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[^{14}C]-β-phenethylamine, its distribution after administration by various routes to cats, and the effects of monoamine oxidase inhibitors

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1 [^{14}C]-β-phenethylamine ([^{14}C]-PEA) was instilled intragastrically, intraduodenally (i.d.) or infused into the portal vein or femoral artery of cats, anaesthetized with chloralose, to investigate its distribution in the body.

2 [^{14}C]-PEA and phenylacetic acid (PAA) accounted for approximately 85% of radioactivity recovered in blood from control cats or those pretreated with deprenyl or mebanazine. Progressively greater portal venous (PV), cranial mesenteric arterial (CMA) and PV-CMA concentrations of PEA and PAA were observed with increase in amount of PEA instilled intraduodenally (i.d.); PAA predominated over PEA, more so in CMA than PV blood. Radioactivity was not recovered from blood following intragastric instillation of PEA.

3 When histamine 1.7 μmol kg^{-1}, i.d., was combined with PEA 1.7 μmol kg^{-1}, i.d., or tyramine 8.5 μmol kg^{-1}, i.d., was combined with PEA 8.5 μmol kg^{-1}, i.d., PV-CMA values for PEA were significantly augmented.

4 Arterial concentrations of PEA were increased 3.5 to 5 fold compared to controls by pretreatment with mebanazine or deprenyl plus clorgyline; arterial concentrations of PAA were reduced. PEA blood concentrations were not significantly altered by clorgyline or deprenyl pretreatment.

5 Infusion of PEA 680, 1020 or 1360 nmol kg^{-1} min^{-1} for 20 min into the portal vein raised blood pressure 60 to 100 mmHg (at a PEA concentration of ca, 2 nmol ml^{-1}) but lacked effect on the nictitating membrane despite peak arterial PEA concentrations of 20 nmol ml^{-1}; in cats pretreated with mebanazine or clorgyline plus deprenyl, half-maximum contraction of the nictitating membrane occurred with arterial PEA concentrations of 4.8 to 9 nmol ml^{-1}. In cats pretreated with mebanazine or deprenyl plus clorgyline, half maximum contraction of the nictitating membrane was elicited also by intraduodenal PEA 8.5 μmol kg^{-1} at arterial PEA concentrations of ca. 2 nmol ml^{-1}, despite lack of effect of PEA 17 μmol kg^{-1}, i.d., in control cats with a peak arterial PEA concentration of 1.8 nmol ml^{-1}.

6 [^{14}C]-PEA and PAA were recovered from liver, kidney, distal small intestine, lung, arterial vessel walls, skeletal muscle, brain, foetus and amniotic liquor, after PEA instilled i.d., overall concentration of PEA exceeding that of PAA except in the kidney. The combined amount of PEA and PAA in kidney was 7 to 20 fold that in other tissues. PEA content of tissues was significantly elevated and that of PAA diminished by pretreatment with deprenyl plus clorgyline, and to a lesser extent after mebanazine.

Introduction

β-Phenethylamine (PEA) is a sympathomimetic amine present in foods such as cheese (Asatoor *et al.*, 1963) red wine and chocolate (quoted by Sandler *et al.*, 1974). Chocolate is the commonest food precipitant for migraine, and ingestion of small doses of PEA (3 mg) elicited headache in migraineurs (Sandler *et al.*, 1974). Although less potent as a pressor agent than another dietary amine, tyramine, its ingestion in cheese could also have contributed in a minority of the 'hypertensive crises' affecting subjects treated with MAO inhibitors and who ingested cheese. β-Phenethylamine has been considered a specific substrate for MAO B (Yang & Neff, 1973; Neff & Yang, 1974).

The present experiments with [^{14}C]-β-phenethylamine ([^{14}C]-PEA) administered by various routes to cats, revealed a dissimilar pattern of absorption, distribution and tissue concentrations compared to tyramine. Indeed, since these tissue concentrations

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were substantially larger than those following identical doses of tyramine, β-phenethylamine emerges as potentially the most relevant to migraine of the three dietary amines examined, the others being histamine (Imrie *et al.*, 1978) and tyramine (Garcha *et al.*, 1983a).

A preliminary account of the results has been communicated to the British Pharmacological Society (Garcha *et al.*, 1983b).

Methods

Experiments were performed on cats (2–4.5 kg) of either sex. Food, apart from milk and water was withheld overnight (18 h). Cats were anaesthetized with chloralose (80 mg kg^{-1}, i.v.), the trachea cannulated and the animal artificially ventilated; also cannulated were a femoral vein for intravenous injections, and a femoral artery for recording blood pressure via a transducer and pen recorder. [^{14}C]-PEA (5 μCi) and non-radioactive PEA hydrochloride (1.7, 8.5 or 17 μmol kg^{-1}) was instilled into the duodenum.

Instillation of phenethylamine into the small intestine

The procedure was as described previously for histamine (Imrie *et al.*, 1978). Briefly, drugs including [^{14}C]-PEA in 10 ml saline (0.9% w/v) were instilled via a cannula tied anterogradely into the duodenum; a more distal cannula, inserted retrogradely into the jejunum, allowed the lumen contents to be washed through and collected at the end of the experiments; this length of intestine weighed 40 ± 5 g. Blood was removed at 5 or 10 min intervals via scalp infusion needles (butterfly-23 0.6 mm, Abbott Ireland, Sligo, Eire), cemented with cyanoacrylate adhesive into the greater portal vein (PV) and cranial mesenteric artery (CMA), the needle tips disposed so that blood flow was uninterrupted. A non-cannulated length of small intestine immediately distal to the second cannula was used to measure the uptake of ^{14}C-compounds from arterial blood (Imrie *et al.*, 1978). MAO inhibitors were given intraduodenally (i.d.) 90 min before [^{14}C]-PEA. Concentrations of PEA and phenylacetic acid (PAA) in venous and arterial blood were used to calculate PV-CMA values for each compound. (Mean values were obtained by averaging the summed total for the 12 samples taken 5–60 min after instilling PEA). These values do not necessarily reflect total absorption. For example, PEA present in arterial blood may subsequently be removed by uptake into the intestinal wall or metabolized during its circulation through the intestinal vasculature.

Liver by-pass experiments

Cats were prepared as above but the spleen removed, and a cannula containing heparin-saline (0.1 mg ml^{-1}) tied at one end into the proximal stump of the splenic vein and at the other end into a jugular vein. Heparin (10 mg kg^{-1}) was injected into a femoral vein, PEA instilled intraduodenally and the portal vein occluded with a bulldog clip, carefully avoiding the hepatic artery; blood from the intestines then passed via the cannula into the systemic circulation. Blood for assay was removed from the CMA and from the portal vein distal to the occlusion..

Infusion of phenethylamine

Radioactive PEA was infused, either 5 μCi with PEA 680, 1020 or 1360 nmol kg^{-1} min^{-1} for 20 min (approx 3.5 ml) via a cannula cemented into the portal vein, or 5 μCi with PEA 34 nmol kg^{-1} min^{-1} for 20 min into a femoral artery in the direction of flow.

Chromatography and scintillation counting

Blood samples (approx. 1.0 ml) were treated with concentrated perchloric acid and vortexed for 10 s. The supernatant fluid (200 μl) from such blood (and tissue) samples, together with reference compounds PEA (75 μg) and (PAA 150μg) were applied for descending chromatography as described by Thomas & Marley (1978). The solvent system was *n*-butanol-propan-2-ol-ammonia-water (3-1-1-1, v/v/v/v) as devised by Tacker *et al.* (1970). Metabolites were located by spraying the paper with bromophenol blue in methanol. Radioactive PEA and PAA were quantitated by direct counting of the appropriate paper areas after immersion in 10 ml of NE 233 scintillation fluid and measured in a NE 8312 scintillation spectrometer (Nuclear Enterprises, Edinburgh) with an efficiency of 30%.

Nictitating membrane

Contractions of the membrane, after enucleation of the eyeball and excision of the ipsilateral superior cervical and vagal nodose ganglia, were recorded by an isotonic transducer (3 g load) and a pen-recorder.

Amine oxidation in vitro

Portions of the liver, distal small intestine and kidney were removed at the conclusion of experiments using combined deprenyl and clorgyline, and separately homogenized at 20,000 r.p.m. for three 5 s periods in 3 vol. ice cold KCl (1.15%) with an Ultra Turrax, Type TP 18/2 (Janke & Kuntel, Stanjen i.Br., G.F.R.), PEA, tyramine and 5-hydroxytryptamine oxidation were measured in the different tissues using ^{14}C-substrates at a final concentration of 1 mmol, except for PEA which was 0.2 mmol, the products being isolated by

ion-exchange chromatography (Tipton & Youdim, 1976).

Histology

Sections of small intestine from the instillation site were fixed in sodium phosphate buffer (0.075 M, pH 7) containing formalin (10%) and then embedded in paraffin wax; 6 mm sections were cut on a Reichert rotary microtome and alternate sections stained with haematoxylin/eosin and Masson's trichrome, the latter staining for connective tissue fibres and mucin.

Drugs

Drugs used were 5-hydroxy [side chain-2-^{14}C] tryptamine creatinine sulphate (58 mCi mmol^{-1}), The Radiochemical Centre, Amersham, and 2-phenyl [1-^{14}C]-ethylamine hydrochloride (60 mCi mmol^{-1}), New England Nuclear, Dreiech, F.R.G.; the hydrochlorides of β-phenethylamine, tryptamine and tyramine (Sigma); histamine dihydrochloride (Sigma); clorgyline hydrochloride (May & Baker); (−)-deprenyl hydrochloride (from Professor Merton Sandler) and mebanazine oxalate (I.C.I.). Other chemicals were of the highest quality commercially available.

Results

Instillation of phenethylamine into the stomach

During the 2 h that PEA (8.5 μmol kg^{-1}) was in the stomach (2 cats), radioactivity could not be detected in portal or CMA blood. However, on displacing the stomach contents into the duodenum/jejunum, radioactivity appeared within 5 min in the portal venous and CMA blood.

Instillation of phenethylamine into the small intestine

PEA and PAA constituted 88% ± 6.3% ($n = 7$) of radioactivity recovered in blood following each of the three doses instilled i.d. (1.7, 8.5 or 17 μmol kg^{-1}).

Portal venous concentrations of [^{14}C]-PEA and PAA

PEA was detected in PV blood 5 min after i.d. instillation following even the small dose, with peak concentrations for the three doses of 1.1, 4 and 7.6 nmol ml^{-1} respectively at 5 to 10 min, and remained present in samples removed over the 60 min experiments (Figure 1a-c). The means of cumulated PEA concentrations (see Methods and legend Table 1, for details) were significantly greater ($P < 0.01$) for the 8.5 and 17 μmol kg^{-1} compared to the small dose (Table 1). PAA concentration exceeded that of PEA (Figure 1) by a ratio of 4:1 for mean values after PEA

Table 1 Blood concentrations of [^{14}]-β-phenethylamine (PEA) and phenylacetic acid (PAA) in control cats and those given monoamine oxidase inhibitors 90 min previously

		1.7 μmol kg^{-1} with 5 μCi			8.5 μmol kg^{-1} with 5 μCi			17 μmol kg^{-1} with 5 μCi		
		pmol ml^{-1}			pmol ml^{-1}			pmol ml^{-1}		
		PEA	*PAA*	*n*	*PEA*	*PAA*	*n*	*PEA*	*PAA*	*n*
Controls	PV	295 ± 31	1262 ± 209	4	2744 ± 414**	5271 ± 747**	7	6351 ± 904**	8166 ± 917**	5
	CMA	113 ± 20	866 ± 54		707 ± 76***	3954 ± 817*		1157 ± 301*	5181 ± 1037*	
Deprenyl (4.5 μmol kg^{-1})	PV	400 ± 86	620 ± 107	4	1921 ± 410	3765 ± 367	3			
	CMA	121 ± 72	260 ± 118*		562 ± 55	4216 ± 749				
Clorgyline (24.5 μmol kg^{-1})	PV				1939 ± 881	2332 ± 563	3			
	CMA				807 ± 307	3309 ± 592				
Deprenyl (4.5 μmol kg^{-1}) plus Clorgyline (24.5 μmol kg^{-1})	PV	975 ± 147***	848 ± 59	4	7825 ± 800**	2593 ± 357*	5			
	CMA	370 ± 57**	629 ± 81		2685 ± 334***	2013 ± 895				
Mebanazine (40 μmol kg^{-1})	PV				6546 ± 890*	2070 ± 445*	5			
	CMA				3376 ± 629***	2131 ± 467				

Values are mean ± s.e.mean; mean is derived from totalled 5 min samples over 60 min for each group of cats. Control values are compared statistically with those after PEA, 1.7 μmol kg^{-1}; values after MAO inhibitors are compared statistically with those for related control dose of PEA (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ for this and other Tables).

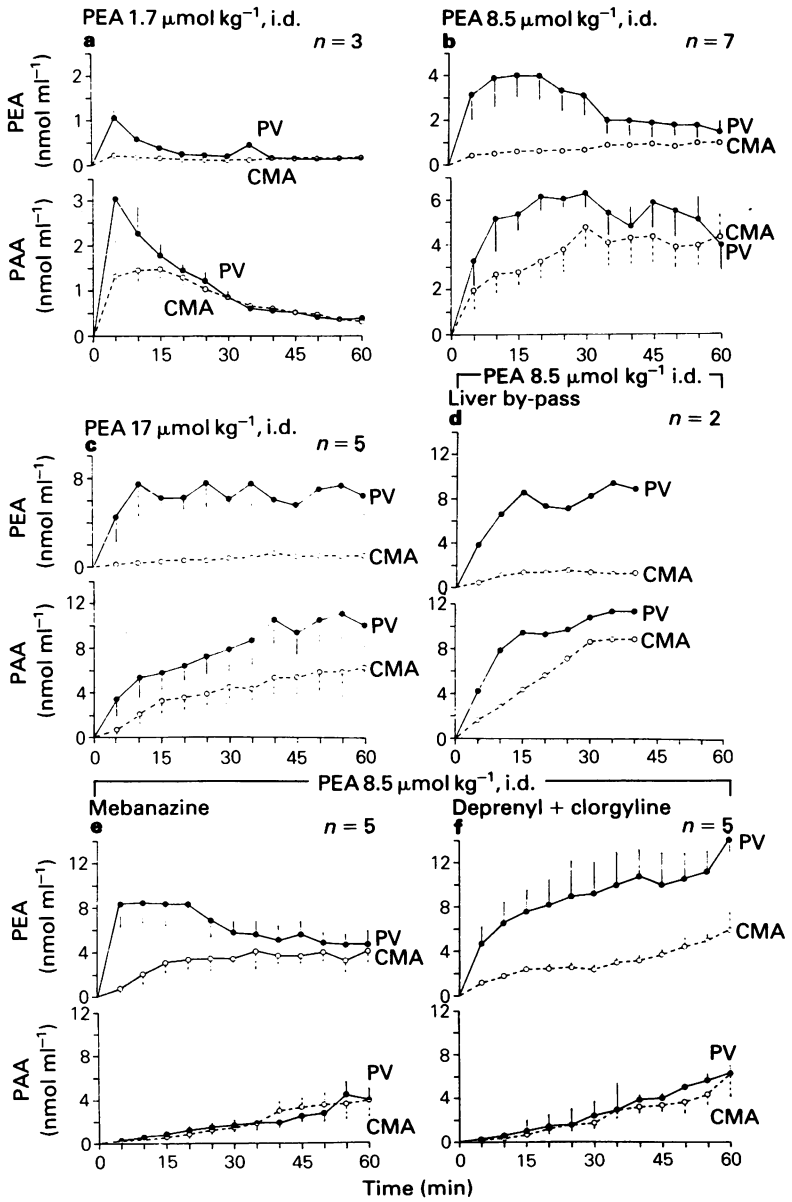

Figure 1 Mean concentrations (nmol ml^{-1}) of [^{14}C]-β-phenethylamine (PEA) and phenylacetic acid (PAA) in portal venous (PV, ●) and cranial mesenteric blood (CMA, 0) in control cats given PEA i.d. (each with 5 μCi); (a) 1.7 μmol kg^{-1}; (b) 8.5 μmol kg^{-1}, i.d. and (c) 17 μmol kg^{-1}. Cats (d) to (e), given PEA, 8.5 μmol kg^{-1}, i.d. (each with 5 μCi) but pretreatment (e) with mebanazine (40 μmol kg^{-1} i.d.) or (f) deprenyl (4.5 μmol kg^{-1}, i.d.) plus clorgyline (24.5 μmol kg^{-1} i.d.) 90 min previously and in (d), the portal vein was clamped and blood from the intestines diverted to a jugular vein. Vertical lines are s.e.means. *n* = number of cats (as in Figure 2).

1.7 μmol kg^{-1}, the ratio declining to 1.3:1 after the large dose (Table 1).

Arterial concentrations of [^{14}C]-PEA and PAA PEA was detected in CMA blood 5 min after its i.d. instillation with each of the 3 doses used (Figure 1a-c) but the concentrations were smaller than in PV blood. Mean PEA concentrations were elevated with increase in dose, significantly for the 8.5 ($P<0.001$) and 17 μmol kg^{-1} ($P<0.05$) doses compared to that for PEA, 1.7 μmol kg^{-1} (Table 1). PAA concentrations exceeded those for PEA (Figure 1) to a greater extent than in portal blood, the ratio of acid:amine for values being about 8:1 after PEA 1.7 μmol kg^{-1} and 4.5:1 following the large dose (Table 1).

PV-CMA differences These were calculated in pmol-ml^{-1}kg^{-1}, being values for PEA absorbed prior to distribution in the body and therefore related to the amount instilled. These values were significantly greater for both the 8.5 ($P<0.001$) and the 17 μmol kg^{-1} ($P<0.01$) doses (631 and 1858 pmol ml^{-1}kg^{-1} respectively) compared to 62 pmol ml^{-1}kg^{-1} after PEA, 1.7 μmol kg^{-1} i.d.

Instillation of PEA with other amines The means of cumulated PEA or PAA concentrations were not significantly altered by combination of PEA (1.7 μmol kg^{-1}) with histamine (1.7 μmol kg^{-1}, i.d.) (3 cats), or PEA (8.5 μmol kg^{-1}, i.d.) with tryptamine (8.5 μmol kg^{-1}, i.d.) (3 cats) or tyramine

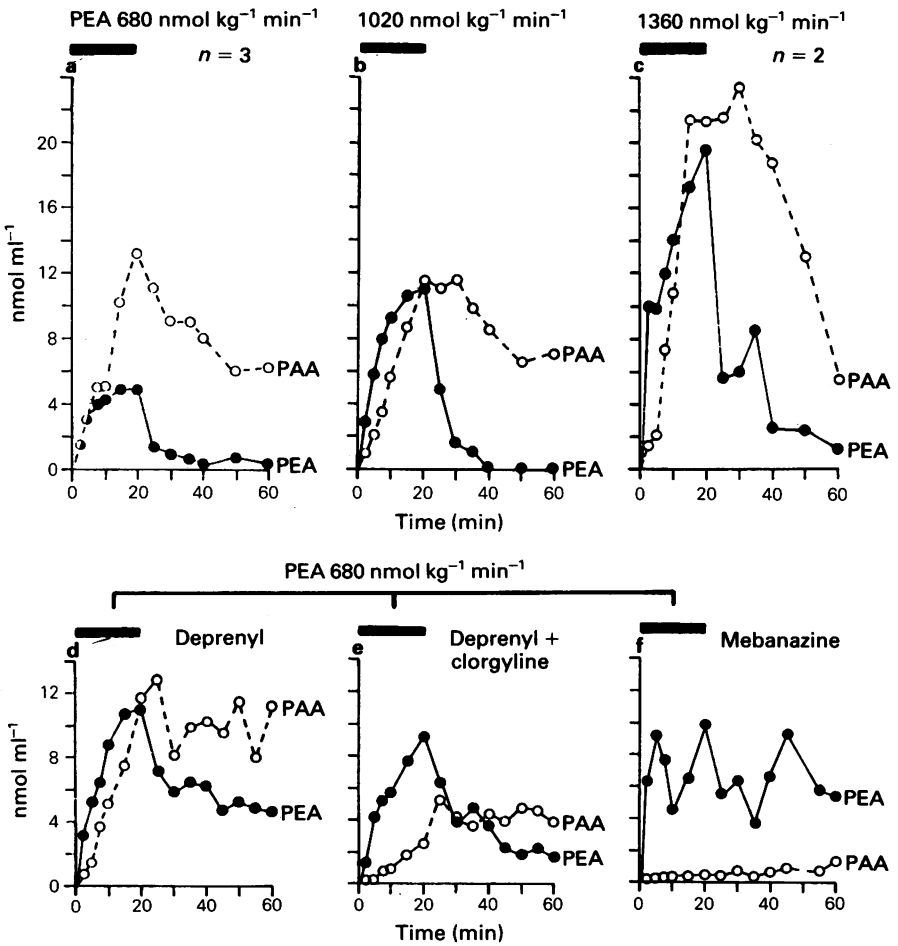

Figure 2 Concentrations of β-phenethylamine (PEA) and phenylacetic acid (PAA) in cranial mesenteric arterial blood from 9 cats infused over the initial 20 min of experimens (black bar) with PEA, 680 nmol kg^{-1}min^{-1} (a,d,e,f), PEA, 1020 nmol kg^{-1}min^{-1} (b) or PEA 1360 nmol kg^{-1}min^{-1} (c). In addition, pretreatment occurred 90 min previously in (d) with deprenyl (4.5 μmol kg^{-1}, i.d.), (e) with deprenyl (4.5 μmol kg^{-1}, i.d.) plus clorgyline (24.5 μmol kg^{-1}, i.d.) and in (f) with mebanazine (40 μmol kg^{-1}, i.d.)

($8.5\,\mu mol\,kg^{-1}$, i.d.) (3 cats). However, PV-CMA differences were significantly elevated; thus for PEA $1.7\,\mu mol\,kg^{-1}$ combined with histamine, the mean value increased from a control of 62 to 152 pmol $ml^{-1}kg^{-1}$ ($P<0.05$); and for PEA $8.5\,\mu mol\,kg^{-1}$ combined with tyramine, the mean value was raised from 631 to 1577 pmol $ml^{-1}kg^{-1}$ ($P<0.05$).

Instillations of PEA into the small intestine after liver by-pass In these experiments the liver was excluded from the circulation by portal vein occlusion (see Methods). Following PEA ($8.5\,\mu mol\,kg^{-1}$ i.d.), blood concentrations of PAA still exceeded those of PEA, despite peak PEA mean concentrations in PV and CMA blood being doubled compared to control cats (contrast Figure 1d with 1b); however, the ratio of mean cumulated acid:amine was reduced, i.e. to 1.1:1 and 4.5:1 respectively in portal venous and CMA blood compared with 2:1 and 5.5:1 respectively for control cats.

Infusions of phenethylamine

Infusion into portal vein Infusions of PEA (680, 1020 or 1360 $nmol\,kg^{-1}min^{-1}$) for 20 min into the portal vein of 6 cats led to peak concentrations of PEA in blood removed from the cranial mesenteric artery of 5, 11 and 19.4 $nmol\,ml^{-1}$ respectively at 20 min (Figure 2a, b, c). The rate of increase of PAA and its peak arterial concentration greatly exceeded that of PEA with infusion of PEA ($680\,nmol\,kg^{-1}min^{-1}$) but paralleled that of PEA with infusions of larger amounts, suggesting that with the larger amounts deamination mechanisms were saturated. As shown in Figure 3a, marked pressor responses were elicited and sustained over the 20 min with these infusions, being evident at an arterial PEA concentration of $2\,nmol\,ml^{-1}$, although nictitating membrane response was absent or minimal.

Intra-arterial infusion In 4 cats, PEA (34 $nmol\,kg^{-1}min^{-1}$) was infused for 20 min into a femoral artery. Whereas the arterial concentration of PEA (Figure 4a) was virtually identical in intact and in abdominally eviscerated cats, i.e. those in which intestinal and hepatic deamination could not occur, that of PAA was smaller and its concentration rose more slowly and reached a smaller peak in eviscerated than in intact cats (Figure 4b); overall, the area under the curve for PAA in eviscerated cats was half that in intact cats. Thus tissues other than the intestine and liver were capable of deaminating PEA, albeit less effectively.

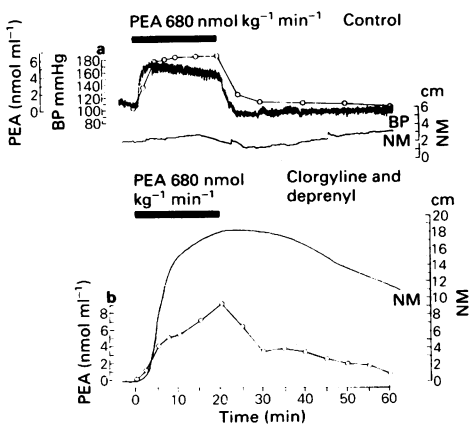

Figure 3 Relation of arterial concentration ($nmol\,ml^{-1}$) of β-phenethylamine PEA (○) to blood pressure (BP) and tone of nictitating membrane (NM) during and following infusion of PEA, 680 $nmol\,kg^{-1}min^{-1}$ over 20 min (black bar). (a) Control cat; (b) cat given clorgyline ($24.5\,\mu mol\,kg^{-1}$, i.d.) plus deprenyl ($4.5\,\mu mol\,kg^{-1}$, i.d.) 90 min previously.

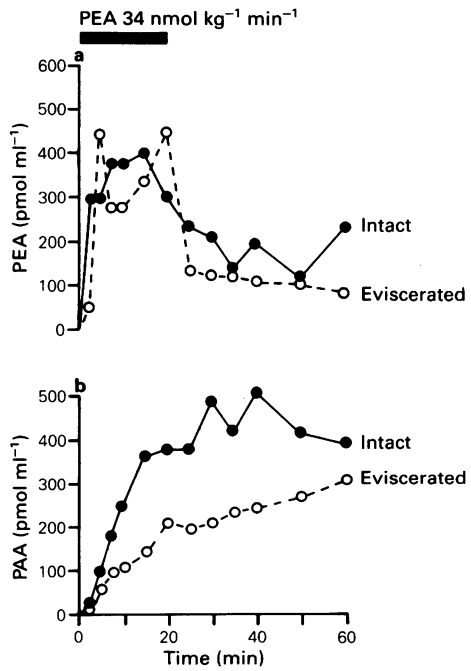

Figure 4 Mean concentration ($pmol\,ml^{-1}$) of β-phenethylamine (PEA, ●) and phenylacetic acid (PAA, ○) in cranial mesenteric blood in 2 intact cats and 2 abdominally eviscerated cats and after infusion of PEA 34 $nmol\,kg^{-1}min^{-1}$ for 20 min (black bar) into a femoral artery, in the direction of flow.

Instillation of PEA into the small intestine after pretreatment with monoamine oxidase inhibitors

Following each of the PEA doses given i.d., PEA and PAA constituted 85 ± 8.33% ($n = 7$) of radioactivity recovered from blood in cats pretreated with deprenyl or mebanazine (doses in Table 1).

Mean peak concentrations of PEA in PV and CMA blood were at least doubled following its administration (PEA 8.5 μmol kg^{-1}) in cats pretreated with mebanazine or clorgyline plus deprenyl, compared to control cats (compare Figures 1e, f with Figure 1b); concentrations of PAA were substantially reduced. The means of cumulated PEA values following this dose were significantly increased – 2.5 to 5 fold in portal ($P < 0.05$ to $P < 0.01$) and arterial blood ($P < 0.001$) by such pretreatment, whereas the reduction in PAA was significant ($P < 0.05$) only for PV samples (Table 1). The mean cumulated PEA value in PV and CMA blood was significantly increased ($P < 0.01$) in cats pretreated with clorgyline plus deprenyl following PEA, 1.7 μmol kg^{-1} (Table 1). In contrast, pretreatment with deprenyl or clorgyline alone (Table 1) had little significant effect on amine or metabolite concentrations following PEA 1.7 or 8.5 μmol kg^{-1}, i.d.

Contraction of the nictitating membrane was not obtained in the control experiments even following PEA (17 μmol kg^{-1}, i.d.) when arterial concentration of PEA reached 1.8 μmol ml^{-1}, nor in cats pretreated with deprenyl plus clorgyline and given PEA (1.7 μmol kg^{-1}, i.d.) or in clorgyline pretreated cats given PEA, 8.5 μmol kg^{-1}, i.d. However, intense contraction of the nictitating membrane ensued after PEA (8.5 μmol kg^{-1}, i.d.) in cats pretreated with mebanazine, or deprenyl plus clorgyline; half maximal contraction occurred at arterial PEA concentrations of about 2 nmol ml^{-1}. A small contraction of the nictitating membrane was obtained in 1 of 3 cats pretreated with deprenyl and given PEA (8.5 μmol kg^{-1}, i.d.).

PV-CMA differences Except in cats pretreated with clorgyline or deprenyl, for which these mean values were reduced, there was a significant increase in the PV-CMA difference for PEA after the MAO inhibitors. This increase ($P < 0.01$) was also seen in cats pretreated with combined deprenyl and clorgyline for both the PEA 1.7 and 8.5 μmol kg^{-1} doses (mean values of 242 and 2379 pmol ml^{-1} kg^{-1} compared to respective control values of 62 and 631 pmol ml^{-1} kg^{-1}) and for cats pretreated with mebanazine (mean value of 1233 compared to the control value of 631 pmol ml^{-1} kg^{-1}, $P < 0.05$).

Infusion of PEA into the portal vein after pretreatment with monoamine oxidase inhibitors PEA (680 nmol kg^{-1} min^{-1}) was infused intraportally to 3 cats given

Table 2 Concentrations (nmol g^{-1}) of [^{14}C]-β-phenethylamine (PEA) and phenylacetic acid (PAA) in the liver from control cats and those pretreated with monoamine oxidase inhibitors approx. 150 min previously

		1.7 μmol kg^{-1} with 5 μCi nmol g^{-1}	n	8.5 μmol kg^{-1} with 5 μCi nmol g^{-1}	n	17 μmol kg^{-1} with 5 μCi nmol g^{-1}	n
Controls	PEA	0.56 ± 0.11	4	6.83 ± 1.37*	7	9.69 ± 2.21**	5
	PAA	0.52 ± 0.20		3.72 ± 0.96*		6.32 ± 2	
Deprenyl (4.5 μmol kg^{-1}) Clorgyline (24.5 μmol kg^{-1})	PEA	1.47 ± 0.38*	3	5.28 ± 1.31	6	4.9	1
	PAA	1.00 ± 0.09		3.62 ± 1.1		15.1	
	PEA			2.27 ± 1.08	4		
	PAA			6.31 ± 0.94			
Deprenyl (4.5 μmol kg^{-1}) plus Clorgyline (24.5 μmol kg^{-1})	PEA	3.10 ± 0.47*	4	30.98 ± 6.07**	5		
	PAA	1.50 ± 0.3		8.14 ± 1.61*			
Mebanazine (40 μmol kg^{-1})	PEA			28.28 ± 7.08*	5		
	PAA			3.31 ± 1			

Values are mean ± s.e.mean. Statistical conventions as in Table 1.

an MAO inhibitor i.d. 90 min previously (for doses see legend Figure 2). Thus following deprenyl, clorgyline combined with deprenyl, or mebanazine, peak PEA arterial concentrations were markedly enhanced to 11, 9.2 and 10 nmol ml^{-1} respectively (compare Figures 2d,e,f with Figure 2a). PAA was reduced compared to the control infusion (Figure 2a), most after mebanazine and least after deprenyl. In contrast to the control cats, contraction of the nictitating membrane ensued (compare Figure 3b,3a), half maximum contraction being present with PEA concentrations (nmol ml^{-1}) of 8.5 (deprenyl), 4.8 (deprenyl plus clorgyline) and 9 (mebanazine) respectively.

Tissue concentrations of PEA and PAA after intraduodenal instillation of PEA

Liver The PEA mean concentration (nmol g^{-1} wet tissue) rose 12 fold ($P<0.05$) after the 8.5 μmol kg^{-1} dose compared to that following the small dose and 17 fold ($P<0.01$) with the largest dose and it accounted for more (65%) of the recovered ^{14}C-compounds with the larger doses than with the smaller (50%). Both the concentration and proportion of PEA were increased after combined clorgyline with deprenyl (5 fold; $P<0.01$) or mebanazine (4.5 fold; $P<0.05$) pretreatment, and following PEA 8.5 μmol kg^{-1}, i.d. (Table 2). Tissue concentrations of PEA and PAA after PEA (8.5 μmol kg^{-1}, i.d.) were not significantly changed by deprenyl or clorgyline pretreatment.

Jejunum ^{14}C-compounds were distributed to the jejunum after hepatic metabolism and as a consequence of uptake from arterial blood. As with the liver, both the proportion of PEA and its concentration were significantly elevated ($P<0.01$) with the larger doses of PEA, compared with the smallest. Absolute amounts of acid were significantly increased (Table 3). Deprenyl, clorgyline or mebanazine pretreatment did not significantly change PEA concentration (not shown) in jejunum or kidney. However, in cats pretreated with combined deprenyl and clorgyline, PEA concentration was significantly elevated ($P<0.05$) following PEA 8.5 μmol kg^{-1}, i.d.

Kidney In contrast to other tissues, the concentration of PAA exceeded that of PEA in the kidney of control cats (Table 3) by as much as 4 to 18 fold with the three different i.d. doses of PEA. Noteworthy also were the substantially larger combined PEA and PAA concentrations (range 19.1 to 115.6 nmol g^{-1}) compared to those for the liver (1.08 to 16.01 nmol g^{-1}) or jejunum (1.07 to 14.33 nmol g^{-1}). While the concentration of PEA was significantly increased ($P<0.01$) after PEA (8.5 or 17 μmol kg^{-1}, i.d.) compared to that after the small dose, its concentration after the 8.5 μmol kg^{-1}, i.d. dose was significantly increased also ($P<0.05$)

Table 3 Concentrations (nmol g^{-1}) of [^{14}C]-β-phenethylamine (PEA) and phenylacetic acid (PAA) in the jejunum and kidney from control cats and those pretreated with monoamine oxidase inhibitors approx. 150 min previously

		1.7 μmol kg^{-1} with 5 μCi nmol g^{-1}	*n*	8.5 μmol kg^{-1} with 5 μCi nmol g^{-1}	*n*	17 μmol kg^{-1} with 5 μCi nmol g^{-1}	*n*
Jejunum							
Controls	PEA	0.42 ± 0.04	4	5.53 ± 0.83**	6	9.83 ± 1.92**	5
	PAA	0.65 ± 0.12		3.35 ± 0.36***		4.50 ± 0.69**	
Deprenyl (4.5 μmolLkg^{-1}) plus Clorgyline (24.5 μmol kg^{-1})	PEA	0.44 ± 0.09	4	14.43 ± 2.36*	5		
	PAA	0.94 ± 0.14		5.57 ± 0.95			
Kidney							
Controls	PEA	1.05 ± 0.21	4	12.35 ± 2.14**	7	11.89 ± 2.3**	5
	PAA	18.05 ± 4.75		48.14 ± 7.61*		103.77 ± 19.8*	
Deprenyl (4.5 μmol kg^{-1}) plus Clorgyline (24.5 μmol kg^{-1})	PEA	1.47 ± 0.24	4	21.29 ± 3.03*	5		
	PAA	26.61 ± 4.21		60.03 ± 10.49			

Values are mean ± s.e.mean. Statistical conventions as in Table 1.

following pretreatment with combined deprenyl and clorgyline.

Other tissues Mean values for PEA and PAA in other tissues and body fluids following PEA (8.5 μmol kg^{-1}, i.d.) are shown in Table 4A. PEA concentrations were substantially lower, (between 15 and 40%) than those in liver, intestine or kidney. PEA was recovered from the brain, foetus and amniotic liquor. Following PEA (17 μmol kg^{-1}, i.d.), PEA concentrations in other tissues (1 cat) were respectively: lung, 11.7 nmol g^{-1}: skeletal muscle, 3.3 nmol g^{-1}; aorta, 1.7 nmol g^{-1}; and muscle-walled arteries, 1 nmol g^{-1}.

Tissue concentrations of PEA and PAA after infusion of PEA into the portal vein

Tissue concentrations of PEA and PAA (Table 4B) following infusion of PEA (680 μmol kg^{-1} min^{-1}) into the portal vein were more variable and the relative values differed from those after i.d. instillation, except that again, concentrations were largest in the kidney. PEA concentration in arterial vessel walls was similar to that in skeletal muscle. PEA concentrations were generally increased following pretreatment with deprenyl or clorgyline plus deprenyl.

Intestinal washings

In control cats, the residue of PEA washed from the lumen of the cannulated proximal intestine at the end of experiments (90 min after instillation) accounted for 1 to 19% of that instilled, with means of 4.3, 6.4 and 11.3% respectively for the 1.7, 8.5 and 17 μmol kg^{-1} doses; the range was wider following MAO inhibitors, 2 to 49.7%.

Table 4 (A) Mean concentrations (nmol g^{-1}) of [^{14}C]-β-phenethylamine (PEA) and phenylacetic acid (PAA) in tissues and amniotic liquor following PEA (8.5 μmol kg^{-1}, i.d.) given to control cats and those pretreated with monoamine oxidase inhibitors approx. 150 min previously
(B) As above except that instead of i.d. instillation, PEA 680 nmol $kg^{-1}min^{-1}$ was infused into the portal vein for 20 min

A

	Controls $n = 4$		*Deprenyl* $n = 3$		*Deprenyl plus clorgyline* $n = 1$		*Mebanazine* $n = 5$	
	PEA	*PAA*	*PEA*	*PAA*	*PEA*	*PAA*	*PEA*	*PAA*
Skeletal muscle	1.38	1.4			6	1.69	4.21	1.27
Lung	1.67	3.99	6.1	2.28			35.27	3.72
Aorta	1.12	3.38(1)						
Muscle-walled arteries	2.12	3.88(1)						
Cerebrum	2.24	1.12(2)						
Cerebellum	0.99	0.72(2)						
Foetus (pooled $n = 5$)	1.29	0.16(1)						
Amniotic liquor	0.22	0.81(1)						

B

	Controls $n = 2$		*Deprenyl* $n = 1$		*Clorgyline plus Deprenyl* $n = 1$	
	PEA	*PAA*	*PEA*	*PAA*	*PEA*	*PAA*
Liver	ND	5.98	6.83	14.23	0.54	6.32
Kidney	8.52	17.5	7.78	207.35	7.20	166
Skeletal muscle	0.75	3.05	3.23	4.27	0.18	0.36
Lung	0.55	5.15	4.46	18.80	10.65	4.15
Aorta	0.75	6.9				
Muscle-walled arteries	0.65	3.5				
Cerebrum	0.99	1.98(1)				
Cerebellum	3.18	1.39(1)				

Mean values are shown.
Doses are as in Table 1 to 3. n = is given at head of column; exceptions to these numbers are shown in parentheses.
ND = not detected.

In vitro *inhibition of monoamine oxidase*

The combined clorgyline and deprenyl dose effectively impaired oxidation of 5-hydroxytryptamine (MAO A) and β-phenethylamine (MAO B) by homogenates of liver and distal small intestine: the inhibition for liver ($n = 6$) was 76% (MAO A) and 95% (MAO B); for distal small intestine ($n = 4$), 70% (MAO A) and 77.2% (MAO B).

Histology

The epithelium of intestine which received instillates of saline, PEA or PEA combined with histamine, tryptamine or tyramine appeared identical to control sections of intestine.

Discussion

Little is known concerning intestinal absorption of PEA, although failure to obtain sympathomimetic effects after 30 μmol kg^{-1} given i.d. to rats implied lack of absorption or efficient hepatic metabolism (Blackwell & Marley, 1966). Serial plasma and tissue concentrations of PEA have been determined but after injection of much larger doses (270 μmol kg^{-1}) i.p or i.v. respectively, to rats (Cohen *et al.*, 1974) and dogs (Shannon *et al.*, 1982). In the present experiments with cats, PEA was detected in tissues as well as portal venous and arterial blood following intraduodenal administration of amounts approx. 1/150th those just described.

β-Phenethylamine is lipid-soluble with a partition coefficient of 1 between water and oleyl alcohol compared to 0.21 for its 4-hydroxylated water-soluble analogue, tyramine (Dewhurst & Marley, 1965). This could account for its rapid absorption with a peak concentration in portal blood 5 to 10 min after intraduodenal instillation compared with one at 60–90 min after i.d. tyramine (Garcha *et al.*, 1983a), although at the end of experiments (60–90 min) there were approximately similar amounts of tyramine and PEA (2 to 19% of total dose) remaining in the lumen of the proximal intestine.

While the total radioactivities recovered from blood after tyramine and PEA were similar (ca.85%), the ratios of the principal acid metabolite to the parent amine in portal blood differed substantially, namely 20:1 for *p*-hydroxyphenylacetic acid (pHPA) and tyramine (with the 1.7 μmol kg^{-1}, i.d. dose) but merely 4:1 for PEA and PAA. Thus intestinal MAO has less influence on intraduodenally administered PEA than on i.d. tyramine. The ratio of the acid metabolites to the parent amine declined with increase in amount instilled, suggesting that intestine deaminating mechanisms could be saturated, or that other routes of absorption were enhanced. Fortunately, deamination by the liver and other tissues was efficient, as evidenced by the much smaller arterial than portal blood concentrations of PEA. That other tissues besides liver effectively deaminated PEA, was clear from the substantial amounts of PAA formed in experiments in which PEA was either given intraduodenally to cats with the liver by-passed, or given intra-arterially to eviscerated cats which lacked both hepatic and intestinal deaminating capability.

PEA was recovered from tissues or other body fluids examined (liver, lung, gut, kidney, brain, skeletal muscle, arterial vessel walls, foetus, amniotic liquor). The concentrations in some tissues were unexpectedly large and, kidney excepted, PEA was usually in excess of PAA, further indicating sequestration of PEA remote from MAO. The results again contrast with those following intraduodenal instillation of tyramine when pHPA invariably predominated (Garcha *et al.*, 1983a). The large concentrations of PEA and PAA in liver, jejunum or kidney, which taken alone would have implied 100% absorption of PEA, contrasted with much smaller concentrations in other tissues, such as skeletal muscle which contribute proportionately greater to body weight. The large hepatic concentration of PEA could be atttributed to the vascularity of the liver and to the fact that the organ is the first exposed to PEA after absorption. The excess of PAA over PEA and the larger concentration of PEA in the kidney was attributed to the latter's excretory role, particularly of polar substances. In general, relatively polar lipid insoluble metabolites e.g. PAA, are more readily secreted by the renal proximal tubules than are the original non-polar lipid-soluble foreign compounds (Parke, 1968) and are also less likely to be reabsorbed from the tubules (Brodie, 1964). PEA concentration in the aorta or its muscle-walled branches was no greater than in other tissues, militating against a predilection for blood vessels as an explanation for the vascular phenomen in food-induced migraine. Penetration of PEA to the brain and to the foetus could be at least partly ascribed to its lipid solubility.

Several foods contain more than one amine (Marley & Blackwell, 1970) which could compete with or enhance absorption of each other. In the present experiments, PV-CMA values for PEA were significantly elevated with combinations of PEA instilled i.d. with histamine or tyramine, although cumulative PEA blood values were not significantly altered.

Blood concentration of PEA following its i.d. instillation was significantly elevated by prior inhibition of MAO A and B, arterial concentrations increasing relatively more than those in portal blood; blood cencentrations of PAA were reduced. In comparison, tyramine concentrations in portal venous and arterial blood were raised equally after its i.d. instillation in

cats with MOA A and B inhibition (Garcha *et al.*, 1983a), again reflecting a greater role of the intestine in deaminating tyramine than PEA. MAO B constitutes 43% of the MAO activity in cats' intestine (Squires, 1972), yet blood concentrations of PEA were not significantly altered in cats pretreated with deprenyl. This could be for a variety of reasons e.g. MAO B inhibition at the instillation site was only 33% with the dose of deprenyl cited (Garcha *et al.*, 1983a); PEA can also serve as a type A, type B or dual substrate depending on the organ examined (Lyles & Callingham, 1973; Dial & Clarke, 1978), or on the substrate concentration. A proportion of the instilled PEA was probably metabolized by intestinal MAO A, although blood concentrations of PEA following its i.d. administration were not increased by clorgyline pretreatment, despite a 66% inhibition of MAO A at the instillation site (see Garcha *et al.*, 1983a).

Nictitating membrane contraction was not obtained after i.d. instillation or portal vein infusion of PEA in the absence of MAO inhibition, even when PEA arterial concentrations reached 20 nmol ml^{-1}; however, for portal vein infusions after MAO inhibition, marked pressor responses were elicited with PEA concentrations of 3 nmol ml^{-1}. Moreover, after inhibition of MAO A and B by mebanazine, or by deprenyl plus clorgyline, half maximum contraction of the nictitating membrane was evoked after intraduodenal PEA with arterial PEA concentration of about 2 nmol ml^{-1}. Similar findings applied with tyramine (Garcha *et al.*, 1983a). Surprisingly, when PEA was given by intraportal infusion to cats pretreated with mebanazine or clorgyline plus deprenyl, larger arterial concentrations of PEA appeared to be necessary to elicit half maximum contractions of the nictitating membrane (9 to 11 nmol ml^{-1}). Presumably with its more gradual absorption from the intestine, plasma concentrations of PEA came into equilibrium with those in even relatively avascular tissues such as the nictitating membrane, whereas with portal vein infusions the rate of increase of PEA in arterial blood was too swift for equilibrium concentrations with tissues to be achieved before contraction of the membrane ensued. Otherwise the results accorded with Knoll's (1981) finding that amounts of PEA, ineffective on the cat's nictitating membrane following an MAO A inhibitor, contracted the membrane after an MAO B inhibitor; nevertheless, its effects on the membrane were even more marked after inhibition of both MAO A and B.

Apart from showing that ingested PEA reaches the arterial circulation and tissues, the results shed little light on its role in migraine. Thus there was no evidence that PEA was sequestered preferentially in larger blood vessels. And while vascular phenomena were elicited on infusing PEA with arterial concentrations which were ineffective on other sympathetically innervated smooth muscle such as the nictitating membrane except after MAO inhibition, these concentrations were many times greater than those accruing after i.d. instillation of PEA, 1.7 μmol kg^{-1}; yet migraine has been elicited in migraineurs with 3 mg of ingested PEA i.e. 0.25 μmol kg^{-1}. Other mechanisms, such as release of vasoactive substances from sites where β-phenethylamine is concentrated, could be involved; certainly histamine is released from tissues by a variety of organic bases, including β-phenethylamine (MacIntosh & Paton, 1949; Paton, 1958).

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