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THE FATE OF BIOGENIC MONOAMINES IN PERFUSED RABBIT LUNG

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1 Inactivation of β-phenylethylamine and several of its derivatives was studied in a preparation of rabbit lung perfused with Krebs physiological medium at 37°C. Inactivation or removal of these compounds was calculated as the difference between the concentration of each amine in the perfusion medium and the effluent, collected separately from each lung. The extent of amine metabolic degradation was also measured, by column chromatography, in lung effluent.

2 With this technique the magnitude of amine removal as a function of concentration was determined and an apparent K_m and V_{max} of removal were calculated for each amine.

3 Percentage removal was highest with phenylethylamine (95%), and decreased, apparently in relation to increasing phenyl- and side chain-hydroxylation (and therefore likely increased hydrophilicity), with 5-hydroxytryptamine (64%), tyramine (53%), octopamine (35%), dopamine (32%) and noradrenaline (23%).

4 Inactivation of each amine could be accounted for by metabolic degradation to deaminated products, which appeared in lung effluent within 90 s of beginning amine perfusion.

5 When intrapulmonary metabolism of phenylethylamine was inhibited by simultaneous perfusion with semicarbazide (10 mM) and pargyline (10 μM), the removal rate was unaltered, establishing that uptake of the amine from the vascular space is not dependent on metabolism at least for 4 min infusions.

Introduction

After they leave the vascular space of perfused rabbit lung, 5-hydroxytryptamine (5-HT), noradrenaline (NA) and phenylethylamine are degraded by monoamine oxidase (MAO) and, in the case of NA, also by catechol-*O*-methyltransferase (COMT) (Hughes, Gillis & Bloom, 1969; Gillis & Iwasawa, 1972; Roth & Gillis, 1975). Three forms of MAO have been distinguished in the perfused lung. These are the A and B forms of the mitochondrial oxidase and the 'plasma' amine oxidase (Roth & Gillis, 1975). During a single passage of [^{14}C]-phenylethylamine through rabbit lung almost 90% of the radioactivity appearing in the effluent was associated with the product of deamination, phenylethylacetic acid; little retention of phenylethylamine or metabolite was evident in these lungs (Roth & Gillis, 1975).

There is at present little information available concerning those structural features which are important for removal (i.e., transport and/or metabolism) of phenylethylamine derivatives by the lung. Accordingly, we carried out, and describe in this paper, results of experiments in which we compared the rates of removal and extent of metabolic degradation, in the perfused lung, of phenylethylamine, tyramine, octopamine, dopamine, NA and 5-HT.

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Methods

Lung perfusion and chromatography

Experiments were carried out on the rabbit lung perfused *in vitro* as described by Gillis & Iwasawa (1972). This preparation, in effect, creates paired organs for experimental purposes. In brief, left and right lungs were perfused independently at 10 ml/min with physiological medium (pH 7.4; 37°C) for a 15-min period to clear the lungs of blood. The perfusion medium had the following composition (mM): NaCl 118.07, KCl 4.75, $CaCl_2$ 2.54, KH_2PO_4 0.93, $MgSO_4$ 1.19, $NaHCO_3$ 25.0, glucose 11.1 and disodium calcium edetate 0.027. Both lungs were then perfused

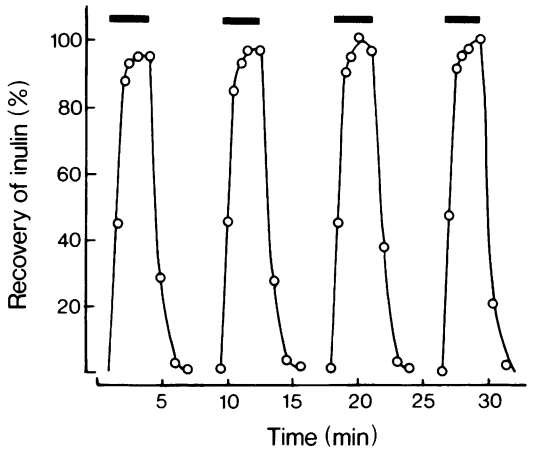

Figure 1 Recovery of [^{14}C]-inulin in effluent of perfused rabbit lung. Lung was perfused at a rate of 10 ml/min for 4 consecutive periods of 4 min with 4×10^5 d/min of [^{14}C]-inulin/ml of Krebs medium. Shown on the ordinate scale is the inulin measured in the effluent (per 30 s) expressed as a percentage of that perfused (per 30 seconds). Horizontal bars show duration of inulin perfusion.

for 10 min with this medium to which one of the amines under study (^{14}C or tritium labelled) had been added. During amine perfusion, the lung effluent was collected for 1 min periods. Total radioactivity in each effluent fraction and in the perfusion medium was measured. Aliquots of lung effluent (0.5 ml) were passed through columns of Bio-Rex 70 (Bio Rad Labs, Richmond, Calif.) cation exchange resin after which the deaminated product was eluted from the columns with 2.5 ml of water (Roth & Gillis, 1975). When effluents containing NA and dopamine were analyzed, columns were subsequently eluted with 3 ml of 2% borate to remove unchanged catecholamine and then with 3 ml of 0.2 M HCl solution to remove *O*-methylated products. The radioactivity in the water, borate and acid washes from the columns was determined by liquid scintillation spectrometry. Removal of amine (per fraction) was calculated as the difference between the radioactivity present as unchanged amine both in the perfusion medium and in each fraction. Total removal is then the cumulative removal during any chosen time period of amine perfusion.

Estimation of initial rates of amine removal

In some experiments we wished to determine the 'initial rates' of amine removal, in order to calculate kinetics for the overall process. Since it was not clear which fractions best reflected the initial rates of removal, it was necessary, in preliminary experiments, to determine the rate of equilibration of an extracellular marker, [^{14}C]-inulin, during 4 successive perfusion periods, each of 4 min duration. Results of a typical experiment (Figure 1) show that the third fraction after beginning inulin perfusion reflects 84–90% recovery of inulin in the effluent (i.e., almost complete equilibration of the inulin space) during each of the four perfusion periods. These data suggested that initial removal of amine should be calculated as [amine infused in 30 s − amine recovered in fraction 3]. In order to increase accuracy of calculated initial removal rate however, we determined the average removal for fractions 3, 4 and 5. Figure 1 also indicates that all inulin within the perfusable space of lung is removed during 3 min of perfusion with inulin-free Krebs solution.

To measure the initial rates of amine removal, lungs were perfused for 4 min with Krebs medium containing one of the ^{14}C- or ^{3}H-labelled amines. Amine perfusion was repeated 3 times with increasing concentrations of the same substrate. Between each period of amine perfusion, lungs were washed for 5 min with amine-free Krebs medium to clear the vascular space of accumulated radioactively-labelled material. Lung effluent fractions were collected for 30 s periods and the third, fourth and fifth fractions (collected as indicated above) after beginning each amine perfusion were used to determine amine removal.

Data derived from these studies were used to calculate the apparent K_m and V_{max} values for removal in left and right perfused lungs of each rabbit. This method eliminates much of the variability in removal among different lung preparations, since removal of each amine, at several different concentrations, is measured in the same lung. Apparent K_m and V_{max} values were determined on a Wang computer by means of a programme supplied by Wang laboratories, Tewkesbury, Mass.

Drugs and isotopes

β-Phenylethylamine hydrochloride-[ethyl-1-^{14}C] (9.86 mCi/mmol), dopamine hydrobromide-[1-^{14}C] (13.69 mCi/mmol), tyramine hydrochloride-[1-^{14}C] (9.2 mCi/mmol), DL-octopamine-[2-^{3}H](N) (3.7 Ci/mmol) were purchased from New England Nuclear, Boston, Mass. (±)-Noradrenaline (±)-bitartrate[carbinol-^{14}C] (35 mCi/mmol) and 5-hydroxytryptamine-[2-^{14}C]creatinine sulphate (59 mCi/mmol) were obtained from Amersham/Searle Corp., Arlington Heights, Ill. The unlabelled amine salts were purchased from Sigma Chemical Co., St. Louis, Mo.

Results

The ability of rabbit lung to deaminate phenylethylamine, 5-HT, tyramine, octopamine,

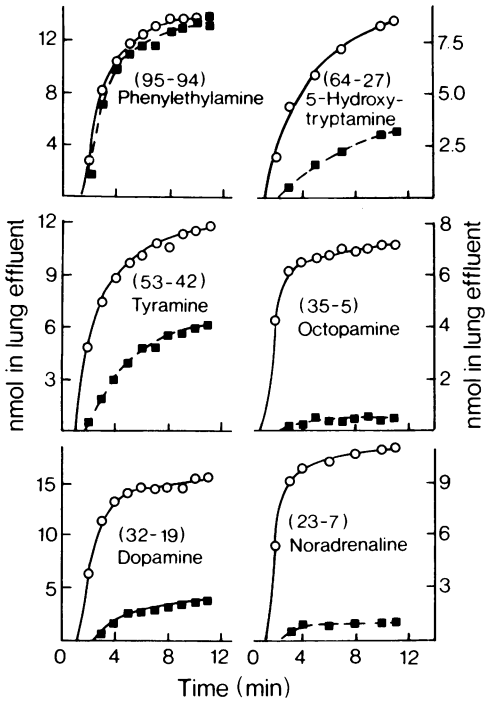

Figure 2 Appearance of amines and metabolites in effluent from lungs perfused with different amines for 10 minutes. Data for amine *and* metabolite (○) and deaminated metabolite alone (■) are presented. For each amine, the percentage total removal and percentage total metabolism during 10 min of perfusion are given in parentheses. The concentrations (μM) of amines used were: phenylethylamine 1.6; tyramine 1.2; dopamine 1.8; 5-hydroxytryptamine 1.4; octopamine 1.0 and noradrenaline 1.5.

dopamine and NA is illustrated in Figure 2. Also indicated in parentheses in the figure, are the (percentage) total removal and total metabolism for each amine. Data presented in Figure 2 are representative of at least 4 similar experiments with each amine. In these experiments rabbit lungs were perfused with 1–2 μM of one of the radioactively-labelled amines for 10 minutes. The percentage removal of phenylethylamine was 95%, the highest observed. This is followed, in decreasing order, by removal of 5-HT (64%), tyramine (53%), octopamine (35%), dopamine (32%) and NA (23%). By comparing the figure for percentage total deaminated product appearing in the effluent with the corresponding value for percentage removal, it can be seen that, among the amines tested, phenylethylamine, tyramine and dopamine were those most effectively deaminated by perfused rabbit lung. In the cases of NA and dopamine, the percentage of radioactivity in

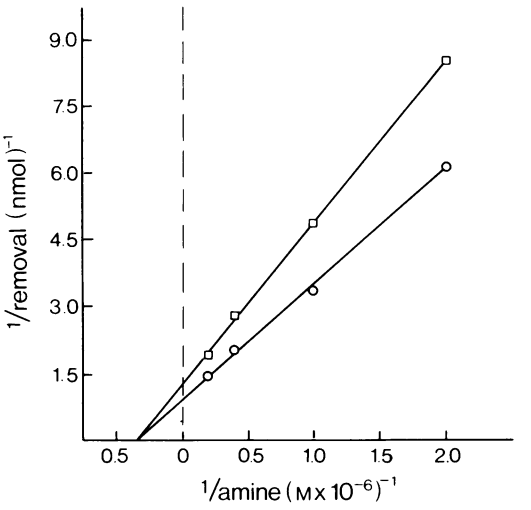

Figure 3 Double reciprocal plot of noradrenaline (□) and 5-hydroxytryptamine (○) removal *vs* concentration.

the acid effluent of columns (COMT metabolites) was always less than 8% of the total.

In experiments designed to estimate initial rates of amine removal, lungs were perfused with different amine concentrations during 4 successive perfusion periods as described in the Methods section. However, it was first necessary to demonstrate that at a given concentration of amine, removal remained constant during each period of perfusion. Table 1 presents the results of two experiments in which lungs were

Table 1 Average removal (per 30 s) during each of four successive periods of [^{14}C]-5-hydroxytryptamine perfusion

Period of amine perfusion	*Amine removal* (nmol/30 s)*	
	Left lung	*Right lung*
Experiment 1		
1	2.431	2.453
2	2.315	2.326
3	2.426	2.461
4	2.442	2.500
Experiment 2		
1	4.509	4.593
2	4.335	4.433
3	4.284	4.440
4	4.274	4.510

5-Hydroxytryptamine concentrations were, experiment 1, 0.56 μM; experiment 2, 1.0 μM.
* Calculated as, $\sum (nmol_{medium} - nmol_{effluent})$ of fractions 3 + 4 + 5.

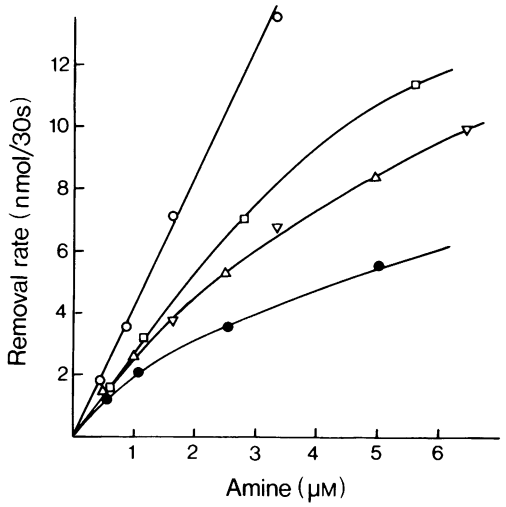

Figure 4 The rates of amine removal as functions of the concentration perfused. Each point represents the mean of data from four lungs. (○) Phenylethylamine; (□) tyramine; (▽) octopamine; (△) dopamine; (●) noradrenaline.

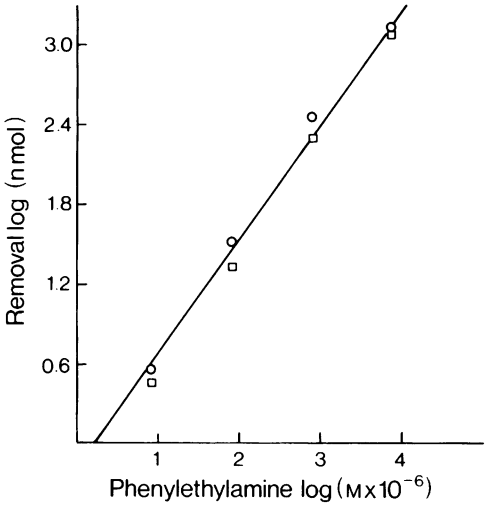

Figure 5 Removal of phenylethylamine in the absence (○) and in the presence (□) of pargyline (10 μM) and semicarbazide (10 mM).

perfused with either 0.56 μM or 1.0 μM [^{14}C]-5-HT during four successive periods as described in the Methods section. It is apparent that the magnitude of removal remained constant during each period of amine perfusion; accordingly, we felt justified in using successive periods of amine perfusion to determine 'initial' removal rates before calculating K_m values for amine transport. Results of a typical experiment in which rabbit lungs were perfused with four different concentrations of 5-HT or NA are illustrated in the Lineweaver-Burk plots in Figure 3. The average K_m (± s.d.) determined for 5-HT and NA was 2.7 μM 1.7 ($n=6$) and 3.2 μM 1.1 ($n=5$), respectively.

Pulmonary removal of the phenylethylamine derivatives, as a function of increasing concentrations perfused is illustrated in Figure 4. It is clear from this figure, and also from the data presented in Figure 2, that over the concentration range used, phenylethylamine was the most extensively removed amine and was followed in decreasing order by tyramine, dopamine, octopamine and NA. Attempts were made to analyze the data of Figure 4 according to the method of Lineweaver & Burk (1934). The 'apparent' K_m and V_{max} values for NA, tyramine, dopamine and octopamine are shown in Table 2. Though linear double reciprocal plots were obtained for each of these amines, the calculated K_m values for the latter three amines were greater than the highest concentration perfused through the lung to estimate those K_m values. However, when in additional experiments, the concentration range of each amine was raised to include that represented by the K_m value shown in Table 2, the K_m then calculated also fell outside this new concentration range used.

Table 2 Apparent K_m and V_{max} for amine removal in perfused rabbit lung

Amine	n	K_m (μM)	V_{max} (nmol/lung per min)
Noradrenaline	5	3.2 ± 1.1	7.6 ± 2.3
Octopamine	4	8.1 ± 1.3	22.6 ± 2.2
Dopamine	4	6.6 ± 0.6	19.4 ± 1.1
Tyramine	4	9.8 ± 0.8	30.0 ± 1.7

n = number of individual determinations.

As shown in Figure 4, removal of phenylethylamine was linear over the relatively narrow range (0.41–3.3 μM) of concentrations used. Phenylethylamine was chosen for additional study since it was much more rapidly and extensively degraded by the perfused lung than the other amines used (see Figure 2). In separate series of experiments in which lungs were perfused with concentrations of phenylethylamine ranging from 0.83 μM to 830 μM, removal was also linear as shown in the log-log plot in Figure 5. At concentrations below 8.3 μM, 76–80% of the radioactivity appearing in fractions three, four and five (collected between 90 and 180 s after beginning perfusion) was deaminated product. The percentage of deaminated product appearing in the effluent was greatly decreased when phenylethylamine was perfused at 83 μM (49 ± 0.7%; $n=6$) and 830 μM

($10 \pm 0.9\%$; $n=6$), suggesting that MAO is likely to be saturated at these concentrations. These results are consistent with data obtained *in vitro* which indicate that the K_m for phenylethylamine deamination by lung mitochondrial MAO is approximately 7 μM (Roth & Gillis, 1974).

In view of the extensive intrapulmonary degradation of phenylethylamine, it was of interest to determine whether inhibition of such metabolism affected initial rate of removal. Therefore, lungs were perfused for 15 min with pargyline (10 μM) and semicarbazide (10 mM) and then with phenylethylamine (0.83–830 μM) and the inhibitors. These concentrations of pargyline and semicarbazide were shown previously (Roth & Gillis, 1975) to inhibit completely the metabolism of phenylethylamine by rabbit lung. Data from these experiments also are presented in Figure 5; it is evident that removal was unaffected by this treatment.

Discussion

The process of amine removal, within the context of experiments involving perfused lung, has been defined (Gillis, 1976) as a reduction in the concentration of amine in lung effluent, when compared to that perfused through lung. Removal thus may, and in many instances does, include a process of transport from the vascular space (as in the case of NA and 5-HT) as well as subsequent metabolic degradation. However, intrapulmonary binding is not a significant component of removal in the case of biogenic amines and structurally related compounds. The biogenic amines and their metabolites leave the lung rapidly (Hughes *et al.*, 1969; Junod, 1972; Nicholas, Strum, Angelo & Junod, 1974). Transport of NA and 5-HT from the vascular space is considered to be the rate-limiting step in overall removal (Alabaster & Bakhle, 1970, 1973; Junod, 1972; Gillis & Iwasawa, 1972). Since little intracellular binding occurs, the rate of loss of amine during constant infusions would, in theory, eventually reach that of inward transport from the vasculature; at this point net removal would be zero. It is apparent, however, from this as well as earlier studies that there is substantial net removal of each amine tested; such removal must ultimately therefore reflect catabolism of the substance perfused and perhaps passive diffusion across endothelial cell membranes.

This is well illustrated by the present experiments with phenylethylamine. It can be seen (Figures 4 and 5) that phenylethylamine is removed to the greatest extent of any of the amines tested. However, over 90% (Figure 2) and at least 75% on the average, of radioactivity in lung effluent is in the form of metabolite. Accordingly, removal in this case seems to reflect largely metabolic degradation. Yet, metabolic inhibition with pargyline and semicarbazide (Figure 5) does not affect total removal. This observation, coupled with the linear relationship between a wide range of phenylethylamine concentrations and removal (Figure 5) suggests the existence of a purely passive process which brings this amine into contact with the forms of MAO responsible for its degradation (Roth & Gillis, 1975).

Noradrenaline represents the opposite extreme. In this case removal is the lowest of any amine used, but seems to involve energy-dependent, temperature and drug-sensitive transport (Bakhle & Vane, 1974; Gillis, 1976). Furthermore, metabolic inhibition does not affect net removal during short-term perfusion (Gillis & Iwasawa, 1972; Alabaster & Bakhle, 1970, 1973; Nicholas *et al.*, 1974).

The ability of rabbit lung to remove phenylethylamine and its structural analogues appears to be related to the hydrophobicity of these amines. Thus phenylethylamine, being the most liphophilic of these compounds, is removed to the greatest degree whereas, NA, with three hydroxyl groups, is removed to the least extent (Figures 2 and 4). Also, in conformity with this explanation, tyramine is more effectively removed than is octopamine or dopamine, while the latter two dihydroxylated amines are removed by lung to a greater extent than is NA. This suggests that for the more lipid soluble amines, passive transport in lung endothelium may play a major role in their removal.

The ability of the perfused rabbit lung to degrade phenylethylamine and structurally related amines is related to both uptake and the form or forms of amine oxidase which are responsible for their deamination. The three amines, phenylethylamine, tyramine and dopamine, which, in relation to percentage removal, are degraded to the greatest extent (Figure 2) are each metabolized by two or more forms of the oxidase. For example, phenylethylamine has been shown to be deaminated by the B form of the mitochondrial oxidase as well as the 'plasma' amine oxidase (Roth & Gillis, 1975; Bakhle & Youdim, 1976). Tyramine and dopamine are degraded by all three forms of amine oxidase identified in the lung vasculature (Neff & Yang, 1974). In contrast, 5-HT, octopamine and NA are deaminated by only the A form of the mitochondrial oxidase (Neff & Yang, 1974), although 5-HT is also reported to be a poor substrate for 'plasma' MAO (McEwen, Cullen & Sober, 1966).

It is noteworthy that we consistently observed metabolism of dopamine by the perfused lung. If the dopamine metabolite is biologically inactive this would imply that there is some loss of dopamine 'activity' during passage through lung. Available information on this possibility is conflicting. Thus, Boileau, Crexells & Biron (1972) found that the pressor effect of dopamine was unaffected by transpulmonary passage in rat, dog or man. However, Nicholas *et al.*

(1974) showed that dopamine was not concentrated by rat lung after a 2 min perfusion of the amine, which could well be explained by lack of binding and rapid metabolism of dopamine removed from the vascular space.

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