Biochemical Pharmacology, Vol. 29, pp. 489–494.

0006-2952/80/0215-0489 $02.00/0

UPTAKE OF β-PHENYLETHYLAMINE IN RAT ISOLATED LUNG*

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(*Received* 1 *August* 1979; *accepted* 7 *September* 1979)

Abstract—^{14}C-β-Phenylethylamine (0.15–200 μM) infused through the pulmonary circulation of rat isolated lungs perfused with Krebs solution was metabolized by a saturable process. Metabolism at 50 μM was inhibited by cold, anoxia, glucose lack or Na-lack, by amine oxidase inhibitors and by a number of other basic drugs taken up by lung. Uptake of PEN consisted of at least two components, a component directly related to concentration and a saturable process reaching V_{max} at 50–100 μM. Uptake was inhibited following inhibition of monoamine oxidase action by deprenyl and other drugs. Inhibition of uptake without inhibition of the enzyme was caused by normetanephrine and dexamethasone. These results lead to the conclusion that PEN metabolism is not limited by uptake and that its uptake resembles that of amphetamine and propranolol but is different from that of 5-hydroxytryptamine and noradrenaline.

Previous studies on β-phenylethylamine (PEN) metabolism in lung [1–3] have shown that this process is unlike that of the two other best studied amines, 5-hydroxytryptamine (5-HT) and noradrenaline (NA), in the same organ. For these amines, the rate-limiting step in metabolism is uptake into the cell [4–6], but the role played by the uptake system in the metabolism of PEN is not clear. In rabbit isolated lung, removal of PEN appears to be non-saturable [7], although in rat lung metabolism does approach saturation [3]. Furthermore, in perfused lung, metabolism of 5-HT, but not that of PEN, was inhibited by desmethylimipramine [3]. We have therefore undertaken a more detailed study of the kinetic and biochemical requirements of PEN uptake and metabolism in rat lung.

MATERIALS AND METHODS

Preparation of isolated lung and perfusion media. The lungs from male rats (Wistar strain, 150–250 g) were prepared as previously described [4]. The standard perfusion medium was warmed (37°) Krebs bicarbonate solution containing (mM): NaCl, 118; KCl, 4.6; $CaCl_2$, 2.54; KH_2PO_4, 1.2; $NaHCO_3$, 25 and glucose, 5, gassed with 95% oxygen and 5% carbon dioxide. The perfusion medium was pumped through the pulmonary circulation at a constant rate (8ml.min^{-1}). After 10–15 min of perfusion, the effluent was free of blood and the lungs were either taken for homogenization or the perfusion continued.

For a Na-free medium, NaCl was replaced by isotonic amounts of sucrose (0.25 M) and $NaHCO_3$ by Tris, the pH of the final solution being adjusted to 7.4 by adding 1N HCl. The only Na contamination was the presence of NaCl and $NaHCO_3$ in the stock solution of PEN. Since the stock solution was diluted 1000 times, the final Na concentration during an infusion of the amine was 0.15 mM. For a K-free medium, KH_2PO_4 and KCl were replaced by the equivalent Na salts in equimolar amount. The presence of KH_2PO_4 and KCl in the stock solution of PEN was again diluted 1000 times. For a glucose-free medium, glucose was simply omitted from the standard Krebs solution. When 'anoxia' was required, the medium was gassed with 95% nitrogen and 5% carbon dioxide. 'Hypothermia' was produced by filling the water bath with an ice–water mixture which was pumped to the heat exchanger, thus cooling the standard Krebs solution entering the pulmonary arterial cannula to 2–4°.

Metabolism and uptake in perfused lungs. After the initial perfusion, PEN metabolism was measured by collecting lung effluent for a total of 30 min, during and after a 3 min infusion (0.4 ml.min^{-1}) of ^{14}C-PEN into the pulmonary arterial cannula. At the end of the 30 min collection time, radioactivity in the effluent had fallen to background levels and a second infusion of PEN was given, in the presence of test drug or medium. Infusion of drugs (0.2ml.min^{-1}) and perfusion of the lung with different media was started 20 min before and continued during the PEN infusion and collection of the lung effluent.

To measure uptake of ^{14}C-PEN, a 3 min infusion of PEN was given through the lungs and 30 sec later perfusion was stopped and the lungs removed from the perfusion system. The effluent from the lung was collected during this period. The lungs were dissected free of the trachea, the remainder of the heart and any extraneous tissue. They were then homogenized in cold (0°) 0.3 M perchloric acid, using two 10 sec bursts of a Polytron homogenizer (PCU-2). The homogenate was centrifuged at 1000 *g* for 20 min and samples of the resulting supernatant, together with samples of lung effluent collected during the infusion, were taken for chromatographic analysis.

* This work was supported by the Medical Research Council.

To check for breakdown of substrate during the extraction procedure, ^{14}C-PEN was added to perfused lungs immediately before homogenization in perchloric acid and the extraction and analysis carried out as usual. Recovery of ^{14}C in the supernatant was 89% and in the effluent 100%. The procedures of homogenization and chromatography resulted in less than 5% breakdown of ^{14}C-PEN.

The PEN infused was a mixture of ^{14}C-PEN (0.25μCi per infusion) and unlabelled PEN to give a final concentration of PEN from 0.15 to 200 μM.

In vitro *assay of monamine oxidase*. Once clear of blood, the lungs were homogenized and prepared as described previously [8]. Metabolism of PEN by the homogenate was assayed in an incubation mixture consisting of: 0.5 ml lung filtrate (approximately 0.4 mg protein, ml^{-1}); 0.5 ml ^{14}C-PEN (10–200 μM) containing approximately 50,000 dpm; and 4 ml of 0.1 M phosphate buffer, pH 7.4. The mixture was incubated at 37° in a shaking water bath for 60 min. In control experiments, buffer and enzyme were pre-incubated for 20 min before addition of the substrate to start the reaction and in test experiments the enzyme was pre-incubated with inhibitor (0.5 ml) and 3.5 ml buffer. The zero time sample (1 ml) was taken immediately after adding the substrate; the 0 and 60 min samples were added to 0.5 ml of 1.5 M perchloric acid at 0° to stop the reaction. Each sample was adjusted to pH 6–6.5 with 3 M potassium hydroxide. Enzyme activity bore a linear relationship to protein concentration and time of incubation. Protein was estimated by the method of Lowry *et al.* [9] using bovine serum albumin as the standard.

Ion-exchange chromatography and measurement of radioactivity. Samples (0.5 ml) of the effluent and the neutralised supernatant from the lung and from the incubation mixtures were applied to columns of Amberlite CG-50 resin [10]. The metabolites of PEN were eluted from the column with water (2 ml) and radioactivity was measured after mixing with Triton–toluene scintillation fluid (PPO, 5g; DMPOPOP, 0.25 g; toluene, 1 litre and Triton X-100, 0.5 litre) using a liquid scintillation counter (Packard 3375). Corrections were made for quenching using sample channels ratio.

Materials. Radioactive PEN, 1-^{14}C-phenylethylamine hydrochloride (51 mCi/mmole), was obtained from New England Nuclear, Frankfurt. The following unlabelled drugs were obtained from Sigma: β-phenylethylamine hydrochloride (PEN); normetanephrine hydrochloride; benzylamine hydrochloride; dopamine hydrochloride; semicarbazide hydrochloride; iodoacetic acid (sodium salt) and (±)-noradrenaline bitartrate. Other drugs used were: 5-hydroxytryptamine creatinine sulphate and histamine acid phosphate (B.D.H.); phenoxybenzamine hydrochloride and (+)-amphetamine sulphate (Smith, Kline & French); methylene blue (GURR's); ascorbic acid (Analar). We acknowledge gratefully the gifts of (−)-deprenyl hydrochloride (Professor M.B.H. Youdim) and desmethylimipramine hydrochloride (DMI) (Geigy). Other chemicals used were of analytical reagent grade. In perfused lung experiments, drugs were dissolved in saline (0.9% NaCl, w/v) except phenoxybenzamine which was dissolved in a small volume of ethanol and then diluted with saline. For use with homogenates, drugs were dissolved in 0.1 M phosphate buffer, pH 7.4 (B.D.H.).

Statistical methods. The significance of differences between means was calculated by Student's *t*-test for paired or unpaired samples and values of $P < 0.05$ were accepted as significant.

RESULTS

Effect of different perfusion media and drugs on the metabolism of ^{14}C-PEN. The metabolism of PEN was measured at two concentrations (0.15 and 50 μM), collecting the lung effluent for a total of 30 min. The low concentration (0.15 μM) used was comparable with concentrations of 5-HT and NA used previously [5,6], and the high concentration (50 μM) used was close to the K_m for PEN metabolism in perfused lung [3]. Table 1 shows the effect of metabolic inhibitors and cation concentration on PEN metabolism. To ensure that stores of endogenous glucose were depleted, the lung was perfused for 60 min instead of the usual 20 min with glucose-free Krebs before testing; this procedure was also fol-

Table 1. Metabolism of ^{14}C-PEN by rat isolated lung under different conditions*

Treatment	0.15 μM PEN Metabolism (%)	0.15 μM PEN Inhibition (%)	50 μM PEN Metabolism (%)	50 μM PEN Inhibition (%)
None (control)	92 ± 1 (31)		67 ± 2 (25)	
Cold (4°)	17 ± 1 (4)	82 ± 1†§	7 ± 0.5 (4)	83 ± 3†‖
Anoxia‡	94 ± 1 (4)	0	54 ± 6 (7)	23 ± 8†
Glucose-free‡	88 ± 2 (3)	0	50 ± 2 (6)	30 ± 5†
Glucose-free + anoxia‡	93 ± 2 (4)	0		
K-free	97 ± 1 (3)	0	66 ± 2 (6)	0
Na-free	69 ± 6 (4)	26 ± 6†	39 ± 1 (6)	33 ± 4†

* The values shown are means (±S.E.) of the number of experiments shown in parentheses. ^{14}C-Phenylethylamine was infused at the concentrations shown for 3 min. Effluent was collected during and after the infusion for a total of 30 min. Effluent radioactivity was analysed by ion exchange chromatography as described in Materials and Methods.

† Significantly different from control values ($P < 0.05$).

‡ Lungs perfused with test drug for 60 min instead of 20 min before infusion of amine.

§ Test infusions made in different lung from controls and compared using unpaired *t*-test.

‖ 100 μM PEN infused.

Table 2. Metabolism of ^{14}C-PEN by rat isolated lung in the presence of amine oxidase inhibitors*

Treatment		0.15 μM PEN Metabolism (%)	0.15 μM PEN Inhibition (%)	50 μM PEN Metabolism (%)	50 μM PEN Inhibition (%)
None (control)		96 ± 3 (4)	—	59 ± 3 (13)	—
Deprenyl	0.5 μM	24 ± 6 (6)	76 ± 7†‡	—	—
Deprenyl	5 μM	—	—	30 ± 1 (3)	52 ± 2†
Semicarbazide	1 mM	90 ± 4 (4)	0	43 ± 4 (7)	28 ± 5†

* The values shown are means (±S.E.) of the number of experiments shown in parentheses. ^{14}C-Phenylethylamine was infused at the concentrations shown for 3 min. Effluent was collected during and after the infusion for a total of 30 min. Effluent radioactivity was analysed by ion exchange chromatography as described in Materials and Methods.

† Significantly different from control values ($P < 0.05$)

‡ Test infusions made in different lungs from controls and compared using unpaired *t*-test

lowed for the 'anoxic' Krebs. Only Na -lack and cold inhibited metabolism at both concentrations. Other drugs tested at the lower concentration of PEN (0.15 μM) were iodoacetate (10 mM), methylene blue (100 μM) [11] and ascorbic acid (100 μM) [12]. They were all without effect on PEN metabolism.

Amine oxidase inhibitors were also investigated (Table 2). Deprenyl inhibited metabolism at both concentrations of substrate, but semicarbazide was effective only at the higher concentration. Table 3 shows the effect of alternative substrates for monoamine oxidase (MAO) and of inhibitors of amine uptake. Each treatment, except for dopamine, inhibited metabolism at the higher substrate concentration (50 μM) as shown, but all were without effect at the lower concentration (0.15 μM).

Uptake of ^{14}C-PEN *and the effect of drugs.* Any drug which alters metabolism can act either on the uptake or on the intracellular enzyme and we have studied the effect of different drugs on the uptake system by measuring PEN uptake after a 3 min infusion. Uptake of PEN in the absence of drugs was calculated from the radioactivity still retained in the lung after the infusion, together with the radioactive metabolite in the effluent collected. Over a wide range of concentrations (0.15–200 μM), there was a decrease in the proportion of radioactivity taken up, but a plateau was not attained (Fig. 1). These results have been analysed as shown in Fig. 2 to provide a diffusion component of uptake and a saturable component with a K_m 25 μM, graphically estimated. When the amount of metabolite in lung and effluent were added together and plotted as a function of substrate concentration, saturation of metabolism was evident (Fig. 3) and from these results, an apparent K_m (67 μM) and V_{max} (1066 nmoles.lung^{-1}.3 min^{-1}) could be calculated for metabolism as distinct from uptake.

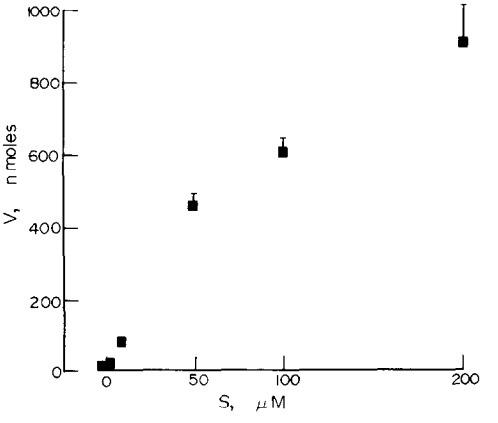

Fig. 1. Uptake of ^{14}C-PEN by rat isolated lung. Uptake was measured as the sum of the radioactivity retained in the lung and the radioactive metabolite in effluent following a 3 min infusion of ^{14}C-PEN and is expressed as nmoles.g lung^{-1}. Each point represents the mean value of 4–8 experiments. The standard error is shown by the vertical bars where this is larger than the symbols used. The concentrations of PEN used were 0.15, 1.0 10, 50, 100 and 200 μM.

Table 3. Metabolism of ^{14}C-PEN by rat isolated lung in the presence of other amines

Treatment		Metabolism (%)	Inhibition (%)
None (control)		59 ± 2 (42)	—
Desmethylimipramine	1 mM	23 ± 3 (5)	61 ± 3 †
Normetanephrine	1 mM	26 ± 5 (8)	49 ± 10†
Dexamethasone	10 μM	20 ± 4 (4)	64 ± 11†
Phenoxybenzamine	100 μM	63 ± 4 (4)	7 ± 2†
Amphetamine	1 mM	18 ± 1 (4)	70 ± 2 †
Benzylamine	1 mM	38 ± 2 (4)	41 ± 5 †
Dopamine	1 mM	64 ± 3 (6)	0
Propranolol	1 mM	33 ± 1 (4)	40 ± 2 †

* The values shown are means (±S.E.) of the number of experiments shown in parentheses. The substrate (^{14}C-PEN: 50 μM) was infused for 3 min and the effluent collected during and after infusion for a total of 30 min. Effluent radioactivity was analysed by ion exchange chromatography as described in Materials and Methods.

† Significantly different from control values ($P < 0.05$).

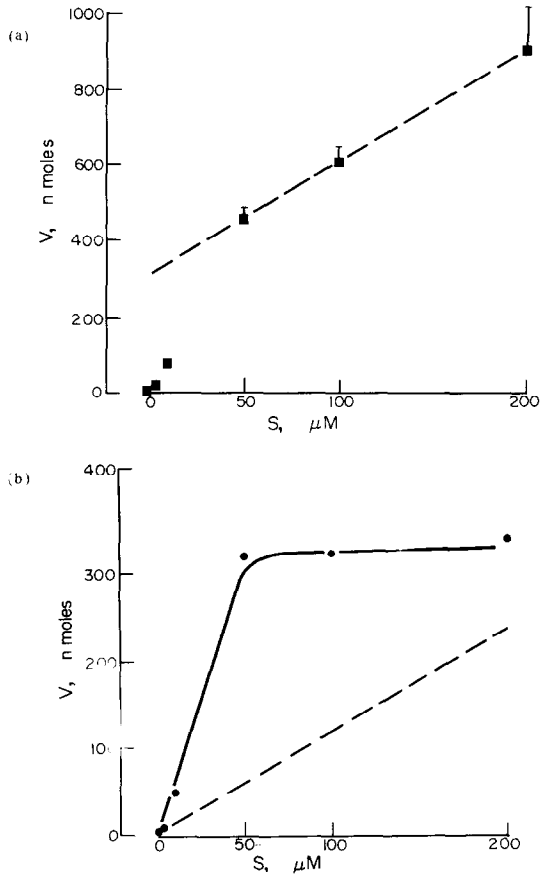

Fig. 2. Analysis of PEN uptake by rat isolated lung. (a) The dotted line through the 3 highest points represents the linear (diffusion) component of the total uptake. From the slope of this line a constant (K_D) was derived and used to calculate the diffusion component at the lower concentrations. Subtraction of the diffusion component from the observed values gave the points in (b). Here the diffusion component is represented by the broken line through the origin and the points joined by the solid curve represent the saturable component of uptake calculated by difference as described above. Note the change in scale of the vertical axis between (a) and (b).

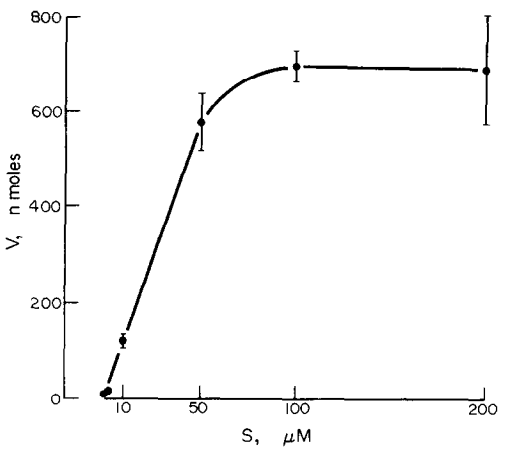

Fig. 3. Metabolism of ^{14}C-PEN by rat isolated lung. Metabolism was measured as the sum of radioactive metabolite in lung and in effluent following a 3 min infusion of ^{14}C-PEN and is expressed as nmoles metabolite.g lung^{-1} formed over the 3 min period. The points shown are the means (with S.E. shown by the bars) of 4–8 experiments. Analysis of this saturable process gave apparent K_m of 67 μM and V_{max} of 1066 nmoles.g lung^{-1}.3 min^{-1}.

The drugs chosen for study on uptake were some of those which inhibited the metabolism of PEN at a concentration of 50 μM measured over 30 min. Deprenyl is known to be an inhibitor of the type of MAO that preferentially metabolizes PEN in rat lung [3]. Accumulation of radioactivity in lung over the range of PEN concentrations was inhibited in the presence of deprenyl (5 μM) by 50–60 per cent (Fig. 4), and this corresponded with a 50–70 per cent reduction in the amount of ^{14}C-metabolite present in the lung and effluent. The other drugs were tested against a single concentration of PEN (50 μM) and the results (Table 4) demonstrate that all decreased accumulation of radioactivity. However, three drugs, normetanephrine, semicarbazide and dexamethasone, did not change the proportion of metabolite in the retained radioactivity, but the others, propranolol, amphetamine, desmethylimipramine (DMI) and benzylamine, decreased the proportion of metabolite, suggesting an inhibition of MAO.

Benzylamine is an alternative substrate for the type of MAO oxidizing PEN, but the other three drugs are not. We therefore examined their effect on rat lung MAO directly.

MAO activity in lung homogenates. In order to determine MAO activity directly, the metabolism of ^{14}C-PEN (10–200 μM) was measured with lung homogenates. The results of these experiments, under control conditions and in the presence of DMI, amphetamine and propranolol, each at 1 mM, are

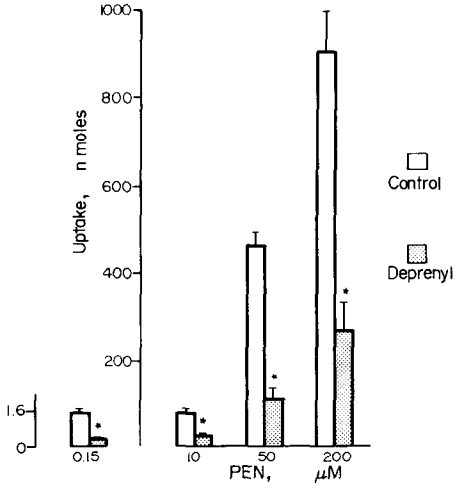

Fig. 4. Inhibition by deprenyl of ^{14}C-PEN uptake in rat isolated lung. The open bars show the mean (±S.E.) uptake in untreated lungs and the stippled bars the uptake following deprenyl treatment. Deprenyl (5 μM) was infused before and during the infusion of ^{14}C-PEN (20 min). At each concentration of PEN, deprenyl decreased significantly ($P<0.05$) the uptake of PEN. Note the change of scale of uptake at the lowest concentration of PEN. The values are from 4–8 experiments at each concentration.

Table 4. Effects of various drugs on uptake of ^{14}C-PEN by rat isolated lung

Treatment	Total ^{14}C uptake† (nmoles. g^{-1})	Metabolite (per cent of total)
None (10)	190 ± 17	64 ± 3
Propranolol (4)	81 ± 5*	47 ± 3*
Amphetamine (6)	70 ± 4*	34 ± 3*
Desmethylimipramine (5)	83 ± 3*	34 ± 8*
Benzylamine (4)	58 ± 13*	26 ± 1*
Normetanephrine (4)	93 ± 11*	71 ± 2
Semicarbazide (8)	105 ± 15*	57 ± 2
Dexamethasone (5)	132 ± 29*	63 ± 2

* Significantly different from control values ($P < 0.05$: unpaired t-test).

† Total uptake represents the sum of (^{14}C-PEN + metabolite) in lung after the 3 min infusion. The values shown are the mean (±S.E.) from the number of experiments shown in brackets for each treatment. ^{14}C-Phenylethylamine (50 μM) was infused for 3 min through the pulmonary circulation of rat lungs. At the end of this time perfusion was stopped and the lungs taken for analysis. The drugs used were infused, in a final concentration of 1 mM, for 20 min before and during the infusion of ^{14}C-PEN.

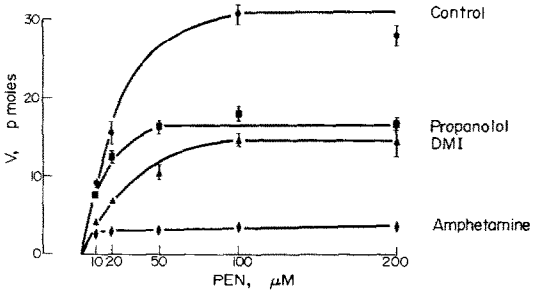

Fig. 5. Effect of amphetamine, desmethylimipramine (DMI) and propranolol on the metabolism of ^{14}C-PEN by rat lung homogenates. Metabolism is expressed as pmoles metabolite produced.min^{-1}.μg protein^{-1} and substrate concentration as μM. Homogenates of rat lung were incubated with different concentrations of ^{14}C-PEN and the reaction mixture analysed as described in Materials and Methods. The drugs, all at 1 mM, were pre-incubated with the homogenate for 30 min before adding substrate. The values are the means (±S.E.) of at least 4 experiments.

shown in Fig. 5. Although all three drugs inhibited PEN metabolism, there were differences in the type of inhibition which were apparent on kinetic analysis of the results (Table 5).

The apparent K_m for PEN under control conditions was 27 μM and the V_{max} was 35 pmoles.min^{-1}.μg protein^{-1}. In the presence of propranolol and amphetamine, both the apparent K_m and the V_{max} fell, indicating uncompetitive inhibition. Another feature of uncompetitive inhibition is that the ratio K_m/V_{max} is unchanged; this is clearly the situation with amphetamine and propranolol where the ratio remained at 0.7 or 0.8. In the presence of DMI, the K_m remained the same but the V_{max} was decreased; thus the K_m/V_{max} ratio was increased.

DISCUSSION

Our experiments have clearly shown that the metabolism of PEN in rat isolated lung was not limited by uptake. This and the nature of the uptake system itself differentiates pulmonary metabolism of this biogenic amine from that of its congeners, 5-HT and noradrenaline.

The first difference, the non-limiting effect of uptake, was most apparent in the experiments with deprenyl. Here inhibition of the enzyme was accompanied by a decrease in the amount of radioactivity taken up by the lung. The finding is in direct contrast to the results obtained with 5-HT and NA where uptake was unaffected by MAO inhibition [5,6,13] and is more like prostaglandin E_2 metabolism in lung which is, under normal conditions, limited by the activity of intracellular prostaglandin dehydrogenase [14]. Three other drugs, amphetamine, propranolol and DMI, probably also inhibited uptake of radioactivity by inhibiting MAO, as demonstrated by their effects on the proportion of metabolite in perfused lung (Table 4) and more

Table 5. Inhibition of rat lung MAO activity *in vitro* using ^{14}C-PEN as substrate

Treatment	K_m (μM)	V_{max} (pmoles . min^{-1} . μg^{-1})	K_m/V_{max} ratio	Character of inhibition
None (control) (4)	27 ± 5	35.0 ± 1	0.8	
Desmethylimipramine (4)	33 ± 6	17.8 ± 2.0*	1.9	Non-competitive
Amphetamine (4)	3 ± 1*	3.6 ± 0.3*	0.8	Uncompetitive
Propranolol (4)	13 ± 2*	19.4 ± 1.1*	0.7	Uncompetitive

* Significantly different from control values ($P < 0.05$). The values shown are the means (±S.E.) from the number of experiments shown in brackets for each treatment. Homogenates of rat lung were prepared as described in Materials and Methods and incubation carried out for 60 min with a range of PEN concentrations (10–200 μM). Drugs were added to the homogenate to yield a final concentration of 1 mM, 20 min before the addition of substrate to start the reaction.

directly by their effects on our preparation of MAO *in vitro*. Amphetamine [15] and DMI [16] are known to be inhibitors of MAO but inhibition by propranolol has not yet been described. The effects of benzylamine on PEN uptake are more difficult to interpret, as this amine could compete both for MAO, for which it is a worse substrate than PEN (K_m 135 μM vs 28 μM for PEN) [3], and possibly for the uptake process.

It was, however, possible to inhibit uptake without affecting MAO activity as shown with normetanephrine, dexamethasone and semicarbazide. All three drugs decreased radioactivity in lung without affecting the proportion of metabolite in lung. The effects of dexamethasone and normetanephrine suggested that an Uptake$_2$-like process was involved in the movement of PEN across the cell membrane. These results also show that although uptake was not normally rate-limiting for metabolism of PEN, it could be made rate-limiting by decreasing uptake. A similar situation exists for prostaglandin metabolism in lung [17–20].

Although we have suggested the involvement of an Uptake$_2$-like process in PEN uptake by lung, this can only be a part of the total uptake. Uptake was measured as radioactivity retained in the lung, together with that appearing as metabolite in the effluent. We added the latter component because, in order to be metabolized, the substrate must have entered the cell during the infusion time. Over the whole concentration range, uptake did not show saturation and we have shown in Fig. 2 one possible analysis of our results. This involves a diffusion component and a saturable component with a K_m value comparable with that reported for Uptake$_2$ in heart [21]. A similar analysis, i.e. a saturable component kinetically distinct at low concentrations and a linearly related diffusion component which becomes rate-determining at higher concentrations, has been made for amphetamine [22]. It is perhaps relevant to point out that amphetamine is α-methyl phenylethylamine and might therefore be the closest non-metabolized analogue of PEN. The uptake in rat lung of propranolol [23] and of oxprenolol [24] probably also comprises two such components.

The second difference between PEN and the monoamines 5-HT and NA is therefore that the uptake process for PEN was not only of higher capacity but that it included a considerable diffusion component. The latter characteristic probably reflects the less polar nature of PEN—it has no hydroxyl groups—and is compatible with the ability of PEN to cross the blood brain barrier—again in contrast to the hydroxylated monoamines [25].

The analogy between PEN uptake and basic drug uptake already drawn is strengthened by the fact that the same type of drugs inhibited uptake of both substrates, for instance, imipramine, DMI, amphetamine and propranolol [22–24]. In the case of non-metabolized substrates like propranolol and amphetamine, inhibition of uptake could only reflect an inhibition of the transfer from medium to tissue. Thus although we have demonstrated that decreased metabolism of PEN led to decreased uptake, we cannot rule out a direct inhibition of the transfer of PEN contributing to the overall effect.

Another similarity between propranolol and PEN uptake was shown by the dependence of both on Na ions and on temperature and is in contrast with that of imipramine which was unaffected by either of these variables [5].

In conclusion, our experiments have shown that metabolism of PEN in lung is not limited by uptake and that uptake involves a considerable proportion of diffusion. Both properties contrast with the fate of 5-HT and noradrenaline. Uptake of PEN is closer in several characteristics to uptake of exogenous compounds like propranolol and amphetamine. However, we still do not know in which cells this uptake occurs, nor why the lung should have this large capacity to inactivate a substrate unlikely to be present in high concentrations.

REFERENCES

1. J. A. Roth and C. N. Gillis, *J. Pharmac. exp. Ther.* **194**, 537 (1975).
2. Y. S. Bakhle and M. B. H. Youdim, *Br. J. Pharmac.* **56**, 125 (1976).
3. Y. S. Bakhle and M. B. H. Youdim, *Br. J. Pharmac.* **65**, 147 (1979).
4. V. A. Alabaster and Y. S. Bakhle, *Br. J. Pharmac.* **40**, 468 (1970).
5. A. F. Junod, *J. Pharmac. exp. Ther.* **183**, 341 (1972).
6. T. E. Nicholas, J. M. Strum, L. S. Angelo and A. F. Junod, *Circulation Res.* **35**, 670 (1974).
7. C. N. Gillis and J. A. Roth, *Br. J. Pharmac.* **59**, 585 (1977).
8. Y. S. Bakhle and R. R. Ben-Harari, *J. Physiol. Lond.* **291**, 11 (1979).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
10. J. Southgate and G. G. S. Collins, *Biochem. Pharmac.* **18**, 2285 (1969).
11. H. Feer, *Med. Exp.* **18**, 55 (1968).
12. I. E. Hughes and J. A. Smith, *J. Pharm. Pharmac.* **30**, 124 (1978).
13. C. N. Gillis and Y. Iwasawa, *J. appl. Physiol.* **33**, 404 (1972).
14. M. W. Anderson and T. E. Eling, *Prostaglandins* **11**, 645 (1976).
15. T. J. Mantle, K. F. Tipton and N. J. Garrett, *Biochem. Pharmac.* **25**, 2073 (1976).
16. J. A. Roth and C. N. Gillis, *Biochem. Pharmac.* **23**, 2537 (1977).
17. L. Z. Bito and R. A. Baroody, *Prostaglandins* **10**, 633 (1975).
18. L. Z. Bito, R. A. Baroody and M. A. Rietz, *Am. J. Physiol.* **232**, E382 (1977).
19. Y. S. Bakhle, S. Jancar and B. J. R. Whittle, *Br. J. Pharmac.* **62**, 275 (1978).
20. Y. S. Bakhle, *Br. J. Pharmac.* **65**, 635 (1979).
21. B. A. Callingham and A. S. V. Burgen, *Molec. Pharmac.* **2**, 37 (1966).
22. M. W. Anderson, T. C. Orton, R. D. Pickett and T. E. Eling, *J. Pharmac. exp. Ther.* **186**, 456 (1974).
23. C. T. Dollery and A. F. Junod, *Br. J. Pharmac.* **57**, 67 (1976).
24. B. A. Hemsworth and J. A. Street, *Br. J. Pharmac.* In press.
25. T. Nakajima, Y. Kakimoto and I. Sano, *J. Pharmac. exp. Ther.* **143**, 319 (1964).