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Intestinal contribution to the presystemic elimination of β-phenethylamine in the rat

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The metabolism of β-phenethylamine (PEA) has been investigated in intestinal, liver and lung homogenates from the rat. Metabolic activity was ranked liver >> lung >> duodenum > jejunum > ileum > colon. Absorption and metabolism of PEA was also studied using in-situ intestinal loops from rats. Absorption of PEA appeared to be a passively mediated process with metabolism occuring in the jejunum, ileum and colon. When isolated loops of jejunum were used, phenylacetic acid was the only metabolite formed. Presystemic elimination of PEA in anaesthetized rats, was largely attributable to the intestine and lung with a relatively small hepatic contribution.

Phenethylamine (PEA) is an endogenous amine that can readily cross the blood brain barrier (Oldendorf 1971) and is postulated to have an important role as a neuromodulator in the central nervous system (Sabelli et al 1978; Patrick 1981). In addition, it is known to be present in dietary substances, e.g. chocolate (Schweitzer et al 1975) and can also be generated by the action of intestinal flora on dietary phenylalanine residues (Sandler 1980). The first step in the metabolism of PEA is oxidative deamination by monoamine oxidase (MAO) to phenylacetaldehyde (PAL). The PAL is then oxidized to phenylacetic acid (PAA) followed by species-dependent conjugation with glutamine or glycine (for review see Sandler 1980). Sandler et al (1976) found the urinary PAA excretion in germ-free rats was only 25% of that in conventionally reared litter mates. From these data, they concluded that a large proportion of the PEA in the body originates from dietary sources. In contrast with this conclusion are reports of very short half-lives for PEA in dogs (1·8–3 min; Cone et al 1978) and the rapid distribution and elimination seen following its intravenous administration to rats (Wu & Boulton 1975).

The present study in rats was designed to investigate the presystemic elimination of PEA and in particular the elimination across the intestinal wall. Homogenate studies were performed to compare intestinal PEA metabolism activity with that in liver and lung. Intestinal metabolism of PEA was also determined utilizing in-situ intestinal loops. The overall importance of the intestine in the presystemic elimination of PEA was evaluated using the method recently described by Cassidy & Houston (1980).

MATERIALS AND METHODS

Animals
Male Wistar rats (220–280 g) were used.

Preparation of tissue homogenates
Homogenates of rat liver, lung, duodenum, jejunum, ileum and colon were freshly prepared at 4°C as described by Ilett et al (1980).

MAO assay
MAO activity was determined in duplicate by a modification of the method of Robinson et al (1968). Incubation mixtures containing the equivalent of 1–5 mg tissue wet weight in 575 μl of 0·05 M phosphate buffer were allowed to equilibrate for 10 min at 37 °C before the addition of 25 μl (0·05 μCi) of radiolabelled [^{14}C]PEA to give the initial concentration of 10^{-5} M in the mixture. Incubation blanks were established by (a) including 10^{-4} M tranylcypromine with the incubation mixture and (b) replacing the prepared homogenate in the incubation mixture with an equal volume of buffer. No difference was observed between these blanks. The reaction was terminated by transferring 0·5 ml aliquots to pasteur pipettes containing 0·5 × 2·5 cm of Amberlite CG 50 cation exchange resin (BDH Chemicals), prepared according to Tipton & Youdim (1976). The

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columns were washed twice with distilled water (1·25 ml) and the entire eluate collected in scintillation vials to which 10 ml of scintillation fluid (340 ml Triton X-100, 660 ml toluene and 6 g 2,5-diphenyloxazole) was added and the radioactivity present quantified in a Packard (model B2450) liquid scintillation counter. The MAO activity was determined under conditions which were linear with respect to time and protein concentration and with not more than 15% of substrate deaminated during incubation.

In-situ loop preparations

Isolated in-situ intestinal loops were prepared by a modification of the method of Iwamoto & Klaassen (1977). The rats were anaesthetized with pentobarbitone sodium (40 mg kg^{-1}) and the trachea cannulated for artificial respiration. Catheters were placed in the jugular vein and carotid artery for replacement of blood volume with heparinized whole blood from donor rats, and collection of systemic arterial blood samples respectively. Jejunal loops were selected from a region 15–30 cm from the pylorus. Isolated loops from the ileum and colon were prepared from sections 2–3 cm proximal and distal to the caecum respectively. After isolating the gut loop and heparinizing the animal (3000 units kg^{-1}) a cannula was inserted into the mesenteric vein to enable collection of the entire venous outflow from the loop. The [^{14}C]PEA was then administered intraluminally in 0·25 ml 0·9% NaCl (saline) containing 0·5 μCi at concentrations of 0·15, 1·91, 4·0, 6·35, 14·0 and 40·0 mM. Blood was collected over 2 min intervals for 20 min into tared vials containing 0.3 mg tranylcypromine. PEA and metabolites in plasma were separated by ion exchange chromatography and quantified as above. Blood concentrations were calculated using the cell to plasma distribution ratio for PEA or PAA. The intestinal loops were maintained at 37 °C.

Determination of metabolites from in-situ loops

Plasma collected from an in-situ loop containing 0·5 μ Ci[^{14}C]PEA (4·0 mM) was mixed with an equal volume of acetonitrile (containing 500 μg ml^{-1} of non-radiolabelled PEA and PAA and 250 μg ml^{-1} of non-radiolabelled PAL) to precipitate proteins, vortexed for 30 s and then centrifuged at 1500 *g* for 10 min. Separation of PEA, PAA and PAL was achieved using a Waters Associates high performance liquid chromatography system consisting of a model 6000 A pump, U6K injection valve, C_{18} μ-Bondapak column (30 cm × 4 mm i.d.) and a model 450 variable wavelength detector set at 254 nm. Operating conditions were: mobile phase 20% acetonitrile in distilled water containing PIC-B6 (Waters Assoc. Aust.; 25 ml $litre^{-1}$), flow rate 1·5 ml min^{-1}, detector sensitivity 0·02 a.u.f.s. and chart speed 0·5 cm min^{-1}. The effluent was collected in appropriate fractions for at least 15 min following sample injection and radioactivity in each fraction was quantified by liquid scintillation spectrometry. Approximate retention times were 4·8, 8·0, 9·4 min for PEA, PAA and PAL respectively. Recovery of injected radioactivity from two determinations was 101·2 and 99·8% respectively.

Red blood cell to plasma distribution ratios

The PEA and PAA cell to plasma distribution ratio was determined by adding 4 ×10^4 and 5·55 × 10^4 d min^{-1} of the respective radiolabelled compound to freshly collected heparinized rat blood. The blood was mixed gently for 20 min. PEA and PAA concentrations in blood and plasma were quantified by liquid scintillation counting and the cell-to-plasma distribution ratio was determined as previously described (Hughes et al 1976). All experiments were carried out at 22 °C. Preliminary experiments showed that distributional equilibrium was attained in less than 10 min.

Metabolism of PEA by rat blood

Heparinized whole blood collected from rats was incubated for 40 min at 37 °C with[^{14}]CPEA (0·18 mCi in 5 ml blood) at initial concentrations of 1 and 5 × 10^{-5} M. PEA and its metabolites in the plasma were separated using the ion exchange chromatographic procedure described above.

Presystemic elimination

[^{14}C]PEA at a dose of 4 μmol ml^{-1} kg^{-1} (32 μCi kg^{-1}) was administered to groups of 4–5 rats by a rapid infusion (45 s) via one of four routes; intra-arterial (i.a.), intravenous (i.v.), intraportal (p.v.), or intraduodenal (oral). Blood samples (200 μl) were collected at 2, 4, 6, 8, 10, 15, 25, 30, 60, 90 and 120 min during which time quantitative whole blood replacement was given via the jugular vein. PEA in blood was quantified by ion exchange chromatography as above. The areas under the blood concentration-time curve (AUC) were calculated using the trapezoidal rule. The fractions of PEA eliminated at the lung, liver and intestine were calculated as described by Cassidy & Houston (1980).

Chemicals

β-Phenethylamine hydrochloride, phenylacetic acid, phenylacetaldehyde, (−)-tranylcypromine (Sigma Chem. Co., St. Louis, MO, USA), [^{14}C]β-phenethylamine hydrochloride sp. act. 48·28–50·0 mCi mmol^{-1} (New England Nuclear, Boston, Mass. USA) and [^{14}C]phenylacetic acid sp. act. 51 mCi mmol^{-1} (Amersham, Aust.) were used. Phenylacetaldehyde was redistilled (195 °C fraction collected) immediately before use.

Data analysis. Results are expressed as mean ± s.e. Differences between means were assessed using Student's *t*-test.

RESULTS

Homogenate studies

The MAO activities for the tissue homogenates are summarized in Table 1. Metabolism was substantially greater in the liver than in the other tissues. The activities of the lung homogenates were also significantly greater than the activity measured in the intestinal tissues.

Table 1. Phenethylamine (10 μM) deamination by whole tissue homogenates from the rat.

Tissue	Rate of deamination (nmol g^{-1}tissue min^{-1})
Liver	181·2 ± 3·4*
Lung	58·0 ± 4·6*
Duodenum	29·5 ± 6·7
Jejunum	26·4 ± 5·5
Ileum	22·2 ± 3·4
Colon	6·8 ± 2·1*

Values expressed as mean ± s.e., n = 3.
Differences relative to the jejunum were assessed by 2-tailed Student's *t*-test (* $P < 0{\cdot}05$).

Metabolites from in-situ loops

PEA and PAA were found to be the only radiolabelled compounds in significant amounts in the venous effluent from the in-situ rat jejunal loops. H.p.l.c. analysis of the plasma showed 54% as PEA, 40% as PAA with the remaining 6% distributed throughout the chromatogram as background activity (Fig. 1).

Red blood cell to plasma distribution ratio for PEA and PAA

The distribution of PEA and PAA was independent of concentration and mean values of 1·57 and 0·73 for PEA and PAA cell to plasma distribution ratio were subsequently used in the conversion of plasma

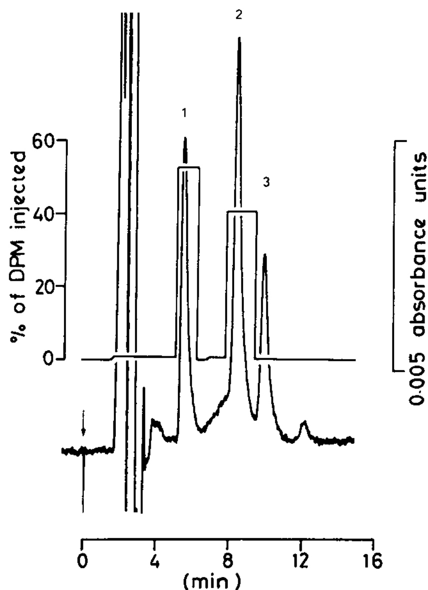

FIG. 1. H.p.l.c. chromatogram of plasma obtained from an in-situ loop experiment. A deproteinated plasma supernatant containing non-labelled PEA, PAA and PAL as a carrier (25 μl) was injected at the arrow and the effluent collected in fractions throughout the chromatogram. Peaks from the h.p.l.c. column were detected by their ultraviolet absorption (1=PEA, 2=PAA, 3=PAL). Radioactivity present in each fraction was quantified by liquid scintillation spectrometry and these data are superimposed on the chromatogram.

PEA or PAA concentrations to their respective blood concentrations. Tranylcypromine (10^{-4} M) did not influence distribution ratios. There was no significant metabolism of PEA when it was added exogenously to whole rat blood.

PEA absorption and metabolism for in-situ intestinal loops

The percent of PEA absorbed from the jejunum is shown in Fig. 2 with the corresponding blood flow rates. From paired data from the 24 individual observations, mean blood flow was found to be significantly correlated (r = 0·74, $P < 0{\cdot}01$) with the mean percent absorbed. The cumulative absorption of PEA (4 × 10^{-3} M in the intraluminal fluid) for in-situ loop preparations from different regions of the gut is shown in Fig. 3. The extent of absorption was greatest in the colon, followed by the ileum and jejunum. There was a short lag phase of approximately 1 min, indicating that transfer of PEA across the intestinal wall was rapid. Preliminary experiments showed that the pH of the intraluminal fluid recovered from in-situ loops (n = 3) at the end of an experiment was 6·4 ±0·1 for the jejunum, 7·7 ±0·1

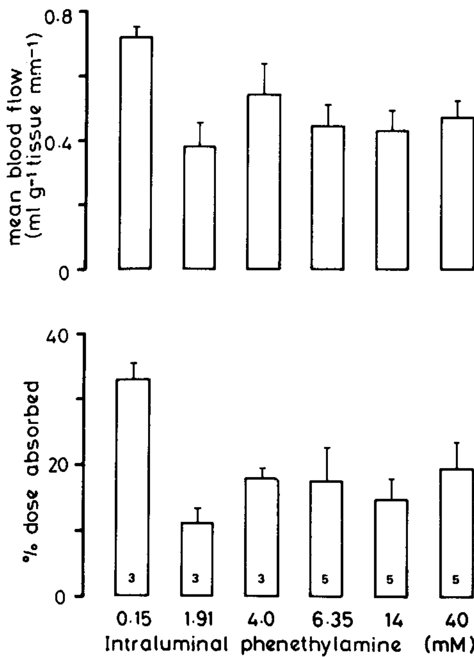

FIG. 2. Mean blood flow (upper panel) and percent of dose absorbed in 20 min (lower panel) versus the concentration of PEA used in the in-situ jejunal loops. Results are expressed as mean ± s.e. and the number of animals (3–5) is shown within the histograms on the lower panel.

for the ileum and 7·8 ±0·1 for the colon. The percent of metabolite in the mesenteric blood from in-situ gut loops is shown in Table 2. At the lowest PEA concentration (0·15 mM) extensive metabolism of PEA had occurred, however, as the PEA concentration increased, a decrease in the extent of metabolism was observed.

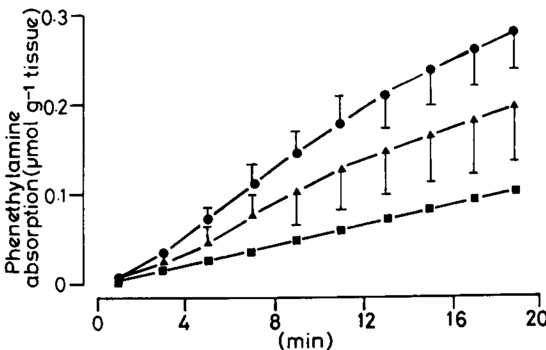

FIG. 3. Cumulative absorption of PEA from isolated in-situ loops of jejunum (■), ileum (▲) and colon (●) with an intraluminal PEA concentration of 4·0 mM. Results are mean ± s.e. of 3 experiments. Errors not shown where less than symbol size.

Table 2. Percent of total radioactivity appearing as metabolite in mesenteric blood from in-situ intestinal preparations.

PEA [mM]	Tissue	Loops In-situ
0·15	Jejunum	78·1 ± 1·7
1·91	Jejunum	49·2 ± 9·0
4·0	Jejunum	36·8 ± 5·0
6·35	Jejunum	25·8 ± 2·7
14·0	Jejunum	19·4 ± 0·7
40·0	Jejunum	11·9 ± 1·5
4·0	Ileum	15·7 ± 1·3
4·0	Colon	7·8 ± 0·7

Results expressed as mean ± s.e., n = 3–5.

Presystemic elimination in anaesthetized rats

The $AUC_{0-30\ min}$ values for PEA blood were 216 ± 61, 109 ± 9, 102 ± 12 and 43 ± 6 (nmol h^{-} ml^{-1}) for the i.a., i.v., p.v. and oral routes of administration respectively. Reliable data could not be obtained after 30 min as the sensitivity limit of the assay was reached at about this time. Using the AUC data and the equations described by Cassidy & Houston (1980) it was calculated that the fractions of PEA eliminated by the major organs of presystemic elimination were 0·5 for the lung, 0·06 for the liver and 0·58 for the intestine.

DISCUSSION

The homogenate studies suggest that the liver would have the major effect on presystemic elimination of PEA with a much smaller approximately equal contribution from the intestine and lungs. These studies also indicated that metabolism of PEA was greater in the small intestine than in the colon and this finding was confirmed with the in-situ loop preparations. However, the extent of metabolism of PEA in the in-situ loops did not increase proportionally with increasing intraluminal PEA concentration, suggesting that saturation of metabolism or restriction of access to the site of metabolism was occurring. Nevertheless, in contrast to the homogenate studies, the in-situ loop preparations indicated that the intestine is capable of substantial presystemic elimination of PEA, particularly at low intraluminal concentrations (e.g. 78% at 0·15 mM).

The detection of PAA as the sole metabolite from the in-situ jejunum indicates that following deamination by MAO, conversion of the intermediate product PAL to the corresponding acid proceeds to completion before transfer from the tissue to the blood compartment. As the recovery of radiolabel was complete with 94% accounted for as either PEA or PAA, gut wall conversion of PEA to other

metabolic products such as an alcohol or a conjugate can be regarded as insignificant. Previous work in rat and guinea-pig brain slices also found that phenethylamines were converted to acid products while β-hydroxylated phenethylamines formed mainly alcohol metabolites (Tipton et al 1977).

The absorption of PEA from the jejunum appears to be a passively mediated process, which is influenced by blood flow. The order of PEA absorption rates from the different gut regions (colon > ileum > jejunum) can be explained in terms of the pH partition hypothesis (Schanker 1960). PEA has a pK_a of 9·83 (Hong & Connors 1968) and the proportion of nonionized drug will therefore increase with the increase in pH from jejunum to ileum to colon. In this case, it would appear that the influence of pH outweighs that of the greater surface area and blood flow found in the upper gut.

The estimate for intestinal elimination of PEA in the whole animal corresponded well with the fraction of an equivalent dose (4 mM) eliminated by the in-situ loops. The large pulmonary fractional elimination of PEA is in agreement with studies in rat isolated perfused lung (Bakhle & Youdim 1979; Ben-Harari & Bakhle 1980) which have demonstrated substantial passive uptake and metabolism of this amine. However, the low estimate for hepatic elimination, despite the high capacity for elimination indicated from the homogenate studies, is a cause for concern. Cassidy & Houston (1980) also found that the liver contributed little toward the presystemic elimination of phenol. However, Shirkey et al (1979) found that phenol was extensively conjugated by isolated hepatocytes and to a lesser extent by isolated intestinal mucosal cells. We would suggest that further investigation of the Cassidy & Houston (1980) method of assessing presystemic elimination is required, using hepatic clearance estimates obtained by alternative methods to verify the apparently low hepatic fractional elimination for PEA found in the present study and also that previously reported for phenol.

Nevertheless, our experiments indicate that presystemic elimination of PEA is substantial, particularly in the gut wall and lung. From the data for the elimination of PEA at the relatively high dose given to our anaesthetized rats, it can be calculated that less than 20% of an intraduodenal dose of PEA would escape presystemic elimination by the gut wall, liver and lung. In view of the extensive metabolism of PEA shown at the lower concentrations used in the in-situ loops and the short plasma half-lives reported for PEA (Cone et al 1978), it would seem unlikely that dietary PEA contributes greatly to body levels of this amine.

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