CCA-387

547.233:542.98

Original Scientific Paper

A New Aspect of the *in Vitro* Metabolism of Some Biogenic Amines

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Received July 2, 1965

Tryptamine, 5-methoxytryptamine, tyramine, histamine, octopamine, dopamine, and noradrenaline were incubated with the »nuclear fraction« of rat liver homogenate. The first four amines were also incubated with rat liver slices. The incubation mixtures were examined by paper chromatography. All substrates were completely metabolised by both tissue preparations except histamine which was left unchanged. After the incubation with the nuclear fraction the major metabolites were always the corresponding arylaldehyde and aryl carboxylic acid having one C-atom less in the side chain than the substrate. Liver slices metabolised the amines to the corresponding aryl acetic acids. These two metabolic pathways, which are both initiated by monoamine oxidase, are discussed.

The metabolic oxidative deamination of the aryl ethylamines has been well established[1–3]. This sequence of events is initiated by monoamine oxidase (MAO); the formed aryl acetaldehyde is then further oxidised by aldehyde oxidase (AO) to the aryl acetic acid.

In a previous paper[4] we have shown that a preparation from rat liver — liver »nuclear fraction« — metabolizes 5-hydroxytryptamine in a different way. In this instance, although the metabolic events are initiated by MAO as in the whole animal[5] or in the liver slices[6], the end product is not 5-hydroxyindole-3-acetic acid but the aldehyde and the acid respectively having one C-atom less in the side chain. We have also shown that these compounds are not formed from 5-hydroxyindole-3-acetic acid but derive, through an unknown reaction, from 5-hydroxyindole-3-acetaldehyde. Since these findings represent a new metabolic pathway of 5-hydroxytryptamine it seemed to us worthwhile to find out whether other biogenic amines undergo the same fate when incubated with the rat liver nuclear fraction.

EXPERIMENTAL

Compounds

5-Methoxytryptamine was prepared according to Kveder and McIsaac[7]; (±)octopamine was a gift from Dr. H. Blaschko; all other compounds were from usual commercial sources or prepared in this laboratory according to appropriate methods.

Tissue preparations and incubation

Rat liver »nuclear fraction« (2 g. of original tissue per experiment) was essentially prepared as described previously[4]. Liver slices (thickness 0,4 mm; 1 g. per experiment) were cut by means of a Stadie-Riggs microtome[8].

For the preparation of tissue as well as for the incubations the Krebs-Ringer bicarbonate buffer[9] was used. The substrates (0.025 mmole) were dissolved in 1 ml. of

isotonic saline and transferred into the incubation flasks. After the addition of the tissue preparations the volumes of the incubation mixtures were made up to 10 ml. with the buffer. The incubations were carried out for 2 hrs., at 37—38°, in an atmosphere of 95% 0_2 and 5% CO_2 with shaking. At the end of the incubation period the nuclear fraction was centrifuged and treated with the deactivated charcoal after the procedure of Dalgliesh as described previously[4]; the liver slices were decanted through glass wool and the filtrate worked up in the same way. The residues after the removal of phenol and evaporation was taken in 0.5 ml. of 30% aqueous ethanol and subjected to the paper chromatography.

Paper chromatography

Descending one-dimensional chromatography on Whatman no. 1. paper was used with *iso*propanol: ammonia: water (10:1:1) (Solvent A), and *n*-butanol:acetic acid:water (65:15:25) (Solvent B) as standard solvents. Dry chromatograms were examined under the ultraviolet light (254 m μ). They were sprayed: a) for indoles with Ehrlich's reagent (2% *p*-dimethylaminobenzaldehyde in 2 *N* HCl) and xanthydrol (0.2% in 95% ethanol; before spraying 10 ml. of conc. HCl were added to 90 ml. of this solution), b) for phenols with Brentamine reagent (0.1% tetrazotised *o*-dianisidine in 50% methanol, followed by the exposure to ammonia vapours) and Pauly's reagent (0.9% sulphanilic acid in 1.5 *N* HCl diazotised with the same volume of 5% $NaNO_2$; after the dipping and the drainage the paper was dipped in 10% Na_2CO_3), c) for amines with ninhydrine (0.2% in ethanol), d) for aldehydes with 2,4-dinitrophenylhydrazine (saturated solution in 2N HCl), and e) for organic acids with methyl red (0.1% in 96% ethanol).

RESULTS AND DISCUSSION

Tryptamine, 5-methoxytryptamine, tyramin, and histamine were incubated both with liver »nuclear fraction« and with liver slices. Octopamine, dopamine and noradrenaline were incubated only with the nuclear fraction, because the corresponding aryl acetic acids that would result from the action of monoamine oxidase (MAO) followed by aldehyde oxidase (AO) were unavailable as chromatographic markers.

All investigated biogenic amines were completely metabolised under the experimental conditions except histamine, which was left mainly unchanged by the liver slices as well as by the nuclear fraction. This was to be expected since histamine is not attacked by MAO, but by histaminase (diamine oxidase).

In Table I are summarised the paper chromatographic characteristics of the used biogenic amines and their metabolites.

The amines that were subjected to the action of both tissue preparations (slices and nuclear fraction) clearly showed the existence of two metabolic pathways. Thus, when either tryptamine, 5-methoxytryptamine or tyramine was incubated with the liver slices, always one major metabolite — the corresponding aryl acetic acid — was found in the incubation mixture. On the contrary, when the above amines were incubated with the nuclear fraction the major metabolites were the corresponding aryl aldehydes and carboxylic acids, having one C-atom less in the side chain than the substrate. Although the substrates were completely digested only traces of corresponding acetic acids were formed and these only in some instances.

In the previous paper[4] we have shown that 5-hydroxyindole-3-aldehyde, one of the major metabolites formed during the incubation of 5-hydroxytryptamine with the nuclear fraction, does not derive from 5-hydroxyindole-3-acetic acid but from 5-hydroxyindole-3-acetaldehyde. There is a good reason to believe that the same pathway is followed in the biogenesis of indole-3-aldehyde, 5-methoxyindole-3-aldehyde and *p*-hydroxybenzaldehyde from tryptamine, 5-methoxytryptamine and tyramine, respectively.

TABLE I

Paper Chromatographic Characteristics of Aryl Ethylamines and Their Metabolites

$$Ar—\underset{\displaystyle R}{\underset{|}{CH}}—CH_2—NH_2$$

Compound	$R_f \times 100$		Colour reactions					
	Solv. A.*	Solv. B.**	Ehrl.[1]	Xanth.[2]	Nin.[3]	DNPH[4]	MR[5]	UV[6]
Ar = indole-3-								
R = H								
— ethylamine (tryptamine)	75	67	++	++	++			
— aldehyde	87	90	+	+		++		Q
— carboxylic acid	54	92	++	++				
— acetic acid	55	93	++	++				
Ar = 5-methoxyindole-3-								
R = H								
— ethylamine (5-methoxytryptamine)	66	65	++	++	++			
— aldehyde	84	89	—	+		++		Q
— carboxylic acid	38	88	++	++				
— acetic acid	43	89	++	++				
Ar = *p*-hydroxyphenyl-			Brent.[7]	Sulph.[8]				
R = H								
— ethylamine (tyramine)	70	59	++	++	++			
— aldehyde	63	90	(+)	(+)		++		Q
— carboxylic acid	30	90	(+)	++			+	
— acetic acid	35	88	++	++			+	
R = OH								
— ethylamine (octopamine)	50	45		+	++			
Ar = 3,4-dihydroxyphenyl-								
R = H								
— ethylamine (dopamine)	22—42	45	+	+	++			
— aldehyde	50—58	84	(+)			++		Q
— carboxylic acid	11—14	78	++				+	
R = OH								
— ethylamine (noradrenaline)	29—38	36		++	++			
Ar = Imidazole-4(5)-								
R = H								
— ethylamine (histamine)	44	13		++	++			
— aldehyde	63	60		(+)		+		Q
— carboxylic acid	27	25		+			+	

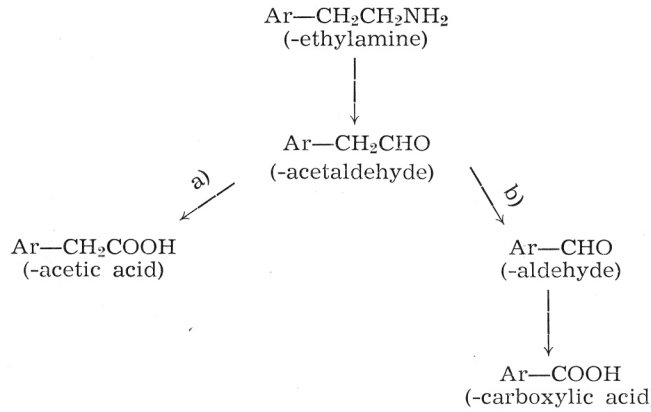
* Solv. A. = *iso*propanol-ammonia-water (10 : 1 : 1);
** Solv. B. = *n*-butanol-acetic acid-water (4 : 1 : 5); [1] Ehrlich's reagent. [2] Xanthydrole. [3] Ninhydrine. [4] 2,4-Dinitrophenylhydrazine. [5] Methyl red. [6] Ultraviolet light. [7] Brentamine. [8] Diazotised sulphanilic acid.
++ = strong, + = weak, (+) = very weak, — = negative, Q = UV quenching.
Remark: In the Solvent A catechol derivatives tend to tailing with decomposition.

The two metabolic pathways of these aryl ethylamines are presented in Scheme I.

When octopamine, dopamine and noradrenaline were incubated with the nuclear fraction, again the degradation of the side chain took place. Thus,

SCHEME I

Metabolism of Aryl Ethylamines



Ar = Indole-3-, 5-hydroxyindole-3-, 5-methoxyindole-3-, *p*-hydroxyphenyl

a) pathway *in vivo* and by liver slices
b) pathway by liver »nuclear fraction«

p-hydroxybenzaldehyde after octopamine, and 3,4-dihydroxybenzaldehyde after both catechol amines were detected in the incubation mixture.

Investigating the metabolism of noradrenaline, Dirscherl and his collaborators[10,11] have found that rat and human liver slices and homogenates are able to convert 3-methoxy-4-hydroxymandelic acid to 3-methoxy-4-hydroxybenzaldehyde (vanillin). At first sight this finding seems to disagree with the above concept of the two metabolic pathways. However, if the hydroxylation of the β-C atom of the side chain is taken as a necessary precedent to its splitting, all the results can be explained. In favor of this is the fact that aryl ethylamines, bearing a hydroxyl group on the β-C atom of the side chain (octopamine, noradrenaline) when incubated with the nuclear fraction give the same metabolites as aryl ethylamines without the supstituent in the side chain (tyramine, dopamine). The most probable substrates for the hydroxylation of the side chain seem to us to be the intermediate aryl acetaldehyde because of the possibility of reaction in the enolic form. The aryl acetic acids are poor substrates[4] for such a hydroxylation, but once the side chain is hydroxylated its splitting seems to proceed easily. The enzymic formation of *p*-hydroxybenzaldehyde from tyramine was observed as early as in 1928[12] and this aldehyde is a normal constituent of mammalian urine. The hydroxylation of tyramine to octopamine *in vivo* is a well known fact.

It is interesting to note that also after the incubation of adrenaline with the nuclear fraction 3,4-dihydroxybenzaldehyde was detected in the incubation mixture, although in smaller amounts than after the incubation of noradrenaline. This shows that the nuclear preparation is able to N-demethylate adrenaline to some extent.

Summerizing the results of the experiments presented in this paper we can say that the alternate metabolic pathway of aryl ethylamines, leading to the loss of one C atom from the side chain, is not true for 5-hydroxytryptamine only but it presents a general feature of biogenic amines which undergo the

attack by MAO. Under what conditions and to which extent a particular aryl ethylamine would follow this additional metabolic pathway is still to be found out.

Acknowledgment. We are indebted to Dr. H. Blaschko, Pharmacol. Dept. Univ. of Oxford, for the generous gift of octopamine. Thanks are due to L. Stančić, B. Sc., for the preparation of indolyl and imidazolyl aldehydes and carboxylic acids, and to Dr. D. Keglević for the interest in this work and helpful discussions.

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IZVOD

Novi aspekti metabolizma nekih biogenih amina *in vitro*

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Triptamin, 5-metoksitriptamin, tiramin, histamin, oktopamin, dopamin i noradrenalin inkubirani su sa »nuklearnom frakcijom« homogenata jetre štakora. Prva četiri amina inkubirana su još i sa rezovima jetre štakora. Nakon inkubacije, čišćenja preko deaktiviranog ugljena i koncentracije, inkubacioni mediji ispitani su papirnom kromatografijom. Svi supstrati bili su potpuno metabolizirani osim histamina koji je ostao nepromijenjen. Nakon inkubacije sa nuklearnom frakcijom glavni metaboliti biogenih amina bili su odgovarajući aril aldehidi i karbonske kiseline s jednim C atomom manje u postranom lancu nego supstrat. Rezovi jetre metabolizirali su amine u odgovarajuće aril octene kiseline. Diskutirana su ova dva metabolička puta, a oba su započeta od monoamino oksidaze.

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Primljeno 2 srpnja 1965.