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ONTOGENESIS OF UPTAKE AND DEAMINATION OF 5-HYDROXYTRYPTAMINE, DOPAMINE AND β-PHENYLETHYLAMINE IN ISOLATED PERFUSED LUNG AND LUNG HOMOGENATES FROM RATS

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1 Uptake of 5-hydroxytryptamine (5-HT) and β-phenylethylamine (PEA) was studied in perfused lung from male rats between 10 and 70 days old.

2 Monoamine oxidase (MAO) activity towards 5-HT, PEA and dopamine was studied in homogenate preparations of lung from rats aged between 5 and 80 days.

3 Uptake of 5-HT (10 μM) decreased throughout the age range studied but uptake of PEA (50 μM) increased for the first 30 days and beyond this age it decreased. Metabolites formed for both amines reflected the changes in uptake.

4 MAO activity deaminating 5-HT is well developed by day 10 and reaches its maximum by day 40. For dopamine and PEA, MAO activity remained low until day 20, and then developed rapidly, reaching a maximum by day 40 for dopamine; activity towards PEA did not reach a maximum by day 80.

5 These results show that uptake and MAO activity changes with age and thus the lung responds like other tissues.

6 These results also demonstrate the independent development of uptake and MAO activity towards 5-HT, PEA and dopamine.

Introduction

Age-dependent changes in monoamine oxidase (MAO) activity in various tissues of rat and other species, including man (Blatchford, Holzbauer, Grahame-Smith & Youdim, 1976; Youdim & Holzbauer, 1976) and in uptake of monoamines (Nomura, Naitoh & Segawa, 1972; Kirksey & Slotkin, 1979) have been described. In none of these studies was the lung investigated.

MAO is a mitochondrial enzyme and, consequently, lung metabolism of monoamines comprises both uptake into the cell and deamination by MAO. A striking feature of this lung metabolism is that the enzymatic properties of lung homogenates are not a reliable guide to the properties shown by the perfused lung (Youdim, Bakhle & Ben-Harari, 1979). This is due to the fact that for amines, such as 5-hydroxytryptamine (5-HT), the uptake step, which is absent in homogenates, is rate limiting (Bakhle & Youdim, 1979).

In order to extend our studies into physiological and pathological factors affecting lung metabolism of monoamines (Youdim, Bakhle & Ben-Harari, 1980), the uptake and deamination of 5-HT, β-phenylethylamine (PEA) and dopamine, were studied in homogenates of lung and compared to those in isolated perfused lung from rats at different ages.

A preliminary account of some of this work has been communicated to the Federation of European Biochemical Societies (Ben-Harari & Youdim, 1980).

Methods

The experiments were performed on inbred littermate male rats. The average lung weights for the various ages are listed in Table 1. The rats were killed by rapid decapitation.

Preparation of lung homogenate and assay of monoamine oxidase

Freshly obtained rat lungs were washed with ice-cold 0.32 M sucrose to remove blood. They were homogenized in 0.32 M sucrose (4°C) using two 10 s bursts of a Polytron homogenizer, with cooling between bursts. The homogenate was diluted with 0.32 M sucrose to give a final preparation of 10% (w/v). Samples (5 ml) were placed in plastic tubes and frozen at −20°C. MAO activity could be retained for almost 3 months.

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Table 1 Change in lung weights with age

	Age (days)							
	10	20	25	29	42	48	56	70
Lung wet weight (g)	0.7±0.1(10)	0.8±0.1(10)	0.8±0.05(5)	1.1±0.2(10)	1.2±0.04(10)	1.2±0.05(5)	2.1±0.3(5)	1.5±0.1(10)

Values shown are the mean lung weights from the number of animals shown in parentheses.

All assays of MAO were carried out at pH 7.4 in potassium phosphate buffer (final concentration 0.05 M) at 37°C. MAO activity against different substrates was measured by the radioassay technique described by Tipton & Youdim (1976). Protein concentrations were determined by the colorimetric procedure of Lowry, Rosebrough, Farr & Randall (1951) with bovine serum albumin as standard.

Preparation of perfused lung and measurement of uptake

The lungs were removed and perfused via the pulmonary artery as described previously (Alabaster & Bakhle, 1970) with Krebs solution (mM: $NaHCO_3$ 25, NaCl 120, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2 and glucose 5.6) at 37°C gassed with a 95% O_2 and 5% CO_2 mixture at a constant flow of 8 ml/min. After 10 to 15 min of perfusion, the effluent was free of blood and the perfusion was continued for measurement of uptake.

To measure uptake of ^{14}C-amine, a 3 min infusion of amine was given through the lungs at a rate of 0.4 ml/min into the pulmonary arterial cannula. The amines infused were a mixture of ^{14}C-amine (10^5 d/min per infusion) and unlabelled amine to give a final concentration of 5-HT of 10 μM and a final concentration of PEA of 50 μM. After the end of the infusion, 30 s were allowed before the perfusion was stopped and the lungs removed from the perfusion system. The effluent from the lung was collected during this 3.5 min 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°C) 0.3 M perchloric acid, using two 10 s bursts of a Polytron homogenizer and the homogenate was centrifuged at 1,000 *g* for 20 min. Aliquots of the supernatant were adjusted to pH 6 to 6.5 with 3 M potassium hydroxide. Recoveries of amines in the supernatant were 82% and in the effluent 100%; the procedures of homogenization and chromatography resulted in 2.5% breakdown of the amines.

Ion-exchange chromatography and measurement of radioactivity

Samples of the effluent and the neutralised supernatant from the lung and from the incubation mixtures were applied to columns of Amberlite CG-50 resin to separate amine metabolites from unchanged substrate (Tipton & Youdim, 1976). Radioactivity was measured after mixing with Triton-toluene scintillation fluid (PPO 5 g, DMPOPOP 0.25 g, toluene 1 litre and Triton X-100 0.5 litre) using a liquid scintillation counter (Packard B2450). Corrections were made for quenching by the sample channels ratio.

Materials

[2-^{14}C]-5-HT creatinine sulphate (58 mCi/mmol) and [1-^{14}C]-dopamine hydrochloride (48 mCi//mmol) were obtained from Radiochemical Centre, Amersham and [1-^{14}C]-PEA hydrochloride (48 mCi/mmol) was obtained from New England Nuclear, Frankfurt. The unlabelled substrates were obtained from Sigma. Other chemicals used were of analytical reagent grade.

Statistical methods

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

Results

Uptake in perfused lung

Since MAO is a mitochondrial enzyme the deaminated metabolite in the effluent emerging from the lungs during the 3 min infusion is also derived from amine taken up into the lung. Therefore, uptake of amine was calculated from the total radioactivity still retained in the lung after the infusion, together with the radioactive metabolite collected in the effluent.

The uptake of [^{14}C]-5-HT was studied at a single concentration of 10 μM over the age range 10 to 55 days. Figure 1a shows that uptake of 5-HT progressively decreased throughout this period. In contrast the uptake of PEA, studied at a single concentration of 50 μM over the same age range, showed a bell-shaped type distribution (Figure 1b). Uptake of PEA reached maximum values at days 20 and 30 after which time, at days 50 and 70, uptake fell.

The amount of deaminated metabolites produced

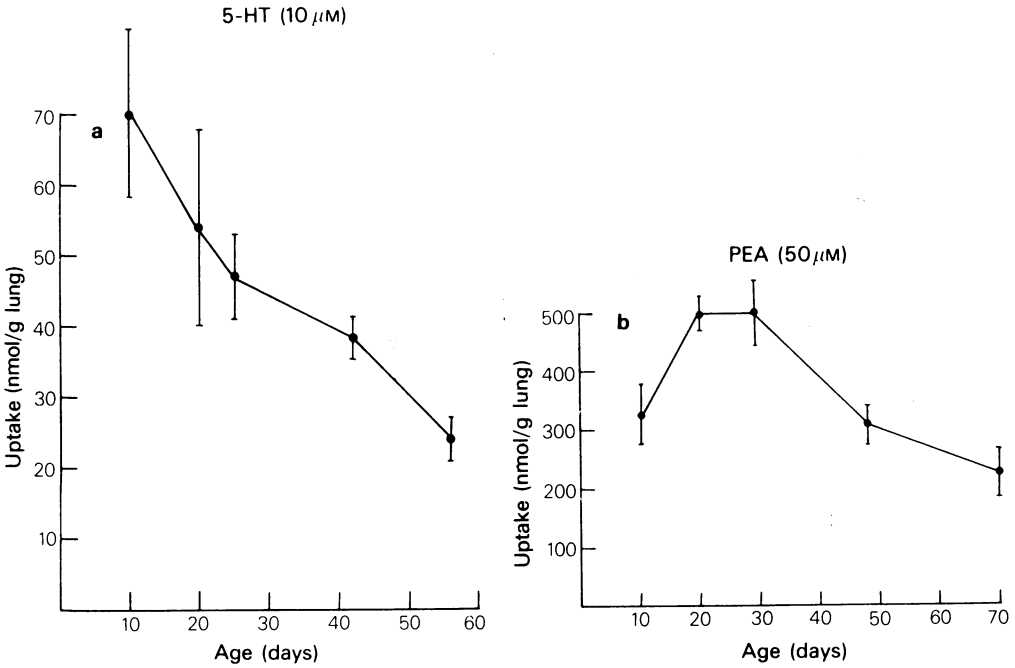

Figure 1 Postnatal development of uptake of 5-hydroxytryptamine (5-HT, a) and β-phenylethylamine (PEA, b) in isolated lungs from litter-mate male rats aged between 10 and 70 days old. Uptake was measured as the sum of radioactivity retained in the lung and the radioactive metabolite in effluent following a 3 min infusion of ^{14}C-amine and is expressed as nmol/g lung. Each point represents the mean value of 4-10 experiments and the standard error is shown by the vertical bars. Mean values are joined by straight lines irrespective of the length of the interval and must not be interpreted as the exact time course at which uptake changes with age.

also changed with the age of the animal (Figure 2a, b). These metabolites are the sum of those in the lung and in the effluent. They were expressed as a percentage of the value obtained at day 10; the value at day 10 was taken as 100%. Values for uptake were also expressed as a percentage of the day 10 value.

Both for 5-HT (Figure 2a) and PEA (Figure 2b) changes in total metabolite correlated closely with the changes in uptake. Alternatively, the changes in the amount of metabolite produced may have been the result of changes in MAO activity. Therefore, MAO activity was examined directly.

Monoamine oxidase activity in lung homogenates

MAO activity, expressed as nmol metabolite produced min^{-1} μg^{-1} protein, was measured with 5-HT, dopamine and PEA as substrates. Figure 3 shows that MAO activity towards 5-HT is well developed by the 10th day and reaches its maximum by the 40th day. For dopamine and PEA, both enzyme activities lagged behind up to the 20th day and then developed rapidly, reaching a maximum by day 40 for dopamine, whereas activity towards PEA did not reach a maximum by day 80.

Discussion

Age-dependent changes in uptake of amines (Kirksey & Slotkin, 1980) and in MAO activity (Blatchford *et al.*, 1976) in various tissues of the rat have been reported. Our results have shown that in lung, uptake and MAO activity also changed with age and thus lung responds like other tissues. Another important feature of our results is the independent development of uptake and MAO activity towards 5-HT, PEA and dopamine.

Uptake of 5-HT progressively decreased with increasing age whereas deamination of 5-HT *in vitro* increased. The uptake in adult rats has been shown to be the rate-limiting step in inactivation of this amine and to be independent of the activity of MAO (Junod, 1972). Therefore, the observation that MAO activity and uptake towards 5-HT developed in opposite directions with increasing age and that the changes in metabolite reflected the changes in uptake, rather than MAO activity, confirms that uptake is rate-limiting for 5-HT metabolism and that the uptake and enzyme system for metabolism of 5-HT develop independently. That uptake is the rate-limiting step in metabolism of 5-HT would appear to

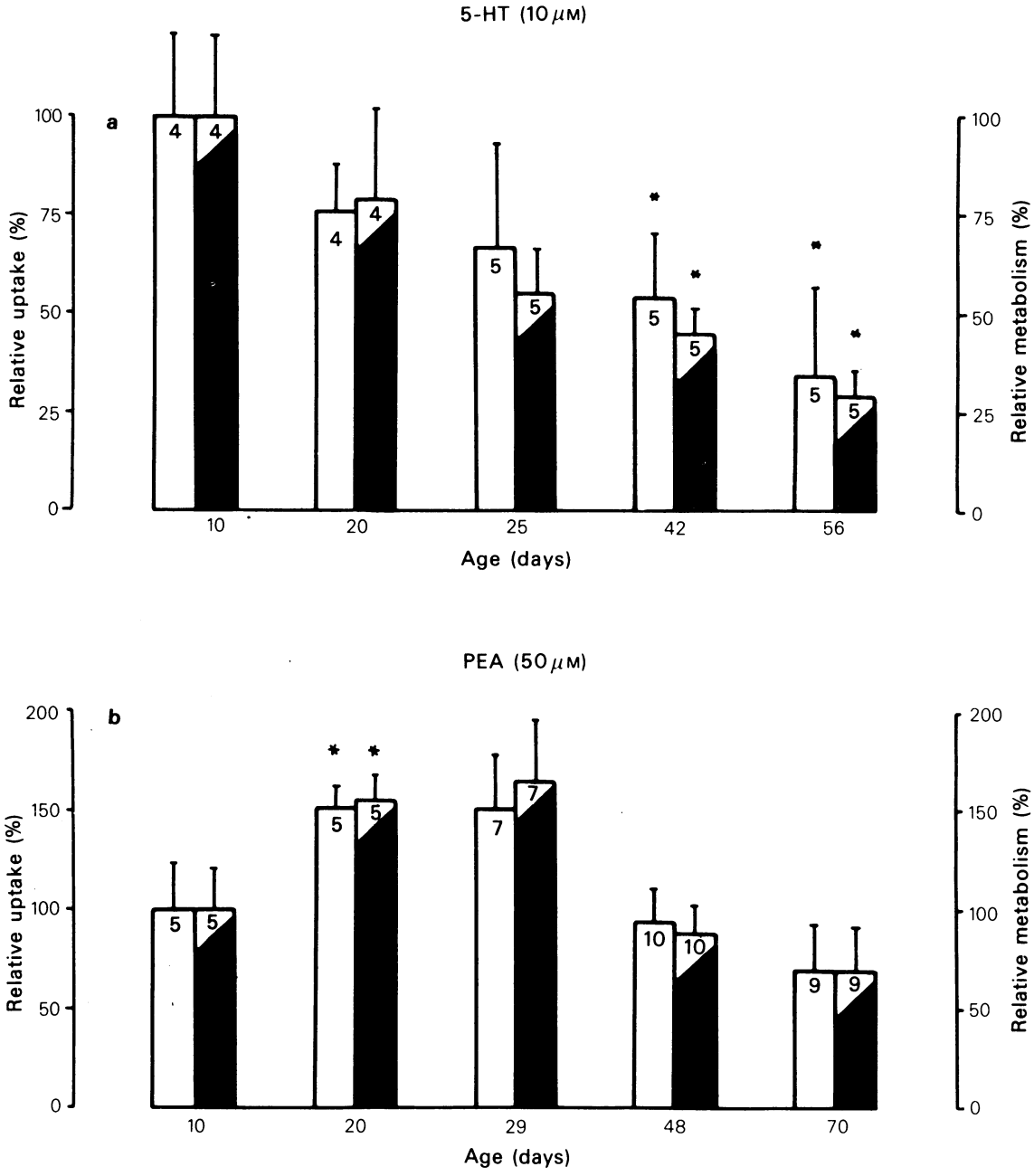

Figure 2 Relative changes in uptake and metabolism of 5-hydroxytryptamine (5-HT, a) and β-phenylethylamine (PEA, b) in isolated lungs from litter-mate male rats aged between 10 and 70 days old. Uptake (open columns) was measured as the sum of radioactivity retained in the lung and the radioactive metabolite in effluent and metabolism (solid columns) as the sum of radioactive metabolite in lung and effluent following a 3 min infusion of ^{14}C-amine. Values for uptake and metabolism are expressed relative to values obtained at 10 days of age (day 10 = 100%). The heights of the columns represent the mean value of the number of experiments indicated by the figure in the column and the vertical lines indicate s.e.mean. *Values significantly different ($P<0.05$) from day 10.

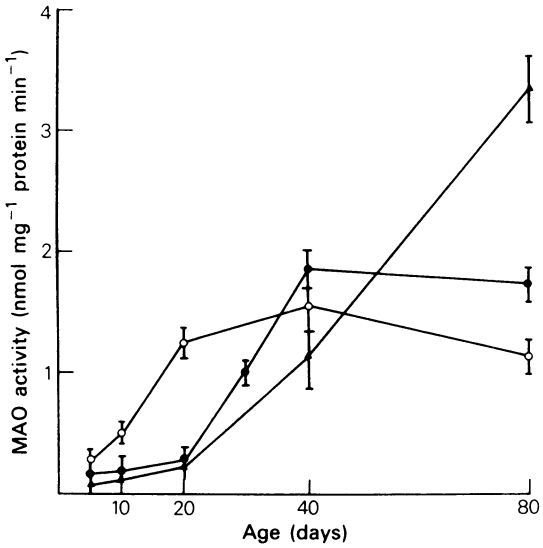

Figure 3 Postnatal development of monoamine oxidase (MAO) activity towards 5-hydroxytryptamine (5-HT, ○) dopamine (●) and β-phenylethylamine (PEA, ▲) in litter-mate male rats aged between 5 and 80 days old. MAO activity was expressed as nmol deaminated metabolite mg^{-1} protein min^{-1}. Mean values are given and the standard error is shown by the vertical lines. Mean values are joined by straight lines irrespective of the length of the interval and must not be interpreted as the exact time course at which MAO activity changes with age.

be strictly true only in comparatively older animals. After about day 40, MAO activity towards 5-HT has fully developed but uptake is still decreasing. Therefore, the low level of uptake would limit the deamination of the substrate. In younger rats, between 5 and 20 days old, MAO activity appears to play a greater part in limiting 5-HT metabolism since it has not fully developed, while values for uptake were the highest found. It is possible that in these younger rats an uptake system for 5-HT does not exist and that it gradually develops with age, thereby explaining the greater role played by MAO.

In adult rats the uptake of PEA comprises both a saturable component with a *K*m of 25 μM, which is identical to the *K*m for MAO metabolizing PEA, and a large passive component (Ben-Harari & Bakhle, 1980). Uptake of PEA is diminished following inhibition of MAO (Ben-Harari & Bakhle, 1980), demonstrating that the rate-limiting step in metabolism of this amine is deamination by MAO. From the results of PEA uptake (Figure 1b) and MAO activity (Figure 3) with increasing age, it appears that until the rats were 30 days old the increase in uptake was a direct result of the increase in MAO activity. After day 30, uptake decreased while MAO activity continued to rise. The reason for this sudden separation of the activities is unknown but clearly demonstrates that it is possible for changes in uptake of PEA to occur without identical changes occurring in MAO activity. In confirmation of this, inhibition of uptake of PEA in lung can also be caused pharmacologically, without inhibition of the enzyme (Ben-Harari & Bakhle, 1980). It cannot be ruled out that the changes in uptake throughout the age range studied were totally independent of MAO activity or may be a resultant of both these factors.

The results obtained *in vitro* and in the perfused lung, both with 5-HT and PEA, clearly demonstrate yet again that enzymatic properties of lung homogenates are not a reliable guide to the metabolic activities exhibited by the perfused lung in isolation (Youdim *et al.*, 1979) and is due to the crucial control exerted by the uptake system in metabolism of these amines.

Enzyme activity towards dopamine and PEA until the 40th day developed almost identically, whereas 5-HT deamination was developed much earlier. After day 40, activity towards PEA developed totally independently of activity towards 5-HT and dopamine. In lung, 5-HT and dopamine are substrates for type A MAO whereas PEA is a substrate for type B MAO (Bakhle & Youdim, 1979). This completely independent development of MAO deaminating 5-HT and PEA confirms the existence of A and B forms of MAO in lung. The observation that in lung, MAO activity metabolizing PEA increased up to 80 days is in contrast to results in most other organs, but agrees with MAO activity in rat heart which did not reach

maximum values by the end of the 4th month of development (Blatchford *et al.*, 1976). These results also suggest that in lung, dopamine is a substrate for MAO-B in younger animals up to 20 days old and becomes a substrate also for MAO-A in older animals. Up to day 20, deamination of dopamine was identical to that of PEA, a MAO-B substrate; after day 20, dopamine deamination increased rapidly to its maximum value at day 40, at which time deamination of 5-HT, a MAO-A substrate, was also maximum.

The precise biochemical mechanism(s) underlying the development of amine uptake and the development and separation of MAO into A and B forms is not known (Singer, von Korff & Murphy, 1979). Mechanisms by which MAO may be affected have been discussed (Youdim & Holzbauer, 1976). The development of PEA uptake may be related to levels of the sex hormone, oestradiol, in blood. In male rats levels of oestradiol in serum have been shown to increase between days 9 and 20 (Döhler & Wuttke, 1975), which corresponds closely with the changes in PEA uptake described. This proposal is supported by the fact that PEA and 5-HT uptake in rat lung is responsive to some factor(s) changing during the oestrous cycle of the rat, the most obvious candidate being oestradiol (Youdim *et al.*, 1979). Since no kinetic studies were performed in these experiments it is not known whether the changes in uptake reflect a change in *Km* or *Vmax*. However, for MAO, in other tissues, such as liver and brain, changes in activity with age have been shown to be a reflection of changes in *Vmax* of the enzyme (Blatchford *et al.*, 1976). Since lung MAO has the same substrate specificities and inhibitor sensitivities as other tissues (Bakhle & Youdim, 1979) and lung demonstrates the same changes with age as other tissues, changes in lung MAO with age are most probably due to variations in *Vmax* rather than *Km*.

At present, the physiological importance of these developmental changes is not clear. The non-uniformity of the development of uptake of 5-HT and PEA indicates that different ontogenetic event(s) may cause the development of these two uptake systems. However, the fact that physiological changes occurring during the developing animal can affect monoamine inactivation in lung, confirms our belief (Youdim *et al.*, 1980) that it has a physiological role. One such physiological role for lung, according to Vane (1969), is to control circulating levels of vasoactive substances in the blood. Thus substances, such as 5-HT, which are inactivated by the lung are regarded as local hormones and substances, such as adrenaline, which pass freely across the lung are regarded as circulating hormones. During foetal life the detoxification of the blood is carried out by the placental-maternal circulation but after birth the newborn must cope with this requirement. The very rapid increase in MAO activity metabolizing 5-HT and its relatively high uptake in lung during the first 20 days after birth may serve to fulfil a detoxifying function by controlling the level of this local hormone in the blood.

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