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Catabolism of epinephrine in control, sensitized and anaphylactic guinea pig lung slices

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There have been many studies on the uptake and metabolism of norepinephrine (NE) in lung [1–5] but few similar investigations of epinephrine (Epi). Ginn and Vane [6] reported that Epi passes through the pulmonary bed of dogs unchanged. More recently, Gillis and Iwasawa [3] showed that 13 per cent of Epi infused is retained in rabbit lung and Mathé *et al.* [7, 8] found evidence for both uptake and metabolism of Epi in isolated, perfused guinea pig lung. Epi is a more effective bronchodilator in asthmatic than in healthy subjects (A. A. Mathé and E. A. Gaensler, manuscript in preparation). Furthermore, Epi inhibits the anaphylactic release of both histamine [9, 10] and prostaglandins [11, 12], and low concentrations of Epi stimulate adenylate cyclase to a greater extent in homogenates from antigen-sensitized than control guinea pig lungs [13, 14]. The aim of this investigation was to study the catabolism of Epi in "healthy" lung slices and to establish whether this catabolism was altered in sensitized and anaphylactic lung slices.

MATERIALS AND METHODS

Animal model. Twelve male Hartley guinea pigs (300–400 g) were randomly divided into two groups. One group was sensitized to ovalbumin (day 1: 0.5 ml i.p. and 0.5 ml s.c., day 3: 0.5 ml i.p., 10 mg ovalbumin/ml of 0.9% NaCl). The control group received the vehicle only. Four weeks later, heparin (1000 I.U.) was injected i.p. and the animals were killed 45 min later. The lungs were removed and perfused through the pulmonary artery for 7 min with Tyrode's solution (20°, pH 7.4, bubbled with 5% CO_2 in O_2).

Slice preparation and incubation. The lungs were sliced in the cold room with a Stadie–Riggs micrometer. Each slice weighed approximately 80 mg and was approximately 0.25 mm thick. Limiting thickness for the free diffusion of O_2 into the slice incubated in air was calculated to be 0.30 mm [15]. Eighteen slices were taken from each lung and three slices (after weighing) distributed at random into six flasks containing 20 ml of Tyrode's solution and 1 mM $Na_2S_2O_5$. The flasks were incubated in a Dubnoff water bath (37°, constant shaking) and after 5 min ovalbumin (final concentration 20 μg/ml) and vehicle (Tyrode's solution) were each added to three samples. After a further 4-min incubation, each of the paired samples (one with and one without ovalbumin) received 100 μl of one of the three different concentrations of Epi (0.2, 2 or 200 μg, 10 μCi/ml). The final Epi concentration in the medium was 1, 10 or 1000 ng/ml, each containing 0.05 μCi/ml. After 5 min of incubation with Epi, the tissue was separated from the media by filtration. The tissues were placed in 5 ml of ice-cold 0.4 M perchloric acid [which contained 10 μg each of Epi, 3,4-dihydroxyphenylglycol (DOPEG), 3,4-dihydroxymandelic acid (DOMA), 3-methoxy-4-hydroxyphenylglycol (MOPEG), 3-methoxy-4-hydroxymandelic acid (VMA) and metanephrine (MN); 10 mg $Na_2S_2O_5$ and 0.2% EDTA], immediately homogenized and centrifuged. The media were acidified with 200 μl of 5 N HCl, and 1 ml of 0.01 M HCl solution containing carriers and antioxidants (10 μg each of Epi, DOPEG, DOMA, MOPEG, VMA and MN; 40 mg $Na_2S_2O_5$ and 4% EDTA) was added. Both tissue supernatants and media were kept frozen until assayed.

Assay for Epi and metabolites. Epi and metabolites were separated by the column chromatographic procedure (Fig. 1) of Cubeddu *et al.* [16]. Preliminary experiments established that this method separated Epi, its *O*-methylated (OM) metabolite (MN), deaminated (DA) metabolites (DOMA and DOPEG) and *O*-methylated + deaminated (OMDA) metabolites (MOPEG and VMA) from each other. When 10 μg of Epi, MN, DOMA, DOPEG, MOPEG or VMA were run separately through the fractionation procedure, more than 90 per cent of each was recovered in the appropriate fraction, when measured by native fluorescence [17]. The spillover of one fraction into another was less than 5 per cent in all cases and except for Epi contributed negligibly to the total counts in each fraction: Epi spillover (or oxidation) was corrected for by incubating without tissue four samples at each concentration of Epi (two with and two without ovalbumin at various times during the experiment). These samples were incubated and processed identically to the experimental samples and the per cent of total Epi counts found in the other fractions was determined and corrected for. A similar correction was made for the lung tissue samples. All the fractions were counted in Triton–toluene on a Beckman LS-250 liquid scintillation counter.

Preliminary experiments. A separate experiment established that the wet weights of control and sensitized lung slices used were not a function of a differential accumulation of fluid during perfusion. After perfusion, lungs were minced and placed in pre-weighed vials. After weighing,

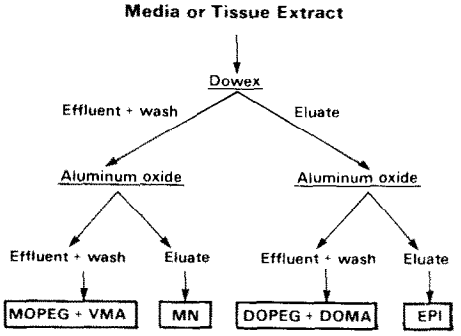

Fig. 1. Schematic representation of epinephrine (Epi) and its metabolites according to the method of Cubeddu *et al.* [16]. Media and tissue supernatants containing carrier substances and antioxidants were applied to Dowex columns at pH 2. After elution with 3 × 4 ml of 1 N HCl, the pH of Dowex effluents and eluates was readjusted to 8.4 and passed over alumina. After elution with 1 × 5 ml of 0.5 N HCl from alumina, all the samples were counted in Triton–toluene on a Beckman LS-250 liquid scintillation counter.

all samples were dried to constant weight with no charring. The wet to dry weight ratio was 4.47 ± 0.05 for six control lungs and 4.43 ± 0.09 for six sensitized lungs.

Additional control and sensitized slices were incubated with [^{14}C]-inulin under the same experimental conditions. Radioactivity was counted in the media and tissue supernatants and the extracellular fluid volume calculated. Approximately 40 per cent of the wet weight of tissue was found to be extracellular in both control and sensitized slices.

Finally, anaphylactic release of histamine, as measured by the method of Shore *et al.* [18], was approximately 35 per cent.

Chemicals. The chemicals used were: DL-epinephrine-L-bitartrate-[7-^{3}H] (N) (sp. act. 11.426 Ci/m-mole), and inulin[carboxyl-^{14}C] (New England Nuclear Corp., Boston); DL-epinephrine, DL-3,4-dihydroxyphenylglycol, DL-metanephrine hydrochloride, DL-3,4-dihydroxymandelic acid, DL-3-methoxy-4-hydroxyphenylglycol, and ovalbumin (Sigma Chemical Co., St. Louis).

Analysis of data. Final results were expressed in pmoles/g wet weight in the tissue and pmoles/ml of medium/g wet weight released into the medium. Each metabolite fraction was analyzed separately by analysis of variance. A block design was employed to determine the effects of dose (Epi concentration in media) and condition (with/without ovalbumin) on control and sensitized tissue. Since each lung provided six samples (one in each cell), the block design allowed us to separate the variability between lungs from the error term. In no case was the variability between lungs significant. A regular factorial design was employed to test for differences between control and sensitized lung tissue, both with/without ovalbumin.

RESULTS

Tissues. Unchanged Epi constituted 80–90 per cent of the radioactivity in lung slices. Metanephrine (that is the *O*-methylated fraction) accounted for the remaining ^{3}H, with less than 0.5 per cent being deaminated products. As shown in Fig. 2, the amount of Epi in the tissue was directly proportional to the Epi concentration in the medium. Sensitization or anaphylactic challenge did not significantly alter these levels. In all cases, the Epi tissue concentration was approximately half of that in the medium.

Media. At the end of the incubation period, the concentration of Epi in the media was diminished by 10 per cent or less. The control, sensitized and anaphylactic tissues all produced the three types of metabolites, indicating that both catecholamine-catabolizing enzymes [monoamine oxidase (MAO) and catechol-*O*-methyl transferase (COMT)] are active in the lung (Table 1). The concentration of the methylated product was markedly higher than the sum of the other two types of catabolic products. At all concentrations of Epi used, 1 g of wet weight tissue catabolized, in 5 min, 20–30 per cent of the Epi presented to it. Table 1 demonstrates that the amount of each of the metabolites produced by control and sensitized tissue is directly proportional to the concentration of Epi used; ten or a thousand times higher Epi concentration in the medium produces a 10- or 1000-fold increase in OM, DA

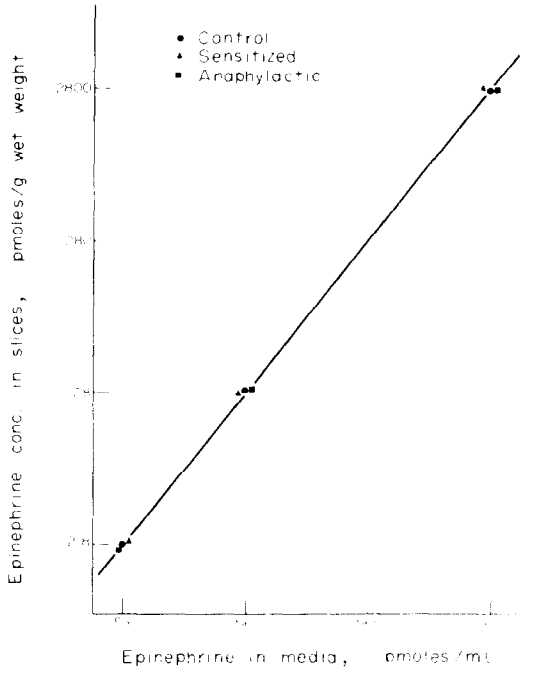

Fig. 2. Epinephrine (Epi) in control (●), sensitized (▲) and anaphylactic (■) guinea pig lung slices after incubation with 5.6×10^{-9}, -10^{-8} or -10^{-6} M Epi. Unchanged Epi in the tissues and media was analyzed as described in Materials and Methods. Since ovalbumin had no effect on control slices, results from these incubations are not presented.

Table 1. Epinephrine metabolites in media after incubation of control (C), sensitized (S) and anaphylactic (A) lung slices with epinephrine (Epi)*

	Epinephrine metabolites								
	Condition (5.6 pmoles Epi/ml)			Condition (56 pmoles Epi/ml)			Condition (5600 pmoles Epi/ml)		
Metabolite fractions	C	S	A	C	S	A	C	S	A
O-methylated	1.22 ± 0.27	0.56 ± 0.16	1.26 ± 0.38	7.46 ± 0.55	12.76 ± 4.08	14.52 ± 1.07	980 ± 51	1240 ± 471	1047 ± 532
Deaminated	0.31 ± 0.09	0.38 ± 0.05	0.39 ± 0.05	5.73 ± 0.70	4.36 ± 0.71	2.89 ± 0.18	480 ± 54	440 ± 39	276 ± 27
O-methylated + deaminated	0.15 ± 0.03	0.09 ± 0.03	0.06 ± 0.02	1.14 ± 0.40	1.32 ± 0.23	0.14 ± 0.09	125 ± 25	100 ± 31	1 ± 0.08

* Guinea pig lung slices were incubated for 5 min with different Epi concentrations. Anaphylaxis was induced by ovalbumin (final concentration 20 μg/ml) added to the media 4 min before Epi. The three metabolite fractions (*O*-methylated, deaminated, and *O*-methylated + deaminated) were separated and assayed as described in Materials and Methods. Values are expressed as mean ± S.E. pmoles metabolite/g of tissue/ml, produced in 5 min. N = six observations/condition.

and OMDA metabolites. Analysis of variance revealed a significant dose effect ($P < 0.01$) in all cases.

O-methylated metabolites (COMT activity). At all Epi concentrations there were no differences in the amount of OM produced in the medium by control compared to sensitized tissues. Ovalbumin added to the control tissue (not shown) or anaphylactic challenge (ovalbumin added to sensitized tissue) did not affect these levels (Table 1).

Deaminated metabolites (MAO activity). Control and sensitized lung tissue produced the same amounts of DA metabolites. Anaphylaxis, however, significantly decreased the DA metabolites (Table 1) in the media (dose × condition, $P < 0.01$). Ovalbumin had no effect on these levels in control lung slices.

O-methylated + deaminated metabolites (COMT + MAO activity). In contrast to the control and sensitized lung tissues which did not differ from each other with respect to OMDA production, antigen-antibody reaction markedly reduced the amount of OMDA metabolites formed (dose × condition, $P < 0.001$). At the highest Epi concentration used in the medium, ovalbumin did cause a reduction in OMDA produced from the control slices. However, the anaphylactic effect (sensitized plus ovalbumin) was significant over and above ($P < 0.05$) this unexplained result.

DISCUSSION

The results demonstrate that guinea pig lung slices are not only capable of catabolizing Epi but do so quite rapidly. Incubated in 20 ml of Tyrode's solution containing 112 nmoles Epi, 1 g each of the control and sensitized tissue can catabolize, in 5 min, 32 and 36 nmoles Epi respectively. These rates are minimal rates since even at this, the highest concentration of Epi used in the medium, neither of the Epi-catabolizing enzymes (COMT and MAO) is saturated and the rate, therefore, is being limited by the supply of substrate. This would suggest that in the intact lung, within this range of Epi concentrations, the mechanism (or rate) by which Epi is removed from the lung circulation may be the limiting step determining the Epi available to the lung tissue. Similarly, in the rat aorta, the concentration of NE metabolites produced was directly proportional to the concentration of NE (3×10^{-6} to 3×10^{-8} M) bathing the tissue [19].

Neuronal (U_1) and extra-neuronal (U_2) uptake processes for catecholamines have been described [20]. The experiments reported here were not designed to define the possible types of uptake in the lung. Nevertheless, although in mammals Epi has only half the affinity of NE for U_1 [20], U_1 should have been demonstrated at the low Epi concentrations used in our experiments. The fact that under these conditions the concentration of Epi in the tissue was always less than that in the medium would indicate that U_1 is of little quantitative importance in our system. This is in agreement with the known paucity of sympathetic innervation of the lung [21] and with the report that pretreatment with 6-hydroxy-dopamine has no effect on the uptake of NE into the rabbit lung [4]. U_2, on the other hand, has a higher affinity for Epi than NE [20] and plays a role in the inactivation of circulating Epi [20]. At these low Epi concentrations, U_2 would probably not be demonstrated, as Epi is not firmly retained and, unless the degradative enzymes are inhibited, is rapidly metabolized [20].

In rat aorta, 85 per cent of the tissue equilibrated with unchanged L-NE [19]. In our experiments, DL-Epi apparently equilibrated with only 50 per cent of the tissue and no differences were found between the control, sensitized and anaphylactic slices (Fig. 2). Experiments with [^{14}C]inulin established that 30–40 per cent of the tissue wet weight was extracellular and equilibration of these spaces with the medium will account for the relatively small amounts of metabolites and most of the Epi in the slices. This is in contrast to the whole lung perfused with Epi or NE where well over 50 per cent of the radioactivity accumulated in the tissue is in the form of metabolites [5, 8, 22]. The results indicate that in the perfused lung there may be a "barrier" to the passage of metabolites to, and of Epi from the vasculature. This barrier is absent in slices where there is apparent free diffusion of Epi into a large extracellular pool. Under such conditions, a small (relative to the extracellular pool), rapidly turning-over, intracellular Epi pool, which was changing in size in response to sensitization or anaphylaxis, would be difficult to measure. Also, the actual size of the intracellular pool may be obscured by stereo-specific transport and enzyme processes. For example, it has been shown that U_1 is stereo-chemically selective [20], and in rat lung MAO is stereo-specific for L-NE, while COMT is not [2, 5].

In our experiments, anaphylaxis did not alter the amount of OM produced. In contrast, the amount of DA produced in the medium from the anaphylactic compared to sensitized and control lung slices was very significantly reduced. The decrease was not apparent at the lowest Epi concentration, since at that concentration it has already been noted that MAO is very far from saturated, and it is conceivable that even an inhibited enzyme is capable of dealing with this low substrate level. As expected, if the DA metabolites are reduced during anaphylaxis, this reduction is also reflected in a decrease in the OMDA metabolites. The greater inhibition of OMDA than DA metabolites is consistent with an inhibited MAO; COMT would have less DA to act on and OM will be subject to an inhibited MAO, both resulting in less OMDA.

The changed catabolic pattern of Epi in anaphylaxis suggests that the antigen-antibody reaction either markedly inhibits MAO or, possibly, decreases the availability of Epi to this enzyme. However, in the latter case, since MN was not changed, two intracellular compartments for intra-cellular Epi, one of which is only available to MAO and the other to COMT, would have to be postulated.

Inhibition of MAO by induction of anaphylaxis could have a number of consequences for the lung. One effect might be to increase the Epi (and NE) available to the target tissues. Since Epi is known to inhibit the release of histamine [9, 10] and prostaglandins [11, 12], this could be an adaptive response. Another possibility is a direct effect on the sympathetic nervous system, e.g. DOPEG is known to turn off the synthesis of NE in sympathetic nerves [23]. A change in catecholamine or catecholamine metabolite level could then affect the sympathetic-parasympathetic prejunctional balance which appears to be operative in the lung [24, 25].

In summary, the data presented suggest that the catabolism of catecholamines is altered in anaphylaxis. Under our experimental conditions, these lung slices were incubated in a medium containing mediators of antigen-antibody reaction, before and during the addition of Epi. The possibility exists that one of these mediators may inhibit MAO or change the amount of Epi available to MAO. Future experiments will be designed to identify the nature of this inhibition and to explore the possible mechanisms whereby this inhibition may influence the lung.

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Mechanism of inhibition of pineal hydroxyindole-*O*-methyltransferase by pyridoxal 5′-phosphate

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Of possible significance relative to the nature of the active site of the enzyme hydroxyindole-*O*-methyltransferase (HIOMT) was the recent report [1] that pyridoxal 5′-phosphate (PLP) inhibited the HIOMT-catalyzed transmethylation of *N*-acetylserotonin to melatonin. Nir *et al.* [1] proposed that this inhibition by PLP could represent a mechanism by which HIOMT activity was regulated in the pineal gland. In addition, Nir *et al.* [1] suggested that PLP inhibited HIOMT through a mechanism involving PLP competing for the *S*-adenosylmethionine (L-SAM) enzymatic binding site.

Interestingly, other examples of PLP inhibition of L-SAM-dependent methyltransferases or vice versa have been reported. For example, Black [2] reported PLP inhibition of the *O*-methylation of norepinephrine by catechol-*O*-methyltransferase. The mechanism of this inhibition was later clarified by our laboratory [3], when it was shown that PLP and norepinephrine underwent a facile chemical reaction resulting in the formation of a tetrahydroisoquinoline adduct. The removal of the norepinephrine by reaction with PLP and the generation of the tetrahydroisoquinoline, which was shown to be a reversible inhibitor of COMT, accounted for the majority of the inhibitory effects observed with PLP. Similar mechanisms have been reported for the inhibition of PLP-dependent enzymes by catecholamines [4–7].

SAM has also been reported by Trewyn *et al.* [8] to be an inhibitor of tyrosine aminotransferase, which is a PLP-dependent enzyme. The mechanism of this inhibition was not completely characterized, but data were presented which suggested a chemical reaction was occurring between SAM and PLP, perhaps involving Schiff base formation, which resulted in the observed inhibition.

In order to determine if PLP inhibited HIOMT by direct interaction with the enzyme (e.g. Schiff base formation with an ϵ-amino group of a lysine residue at the enzyme active site) or by nonenzymatic reaction with either of the substrates (e.g. L-SAM or *N*-acetylserotonin), a study of the mechanism of PLP inhibition of HIOMT was carried out. The present paper describes the results of this study and a mechanism is proposed to account for the PLP inhibition of the HIOMT-catalyzed transmethylation *in vitro*.

MATERIALS AND METHODS

HIOMT was isolated from bovine pineal glands (Pel-Freez Biologicals) according to the procedure of Jackson and Lovenberg [9] and purified through the DEAE-Sephadex chromatography step, which resulted in a 10-fold purification of the enzyme. For the HIOMT assay, [^{14}C]H$_3$-L-SAM (New England Nuclear, 55.0 mCi/mmole) was stored at −20°F. L-SAM iodide and *N*-acetylserotonin (Sigma) were stored as 0.01 M aqueous stock solutions. Enzyme activity was measured by a previously described radiochemical assay [10]. A normal enzyme assay mixture consisted of the following components (in μmoles): water, so that the final volume was 0.25 ml; *N*-acetylserotonin (0.25); SAM (variable); PLP (variable); 0.55 μCi [^{14}C]H$_3$-L-SAM; phosphate buffer, pH 7.9 (10);