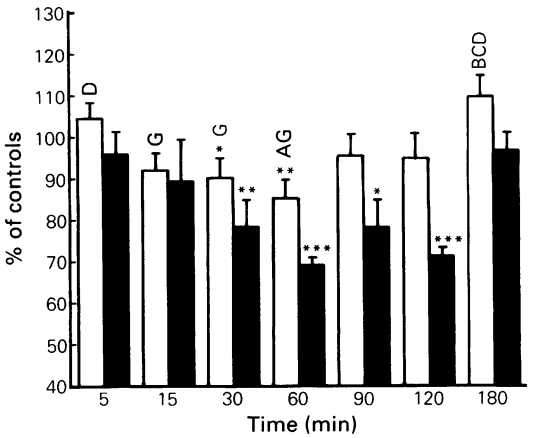

Figure 5 Rat brain noradrenaline (NA) concentrations (% of controls, mean of $n = 5-12$; vertical lines show s.e.mean) after administration of 2-phenylethylamine (PEA, open columns) and N-(2-cyanoethyl)-2-phenylethylamine (CEPEA, solid columns) (each 0.1 mmol kg^{-1}, i.p.). ($*P<0.05$; $**P<0.01$; $***P<0.001$, compared with saline controls at corresponding times by t test). Saline control NA concentrations (pooled from controls killed at different times, ng g^{-1}, mean ± s.e.mean $n = 65$) = 328.3 ± 5.7. Analysis of NA concentrations after PEA and CEPEA administration indicated a significant difference between the effects of drug treatments [$F(1,80) = 16.95$, $P<0.001$] and a significant effect of time [$F(6,80) = 4.96$, $P<0.001$], but no significant interaction between drug treatment and time [$F(6,80) = 0.64$, $P>0.2$]. Concentrations of NA after administration of PEA and CEPEA at 60 and 120 min were significantly different from each other ($P<0.01$) (t test). Multiple mean comparisons were made with the Newman-Keuls test ($\alpha = 0.05$) following analysis of variance. Superscripts denote: A, different from 5 min; B, different from 15 min; C, different from 30 min; D, different from 60 min; E, different from 90 min; F, different from 120 min; G, different from 180 min. The Newman-Keuls test (multiple mean comparisons) indicated no significant between-group differences in NA concentrations after CEPEA treatment.

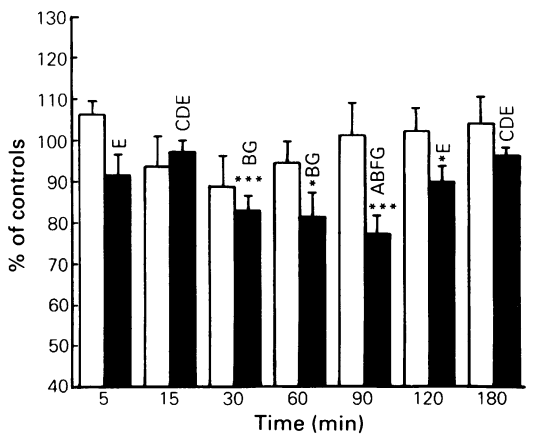

Figure 6 Rat brain dopamine concentrations (% of controls, mean of $n = 5-12$; vertical lines show s.e.mean) after administration of 2-phenylethylamine (PEA, open columns) and N-(2-cyanoethyl)-2-phenylethylamine (CEPEA, solid columns) (each 0.1 mmol kg^{-1}, i.p.). Saline control dopamine concentrations (pooled from controls killed at different times, ng g^{-1}, mean ± s.e.mean, $n = 66$) = 675.2 ± 8.3. Analysis of dopamine concentrations after PEA and CEPEA administration indicated a significant difference between effects of drug treatments [$F(1,80) = 21.98$, $P<0.001$], a significant effect of time [$F(6,80) = 3.63$, $P<0.01$] and a significant interaction between drug treatment and time [$F(6,80) = 2.26$, $P<0.025$]. Concentrations of dopamine after administration of PEA and CEPEA at 5 ($P<0.01$), 60 ($P<0.02$), 90 and 120 min ($P<0.01$) were significantly different from each other (t test). Superscripts and statistical notations are as in Figure 5.

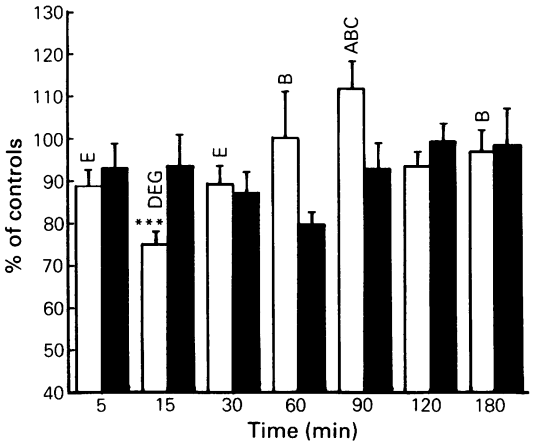

Figure 7 Rat brain 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations (% of controls, mean of $n = 5-12$; s.e.mean shown by vertical lines) after 2-phenylethylamine (PEA, open columns) and N-(2-cyanoethyl)-2-phenylethylamine (CEPEA, solid columns) administration (each 0.1 mmol kg^{-1}, i.p.). Saline control DOPAC concentrations (pooled from controls killed at different times, ng g^{-1}, mean ± s.e.mean, $n = 65$) = 109.6 ± 2.7. Analysis of DOPAC concentrations after PEA and CEPEA administration indicated no significant differences between effects of drug treatments [$F(1,88) = 0.27$, $P > 0.2$], but a significant effect of time [$F(6,68) = 2.76$, $P < 0.025$] and a significant interaction between time and drug treatment [$F(6,88) = 3.49$, $P < 0.01$]. Concentrations of DOPAC after administration of PEA and CEPEA at 15 ($P < 0.001$) and 90 min ($P < 0.05$) were significantly different from each other (t test). Superscripts and statistical notations are as in Figure 5.

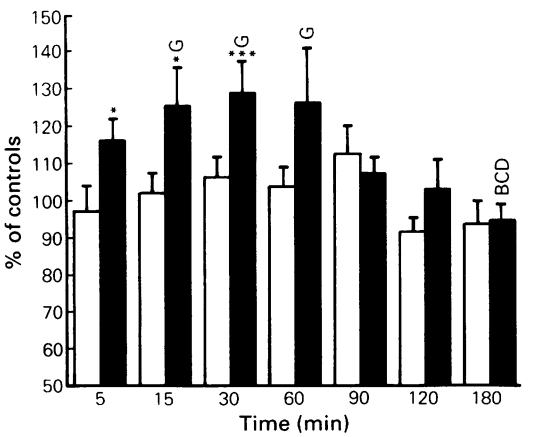

Figure 8 Rat brain homovanillic acid (HVA) concentrations (% of controls, mean of $n = 5-12$; vertical lines show s.e.mean) after administration 2-phenylethylamine (PEA, open columns) and N-(2-cyanoethyl)-2-phenylethylamine (CEPEA, solid columns) (each 0.1 mmol kg^{-1}, i.p.). Saline control HVA concentrations (pooled from controls killed at different times, ng g^{-1}, mean ± s.e.mean, $n = 69$) = 79.1 ± 1.7. Statistical analysis of HVA concentrations after PEA and CEPEA administration indicated significant differences between effects of drug treatments [$F(1,85) = 14.44$, $P < 0.001$], a significant effect of time [$F(6,85) = 3.53$, $P < 0.005$] but no interaction between drug treatment and time ($F(6,85) = 1.39$, $P > 0.2$]. Concentrations of HVA after administration of PEA and CEPEA at 5, 15 ($P < 0.05$) and 30 min ($P < 0.01$) were significantly different from each other (t test). Superscripts and statistical notations are as in Figure 5.

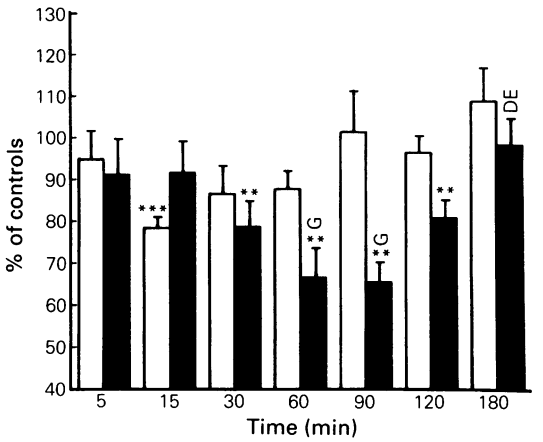

Figure 9 Rat brain 5-hydroxytryptamine (5-HT) concentrations (% of controls, mean of $n = 5-12$, vertical lines show s.e.mean) after administration of 2-phenylethylamine (PEA, open columns) and N-(2-cyanoethyl)-2-phenylethylamine (CEPEA, solid columns) (each 0.1 mmol kg^{-1}, i.p.). Saline control 5-HT concentrations (pooled from controls killed at different times, ng g^{-1} mean ± s.e.mean, $n = 66$) = 435.0 ± 6.5. Analysis of 5-HT concentrations after PEA and CEPEA administration indicated a significant difference between the effects of drug treatments [$F(1,90) = 8.85$, $P < 0.005$], no significant effect of time [$F(6,90) = 2.11$, $P > 0.05$] and no significant interaction between drug treatment and time [$F(6,90) = 1.72$, $P > 0.2$]. Concentrations of 5-HT after administration of PEA and CEPEA at 60 ($P < 0.02$), 90 ($P < 0.01$) and 120 min ($P < 0.01$) were significantly different from each other (t test). Superscripts and statistical notations are as in Figure 5.

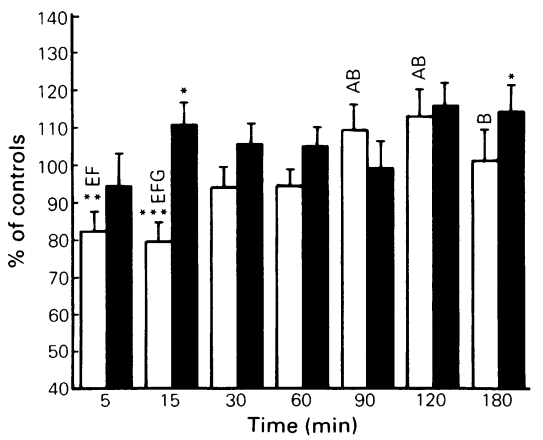

Figure 10 Rat brain 5-hydroxyindole-3-acetic acid (5-HIAA) concentrations (% of controls, mean of $n = 5-12$; vertical lines show s.e.mean) after administration 2-phenylethylamine (PEA, open columns) and N-(2-cyanoethyl)-2-phenylethylamine (CEPEA, solid columns) (each 0.1 mmol kg^{-1}, i.p.). Saline control 5-HIAA concentrations (pooled from controls killed at different times, ng g^{-1}, mean ± s.e.mean, $n = 66$) = 286.1 ± 4.3. Statistical analysis of 5-HIAA concentrations after PEA and CEPEA administration indicated a significant difference between effects of drug treatments [$F(1,86) = 13.14$, $P < 0.001$], a significant effect of time [$F(6,86) = 5.15$, $P < 0.001$] and a significant interaction between drug treatment and time [$F(6,86) = 2.73$, $P < 0.05$]. Concentrations of 5-HIAA after administration of PEA and CEPEA at 15 ($P < 0.001$), 30 and 60 min ($P < 0.05$) were significantly different from each other (t test). Superscripts and statistical notations are as in Figure 5. Newman-Keuls test (multiple mean comparisons) indicated no significant group differences in 5-HIAA concentrations after CEPEA.

Oldendorf & Dewhurst, 1978; Edwards & Antelman, 1978; Karoum *et al.*, 1982). The concentrations of PEA decreased very rapidly in brain and blood and much more slowly in liver. The rapid decrease in brain is possibly due to the excellent substrate characteristics of PEA for MAO enzymes (Yang & Neff, 1973) and/or rapid diffusion into other tissues. Cohen *et al.* (1974) could not detect rat brain PEA concentrations 60 min after i.p. administration of PEA 100 mg kg^{-1} and Wu & Boulton (1975) reported recovery of PEA only after pretreatment with a MAO-inhibitor at 30 min after intravenous administration of radiolabelled PEA. Edwards & Antelman (1978) detected small amounts of PEA in caudate nucleus and the rest of the brain at 60 min after a dose of PEA of 100 mg kg^{-1}, i.p. Wu & Boulton (1975) reported the half-life of elimination of PEA from untreated rat brain to be approximately 39 min (range is 15–60 min) and in different nuclei of brain in the range of 1–5 min. Shannon *et al.* (1982) reported the half-life elimination in dog plasma to be about 5 min at a dose of 5.6–10.0 mg kg^{-1} (i.v.). In the present investigation we observed a rapid decline in PEA levels in brain, with a half-life of about 1.1 min followed by rather slow elimination. The measurements of AUC indicated that more than 90% of the available PEA in brain was eliminated within first 15 min (α-phase), thus suggesting little pharmacokinetic importance of the β-phase. However, it is conceivable that there could be some accumulation after repeated administration, and future investigations on PEA concentrations after long-term administration of CEPEA should include comparisons with animals similarly treated with PEA. The differences between the results of the present investigation and those described previously might be due to differences in doses employed and routes of PEA administration. Recent studies indicate dose-dependent non-linear pharmacokinetics (Shannon *et al.*, 1982) and route of administration-dependent kinetics of elimination for PEA (Garcha *et al.*, 1985).

In contrast to brain and blood, liver levels decreased slowly and the half-life of 27 min is in agreement with earlier reports (Wu & Boulton, 1975). Liver contains quantitatively more MAO activity and it would be expected to metabolize PEA more rapidly than brain. The large amount of PEA available to liver soon after administration might be saturating or inhibiting MAO (Kinemuchi *et al.*, 1982), causing the PEA to attain very high concentrations in other tissues. Despite the differences in peak concentrations and rates of elimination of PEA from brain, blood and liver in our study, the total availability as measured by AUC was not different between any two tissues, indicating fairly uniform distribution in these tissues after administration of PEA.

The administration of CEPEA resulted in gradual increases in PEA concentrations in brain, blood and liver and these concentrations were sustained for longer times in contrast to transient changes after PEA treatment itself. Metabolic N-dealkylation of CEPEA to PEA and the observed MAO inhibition may both be contributing to the elevated concentrations. Both *in vitro* and *ex vivo* studies suggest CEPEA to be a weak MAO inhibitor. The concentrations of PEA in rat brain after CEPEA treatment are much higher than those seen after administration of the MAO inhibitor tranylcypromine (Baker *et al.*, 1986) at doses that almost completely inhibit MAO, and this would suggest that the contribution of MAO inhibition to the sustained elevations of PEA in the present experiments is minimal. The *ex vivo* assay method employed in the present study measures only irreversible inhibition of MAO. Although it is unlikely that reversible MAO inhibition would be an important factor, the technique used would probably give an underestimate of such inhibition. Measurement of PEA concentrations after administration of radiolabelled or stable isotope-labelled CEPEA should reveal the relative contribution of N-dealkylation to the increases in PEA, but these labelled compounds are not available at the moment. However, studies with the N-cyanoethyl analogues of the structurally related phenylethylamines amphetamine and tranylcypromine (Coutts *et al.*, 1986; Nazarali *et al.*, 1987a) suggest that N-dealkylation is a major contributing factor.

In the present study no attempt was made to identify other metabolites of CEPEA, but metabolic studies on N-cyanoethylamphetamine (Fenproporex), which is used as an anorectic agent (Martindale, 1982), suggest N-dealkylation to be a major route of metabolism (Beckett *et al.*, 1972; Coutts *et al.*, 1986). However, it is likely that hydroxylated metabolites may be formed, and future investigations should include a search for these.

The metabolically removed cyanoethyl group would be expected to be converted to cyanoacetaldehyde which further undergoes oxidation to cyanoacetic acid which would probably be excreted as its conjugated form. To our knowledge, no untoward effects due to the cyanoethyl group have been reported (Martindale, 1982).

N-dealkylation reactions for several N-alkylated phenylethylamines have been shown to be mediated by the cytochrome P-450 enzyme system (Duncan *et al.*, 1983). In the present study, there was also a fairly linear dose-response relationship for CEPEA N-dealkylation (Table 3).

Distribution of CEPEA into brain was fairly rapid and concentration-time profiles of CEPEA in brain and liver were superimposable, although the availability of CEPEA was significantly greater in liver than in brain. In both organs, after a rapid distribution phase, CEPEA was eliminated slowly. Such a rapid distribution followed by slower elimination was

observed earlier for 2-N-phenylethylaminealkanenitriles in a scintigraphic study in dog (Winstead *et al.*, 1978).

We have also examined the neurochemical effects of administration of PEA and CEPEA on brain concentrations of NA, dopamine, 5-HT, HVA, DOPAC and 5-HIAA. In order to account for possible circadian or experimental variations in the levels of the neurotransmitters and their metabolites, we included a saline-treated control group at each time interval. Our studies indicated that there were no differences between saline-treated ($n = 65$) and untreated control groups ($n = 25$) in the levels of neurotransmitters and metabolites ($P > 0.05$). Jackson & Smythe (1973) have reported a significant decrease in rat whole brain NA and dopamine but not 5-HT after a 100 mg kg^{-1} (i.p.) dose of PEA. Karoum *et al.* (1982), employing a dose of 20 mg kg^{-1} (i.v.), observed increased NA concentrations in hypothalamus at 7.5 min and decreased levels by 20 min; in the caudate nucleus diametrically opposite effects were observed. Fuxe *et al.* (1967) observed a marked decrease in NA after a 100 mg kg^{-1} dose, but not after a 50 mg kg^{-1} dose. Repeated administration of PEA resulted in profound decreases in rat brain NA concentrations (Jonsson *et al.*, 1966). Decreases in brain NA concentrations were also observed in guinea-pig following doses of PEA of either 100 or 200 mg kg^{-1} (Jackson, 1971). Studies *in vitro* with synaptosomes (Raiteri *et al.*, 1977) and recent electrophysiological data (Lundberg *et al.*, 1986) also support catecholamine-releasing properties of PEA in brain. Furthermore, Fuxe *et al.* (1967) demonstrated that pretreatment of animals with an MAO inhibitor which prevented the metabolism of subsequently injected PEA, caused significant decreases in rat whole brain NA concentrations (compared to MAO inhibitor-treated controls). If sustained concentrations of PEA were responsible for the decreases in NA levels in the study of Fuxe *et al.* (1967), it would be expected that CEPEA treatment would result in marked decreases in brain NA concentrations, in contrast to PEA treatment.

Administration of PEA and CEPEA caused changes in DA metabolism. Several groups (Fuxe *et al.*, 1967; Jackson and Smythe, 1973; Baker *et al.*, 1976; Raiteri *et al.*, 1977; Baker and Yasensky, 1981; Karoum *et al.*, 1982; McQuade & Wood, 1983; Philips & Robson, 1983; Nielsen *et al.*, 1983; Dyck, 1984) have reported dopamine-releasing effects of PEA. Low doses of PEA stimulate, while large doses inhibit, synthesis of dopamine through the release and inhibition of reuptake of dopamine (Roberts & Patrick, 1979; Patrick, 1981; Nielsen *et al.*, 1983). The administration of CEPEA appears to mimic large-dose PEA effects.

PEA was reported to be a weak inhibitor of 5-HT uptake in human platelets (Richter & Smith, 1974), and Jackson & Smythe (1973) observed a significant decrease in 5-HT levels in mouse whole brain and in rat midbrain and cortex after administration of PEA. The initial behavioural effects of PEA seem to be more 5-HTergic than dopaminergic in nature (Dourish, 1981; 1982) although PEA is a better uptake inhibitor of dopamine than of 5-HT (Raiteri *et al.*, 1977) *in vitro*. In rat striatal slices, Dyck (1984) showed significant release of [^{3}H]-5-HT by PEA. Anden *et al.* (1974) reported that some hallucinogenic phenylethylamines decrease 5-HT synthesis through receptor interaction. High concentrations of pyridoxylidene-PEA are known to inhibit 5-hydroxytryptophan decarboxylase, an enzyme responsible for biosynthesis of 5-HT (Loo *et al.*, 1978). With the transient increase of brain PEA after PEA administration, inhibition of 5-HT synthesis is unlikely to contribute to the changes in the 5-HT-ergic system, whereas changes after CEPEA treatment suggest an increase in 5-HT turnover via enhanced 5-HT release.

The administration of the prodrug causes more pronounced decreases in NA, dopamine and 5-HT than does PEA, although in both cases the effects are relatively weak and short-lived. Besides the effect of elevated PEA one has to consider the direct effect of the prodrug itself on these neurotransmitters and metabolites. A series of N-n-alkylated analogues of PEA have been shown to be active at inhibiting 5-HT uptake mechanisms (Suckling *et al.*, 1985), and other N-alkylated analogues of phenylethylamines are known to affect the uptake and release of these biogenic amines (Koe, 1976; Baker *et al.*, 1980). Decreases in brain NA, dopamine and 5-HT levels have also observed after administration of N-(3-chloropropyl)-2-phenylethylamine, another potential prodrug of PEA (Rao *et al.*, 1987). At present uptake and release studies are being conducted to distinguish the effects of prodrug from the PEA formed from it.

In summary, CEPEA administration caused sustained elevation of rat brain, blood and liver PEA concentrations, thus acting as a prodrug. The brain concentrations of PEA obtained after administration of CEPEA are similar to or greater than those reported after administration of MAO inhibitors (Boulton *et al.*, 1973; Philips & Boulton, 1979; Philips *et al.*, 1980; Baker *et al.*, 1984). The administration of CEPEA did not markedly inhibit MAO activity, and this study demonstrates the feasibility of using a prodrug to increase the brain PEA concentrations. Pharmacokinetic and neurochemical analyses were conducted with a view to understanding better the nature of the formation of PEA from CEPEA and to investigate interactions with putative neurotransmitter amines. The prodrug CEPEA appears to be a useful pharmacological tool with which to effect relatively sustained elevations of PEA concentrations, and future studies on chronic administration of this prodrug and

its behavioural effects should provide important information about the actions of PEA in the brain and the possible relationship of PEA to depression and to the actions of antidepressants.

The authors gratefully acknowledge financial assistance from the Provincial Mental Health Advisory Council (PMHAC) and Alberta Heritage Foundation for Medical Research (AHFMR). They wish to thank Dr T.W. Hall and Dr R.G. Micetich for synthesizing CEPEA.HCl, Miss Heather Schmidt for her secretarial assistance in preparing this manuscript and Dr A.J. Greenshaw for his advice statistical analysis. They also thank Mrs James Wong for his advice on and assistance in h.p.l.c. analysis. T.S.R. is a recipient of an AHFMR Studentship award.

References

ANDEN, N.E., CORRODI, H., FUXE, K. & MEEK, J.C. (1974). Hallucinogenic phenylethylamines: Interactions with serotonin turnover and receptors. *Eur. J. Pharmac.*, **25**, 176–184.

BAKER, G.B., BERTOLLINI, A., DEL CARMINE, R., KEANE, P.E. & MARTIN, I.L. (1976). Interaction of β-phenylethylamine with dopamine and noradrenaline in the central nervous system of the rat. *J. Pharm. Pharmac.*, **28**, 456–457.

BAKER, G.B., COUTTS, R.T., BENDERLY, A., CRISTOFOLI, W.A. & DEWHURST, W.G. (1980). The synthesis of N-alkylated *p*-chlorphentermine derivatives and their effects on releasing 5-hydroxytryptamine from rat striatum *in vitro*. *Can. J. Pharm. Sci.*, **15**, 71–74.

BAKER, G.B., LEGATT, D.F. & COUTTS, R.T. (1984). A comparison of the effects of acute and chronic administration of phenelzine and tranylcypromine on brain concentrations of 2-phenylethylamine, *p*-tyramine, and tryptamine in the rat. In *Neurobiology of the Trace Amines*. ed. Boulton, A.A., Baker, G.B., Dewhurst, W.G. & Sandler, M. pp. 277–283. Clifton: Humana Press.

BAKER, G.B., RAO, T.S. & COUTTS, R.T. (1986). Electron-capture gas chromatographic analysis of β-phenylethylamine in tissues and body fluids using pentafluorobenzenesulfonyl chloride for derivatization. *J. Chromatogr. Biomed. Appl.*, **381**, 211–217.

BAKER, G.B. & YASENSKY, D.L. (1981). Interactions of trace amines with dopamine in rat striatum. *Prog. Neuropsychopharmac.*, **5**, 577–580.

BECKETT, A.H., SHENOY, E.V.B. & SALMON, J.A. (1972). The influence of the replacement of the N-ethyl group by the cyanoethyl group on the absorption, distribution and metabolism of (±)-ethylamphetamine in man. *J. Pharm. Pharmac.*, **24**, 194–202.

BOULTON, A.A. & JUORIO, A. (1982). Brain trace amines. In *Handbook of Neurochemistry* ed. Lajtha, A. pp. 189–222. New York: Plenum Press.

BOULTON, A.A. & MILWARD, L. (1971). Separation, detection and quantitation analysis of urinary β-phenylethylamine. *J. Chromatogr.*, **57**, 287–296.

BOULTON, A.A., PHILIPS, S.R. & DURDEN, D.A. (1973). The analysis of certain amines in tissues and body fluids as their dansyl derivatives. *J. Chromatogr.*, **82**, 137–142.

COHEN, I., FISCHER, J.F. & VOGEL, W.H. (1974). Physiological disposition of β-phenylethylamine, 2,4,5-trimethoxyphenylethylamine, 2,3,4,5,6-pentamethoxyphenylethylamine and β-hydroxymescaline in rat brain, liver and plasma. *Psychopharmac. (Berl.)*, **36**, 77–84.

COUTTS, R.T., BAKER, G.B., NAZARALI, A.J., RAO, T.S., MICETICH, R.G. & HALL, T.W. (1985). Prodrugs of trace amines. In *Neuropsychopharmacology of the Trace Amines*. ed. Boulton, A.A., Maitre, L., Bieck, P.R. & Riederer, P. pp. 175–180. Clifton, New Jersey: Humana Press.

COUTTS, R.T., NAZARALI, A.J., BAKER, G.B. & PASUTTO, F.M. (1986). Metabolism and disposition of N-(2-cyanoethyl)amphetamine (Fenproporex) and amphetamine: study in the rat brain. *Can. J. Physiol. Pharmac.*, **64**, 724–728.

DEWHURST, W.G. (1968). New theory of cerebral amine function and its clinical application. *Nature (Lond.)*, **218**, 1130–1133.

DEWHURST, W.G. (1984). Trace amines: the early years. In *Neurobiology of the Trace Amines*. ed. Boulton, A.A., Baker, G.B., Dewhurst, W.G. & Sandler, M. pp. 3–12. Clifton, New Jersey: Humana Press.

DOURISH, C.T. (1981). Behavioral effects of acute and chronic β-phenylehylamine administration in the rat: evidence for the involvement of 5-hydroxytryptamine. *Neuropharmac.*, **20**, 1067–1072.

DOURISH, C.T. (1982). A pharmacological analysis of the hyperactivity syndrome induced by β-phenylethylamine in the mouse. *Br. J. Pharmac.*, **77**, 129–139.

DUNCAN, J., HALLSTROM, G., PAULSEN-SORMAN, U., LINDEKE, B. & CHO, A.K. (1983). Effects of α-carbon substituents on the N-demethylation of N-methyl-2-phenylethylamines by rat liver microsomes. *Drug Metab. Disp.*, **11**, 15–20.

DYCK, L. (1984). The behavioral effects of phenelzine and phenylethylamine may be due to amine release. *Brain Res. Bull.*, **12**, 23–28.

EDWARDS, D.J. & ANTELMAN, S.M. (1978). Phenylethylamine in brain function and dysfunction. Neurochemical and pharmacological studies. In *Non-Catecholic Phenylethylamines, Part I* ed. Mosnaim, A.D. & Wolf, M.E. pp. 21–46. New York: Marcel Dekker.

FUXE, K., GROBECKER, H. & JONSSON, J. (1967). The effect of β-phenylethylamine on central and peripheral monoamine containing neurons. *Eur. J. Pharmac.*, **2**, 202–207.

GARCHA, G., IMRIE, P.R., MARLEY, E. & THOMAS, D.V. (1985). [^{14}C]-β-Phenylethylamine, its distribution after administration by various routes to cats, and the effect of monoamine oxidase inhibitors. *Br. J. Pharmac.*, **86**, 877–888.

GIBALDI, M. & PERRIER, D. (1982). *Pharmacokinetics*. New York: Marcel Dekker.

JACKSON, D.M. (1971). The effect of β-phenylethylamine on noradrenaline concentrations in guinea pig brain. *J. Pharm. Pharmac.*, **23**, 623–624.

JACKSON, D.M. & SMYTHE, D.B. (1973). The distribution of β-phenylethylamine in discrete regions of the rat brain and its effect on brain noradrenaline, dopamine and 5-hydroxytryptamine levels. *Neuropharmac.*, **12**, 663–668.

JONSSON, J., GROBECKER, H. & HOLTZ, P. (1966). Effects of β-phenylethylamine on content and subcellular distribution of norepinephrine in rat heart and brain. *Life Sci.*, **5**, 2235–2246.

KAROUM, F., SPECIALE, S.G., CHUANG, L.W. & WYATT, R.J. (1982). Selective effects of phenylethylamine on central catecholamines: a comparative study with amphetamine. *J. Pharmac. exp. Ther.*, **224**, 432–439.

KINEMUCHI, H., ARAI, Y., ORELAND, L., TIPTON, K.F. & FOWLER, C.J. (1982). Time-dependent inhibition of monoamineoxidase by β-phenylethylamine. *Biochem. Pharmac.*, **23**, 47–56.

KOE, B.K. (1976). Molecular geometry of inhibitors of uptake of catecholamines and serotonin in synaptosomal preparations of rat brain. *J. Pharmac. exp. Ther.*, **199**, 649–661.

LOO, H.Y., JERVIS, G.A. & HORNING, M.G. (1978). Possible role of 2-phenylethylamine in the pathology of the central nervous system. In *Non-Catecholic Phenylethylamines*, Part I. ed. Mosnaim, A.D. & Wolf, M.E. pp. 419–445. New York: Marcel Dekker.

LUNDBERG, P.A., ORELAND, L. & ENGBERG, G. (1986). Inhibition of locus coeruleus neuronal activity by beta-phenylethylamine. *Life Sci.*, **36**, 1889–1896.

MARTINDALE THE EXTRA PHARMACOPOEIA (1982). 28th edition. ed. Reynolds, J.E.F. & Prasad, A.D. p. 68. London: The Pharmaceutical Press.

McQUADE, P.S. & WOOD, P.L. (1983). The effects of β-phenylethylamine on tyramine and dopamine metabolism. *Prog. Neuro-Psychopharmac. Biol. Psychiat.*, **7**, 755–758.

NAZARALI, A.J., BAKER, G.B., COUTTS, R.T. & WONG, J.T.F. (1987a). N-2-(cyanoethyl)tranylcypromine, a potential prodrug of tranylcypromine; its disposition and interaction with catecholamine neurotransmitters in the brain. *Pharmaceut. Res.*, **4**, 16–20.

NAZARALI, A.J., BAKER, G.B., COUTTS, R.T., YEUNG, J.M. & RAO, T.S. (1987b). Rapid analysis of β-phenylethylamine in tissues and body fluids utilizing pentafluorobenzoylation followed by electron capture gas chromatography. *Progr. Neuro-Psychopharmac. Biol. Psychiat.*, **11**, 251–258.

NIELSEN, J.A., CHAPIN, D.S. & MOORE, K.E. (1983). Differential effects of d-amphetamine, β-phenylethylamine, cocaine and methylphenidate on the rate of dopamine synthesis in terminals of nigrostriatal and mesolimbic neurons and on the efflux of dopamine metabolites into cerebroventricular perfusates of rats. *Life Sci.*, **33**, 1899–1907.

OLDENDORF, W.H. & DEWHURST, W.G. (1978). The blood-brain barrier and psychotropic drugs. In *Principles of Psychopharmacology* ed. Clark, W.G. & delGuidice, J. pp. 183–191. New York: Academic Press.

PATRICK, R.L. (1981). Phenylethylamine effects on dopamine synthesis. Structure-activity relationships. *Biochem. Pharmac.*, **30**, 141–146.

PHILIPS, S.R., BAKER, G.B. & McKIM, H.R. (1980). Effects of tranylcypromine on concentrations of some trace amines in the diencephalon and hippocampus of the rat. *Experientia*, **36**, 241–242.

PHILIPS, S.R. & BOULTON, A.A. (1979). The effect of monoamine oxidase inhibitors on some arylalkylamines in rat striatum. *J. Neurochem.*, **33**, 159–167.

PHILIPS, S.R. & ROBSON, A.M. (1983). *In vivo* release of endogenous dopamine from rat caudate nucleus by phenylethylamine. *Neuropharmac.*, **22**, 1297–1301.

RAITERI, M., DEL CARMINE, R., BERTOLLINI, A. & LEVI, G. (1977). Effect of sympathomimetic amines on the synaptosomal transport of noradrenaline, dopamine and 5-hydroxytryptamine. *Eur. J. Pharmac.*, **41**, 133–143.

RAO, T.S., BAKER, G.B. & COUTTS, R.T. (1987). N-(3-Chloropropyl)phenylethylamine as a possible prodrug of β-phenylethylamine: studies in the rat brain. *Progr. Neuro-Psychopharmac. Biol. Psychiat.*, **11**, 301–308.

RICHTER, A. & SMITH, S.E. (1974). Inhibition of human platelet 5-hydroxytryptamine uptake by β-phenylethylamine derivatives. *J. Pharm. Pharmac.*, **26**, 763–770.

ROBERTS, M.M. & PATRICK, R.C. (1979). Amphetamine and phenylethylamine induced alterations in dopamine synthesis regulation in rat brain synaptosomes. *J. Pharmac. exp. Ther.*, **209**, 104–110.

SABELLI, H.C. & MOSNAIM, A.D. (1974). Phenylethylamine hypothesis of affective behavior. *Am. J. Psychiat.*, **131**, 695–699.

SANDLER, M., RUTHVEN, C.R.J., GOODWIN, B.C. & COPPEN, A. (1979). Decreased cerebrospinal fluid concentration of free phenylacetic acid in depressive illness. *Clin. Chim. Acta*, **93**, 169–171.

SHANNON, H.E., CONE, E.J. & YOUSEFNEJAD, D. (1982). Physiologic effects and plasma kinetics of β-phenylethylamine and its N-methyl homolog in dog. *J. Pharmac. exp. Ther.*, **223**, 190–196.

SUCKLING, C.J., BRECKENBRIDGE, R.J., BANSAL, S.S., WILLIAMS, L.C., WALTING, K.J. & IVERSEN, L.L. (1985). Non-selective inhibition of GABA and 5-HT uptake systems in rat brain by N-n-alkylhydroxybenzylamine and N-n-alkylphenylethylamine derivatives. *Biochem. Pharmac.*, **34**, 4173–4177.

USDIN, E. & SANDLER, M., ed. (1976). *Trace Amines and the Brain*. New York: Marcel Dekker.

WINSTEAD, M.B., PODLESNY Jr., E.J. & WINCHELL, H.S. (1978). Preparation and scintigraphic evaluation of carbon-11-labelled 2-N-phenethylaminoalkane-nitriles. *Nucl.-Med.*, **17**, 277–282.

WOLF, M.E. & MOSNAIM, A.D. (1985). Phenylethylamine in neuropsychiatric disorders. *Gen. Pharmac.*, **14**, 385–390.

WU, P.H. & BOULTON, A.A. (1975). Metabolism and disappearance of injected β-phenylethylamine in the rat. *Can. J. Biochem.*, **53**, 42–50.

WURTMAN, R.J. & AXELROD, J. (1963). A sensitive and specific assay for the estimation of monoamine oxidase. *Biochem. Pharmac.*, **12**, 1439–1440.

YANG, H.-Y.T. & NEFF, N.H. (1973). β-Phenylethylamine: a specific substrate for type-B monoamine oxidase of brain. *J. Pharmac. exp. Ther.*, **187**, 365–371.

(Received September 15, 1986.
Revised May 29, 1987.
Accepted June 8, 1987.)