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ENZYMIC OXIDATION OF TRYPTAMINE DERIVATIVES

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It has been known for some time that tryptamine is a substrate of amine oxidase (Blaschko, Richter & Schlossmann, 1937; Pugh & Quastel, 1937). Oxidation by amine oxidase accounts for the metabolic fate of tryptamine in the intact organism and in perfused organs, where β-indoleacetic acid was shown to be produced (Ewins & Laidlaw, 1913; Guggenheim & Loeffler, 1916). These observations have since acquired a new significance, as it is now known that amines closely related to tryptamine are found in animals; these are 5-hydroxytryptamine, which has also been described under the names 'serotonin' and 'enteramine', and its *N*-dimethyl derivative, 'bufotenine', which occurs in the skin secretions of some amphibia, sometimes in association with the primary amine.

That 5-hydroxytryptamine is a substrate of amine oxidase was made likely by observations on the biological inactivation of 'serotonin' and 'enteramine' which have already been fully discussed elsewhere (Blaschko, 1952*a*). Experiments have since been reported which show that the amine is rapidly oxidized by preparations of amine oxidase of mammalian and cephalopod tissues (Freyburger, Graham, Rapport, Seay, Govier, Swoap & Vander Brook, 1952; Blaschko, 1952*b*). Similarly, studies on the inactivation of bufotenine by mammalian tissues suggest that this too is destroyed by amine oxidase (Erspamer, 1946), but experiments on the enzymic oxidation of this compound have not been reported.

In this paper we describe some observations on the enzymic oxidation of tryptamine itself and its two 5-hydroxy derivatives. We have used the manometric method, in order to obtain data which make it possible to compare the rates of oxidation of the different compounds.

MATERIAL AND METHODS

In most experiments, samples of 5-hydroxytryptamine creatinine sulphate from the Abbott Laboratories, Chicago, were used; a few experiments were carried out with the picrate. Two samples of bufotenine were used; one was the picrate, prepared by Wieland, Konz & Mittasch (1934); the other was the base, prepared by Harley-Mason & Jackson (1952).

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The tissues were prepared with a glass homogenizer; the medium was 0·067 M-sodium phosphate buffer of pH 7·4. The mammalian tissues were from freshly killed animals, but some of the cephalopod tissues, which had been collected at Plymouth, had been stored at – 10° C for some time.

EXPERIMENTS

Oxidation of 5-hydroxytryptamine by mammalian tissues

We have compared the rates of oxidation of tyramine, tryptamine and 5-hydroxytryptamine in homogenates of liver and kidney from the guinea-pig, the cat and the pig.

In most of these experiments the homogenates contained 200 mg of fresh tissue per ml.; this homogenate was dialysed for about 16 hr against tap water, and to each ml. of the dialysed preparation 0·2 ml. of an 0·2 M-sodium phosphate buffer of pH 7·4 was added. In the manometric experiments each flask contained 1·0 ml. of the tissue preparation, 0·6 ml. of the 0·067 M-sodium phosphate buffer and, in the side arm, either 0·4 ml. of water or 0·4 ml. of 0·05 M-amine. The potash tube contained 0·2 ml. N-KOH and a filter-paper. The experiments were carried out at 37·5° C; the gas phase was oxygen.

The results of these experiments are summarized in Table 1. In this table, the oxygen consumption with tryptamine and 5-hydroxytryptamine is expressed as a percentage of the oxygen consumption with tyramine.

TABLE 1. Relative rates of oxidation of tyramine, tryptamine and 5-hydroxytryptamine by homogenates of mammalian tissues. Oxygen uptake in μl. with tyramine as substrate in the first 30 min is given in brackets.

	Substrate			
Tissue	Tyramine	Tryptamine	5-Hydroxy-tryptamine	Additions
Guinea-pig liver	100 (136)	60	67	KCN
	100 (275)	84	67	—
	100 (147)	51	55	KCN
Guinea-pig kidney	100 (132)	93	80	—
	100 (154)	98	80	—
Cat liver	100 (138)	11	8	KCN
	100 (195)	16	5	KCN
	100 (217)	23	16	—
Cat kidney	100 (167)	24	9	—
	100 (167)	15	11	—
Pig liver	100 (236)	106	36	—
	100 (246)	107	26	—
Pig kidney	100 (98)	92	20	KCN
	100 (181)	86	36	KCN
	100 (215)	99	33	—

The table also includes a few experiments in which 0·01 M-cyanide was present; in these experiments the potash tube contained a KCN-KOH mixture.

It can be seen that preparations from all the tissues examined oxidized the three amines, but the relative rates at which they were oxidized were different.

Tyramine was always rapidly oxidized; in the guinea-pig and the cat the rate of oxidation of tyramine was higher in the liver than the kidney, but in the pig there was little difference in the rate of oxidation of tyramine in the two tissues. The rate of oxidation of tryptamine was also high in the guinea-pig and the pig, but in the preparation from cat liver and kidney it was low. In the guinea-pig, the rate of oxidation of 5-hydroxytryptamine was very similar to that of tryptamine, but in the pig 5-hydroxytryptamine was oxidized more slowly than tryptamine, and in the cat the rate of oxidation of 5-hydroxytryptamine was low.

Experiments with bufotenine

Very little material was available, and smaller manometer flasks were therefore used. The main compartment contained 0·56 ml. of homogenate, the side arm 0·14 ml. of either water or 0·05 M-substrate, and the potash tube 0·1 ml. N-KOH. In a second experiment, 0·01 M-cyanide was also added.

In each experiment bufotenine was oxidized, but the rate of oxygen uptake was much slower than with 5-hydroxytryptamine. In an experiment with cat liver, 31 μl. were taken up in 30 min with 5-hydroxytryptamine as substrate and 4 μl. with bufotenine; with guinea-pig liver the corresponding figures were 74 μl. of O_2 with 5-hydroxytryptamine and 3 μl. with bufotenine.

Oxidation of 5-hydroxytryptamine by cephalopod tissues

The experiments with tissue preparations from *Octopus vulgaris* were carried out in May 1952, on tissue that had been collected in the preceding autumn and kept at $-10°$ C. It is possible, therefore, that the enzymic activity was a little lower than it would have been in fresh tissue. Table 2 summarizes our

TABLE 2. Oxidation of amines in cephalopod tissues. Oxygen uptake in μl. O_2 during the first 15 min of incubation.

Source of enzyme	Fresh weight of tissue in each flask (mg)	Substrate concn. (M)	Substrate		
			Tyramine	Tryptamine	5-Hydroxy-tryptamine
Loligo forbesii optic lobe	233	0·01	58	34	26
Eusepia officinalis liver	150	0·01	32	50	7
Octopus vulgaris posterior salivary glands					
Expt. 1	333	0·005	41	27	30
Expt. 2	333	0·005	—	50	27

results with cephalopod preparations. In these experiments homogenates of tissue were prepared in 0·067 M-sodium phosphate buffer; these were dialysed against the phosphate buffer overnight. The final preparation, suitably diluted with phosphate buffer, was incubated with substrate at 37·5° C in oxygen.

It has already been reported elsewhere that the optic lobe of *Loligo forbesii*

contains amine oxidase (Blaschko & Himms, 1953). This preparation oxidized all three amines. The table also gives the result with a dialysed preparation of *Eusepia* liver; this organ had been kept frozen for 8 months at $-10°$ C.

A preparation of *Octopus* liver oxidized tyramine and tryptamine at approximately equal rates. It also oxidized 5-hydroxytryptamine, but this amine was used as the picrate and it was found that added picrate markedly slowed down the enzymic oxidation of tyramine and tryptamine by *Octopus* liver. We have therefore not included the experiments with *Octopus* liver in Table 2. We have, however, included two experiments with *Octopus* posterior salivary gland, as picrate did not affect the rate of oxidation of tryptamine by this preparation. In Expt. 1 of Table 2 we used 5-hydroxytryptamine picrate, and tyramine and tryptamine as hydrochlorides but with an equivalent amount of sodium picrate added. In Expt. 2 of Table 2 5-hydroxytryptamine creatinine sulphate was used. The *Octopus* tissues had been kept at $-10°$ C for about two years when these experiments were carried out.

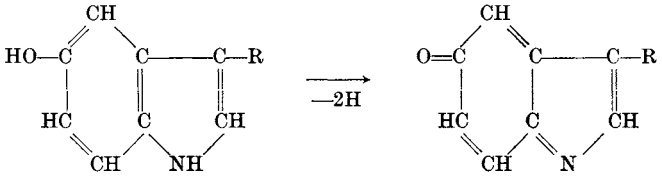
DISCUSSION

The observations reported show that 5-hydroxytryptamine is oxidized by all the tissue preparations which act on tyramine and tryptamine. This supports the conclusion that amine oxidase is the catalyst of the reaction. The main outcome of this study is the finding that the relative rate of oxidation of the amines is not the same in all species; in the cat, both tryptamine and its 5-hydroxy derivative were slowly oxidized. In the pig, tryptamine was oxidized as rapidly as tyramine, but 5-hydroxytryptamine was oxidized at a much lower rate. Similarly, in the one experiment with *Eusepia* liver, the rate of oxidation with 5-hydroxytryptamine was lower than with the two other amines tested.

These observations are of interest in connexion with the question of the physiological significance of amine oxidase. The recognition of 5-hydroxytryptamine as a widely distributed constituent of vertebrate and molluscan tissues suggests that the biological inactivation of this amine is one of the functions of amine oxidase. It is of interest, therefore, that in the cat 5-hydroxytryptamine is rather slowly oxidized by amine oxidase. This is not explained by a lack of sensitivity of the cat tissues to the amine; on the contrary, one might consider the possibility that the very high sensitivity of the cat's bronchial muscles to 5-hydroxytryptamine is connected with the slow removal of the amine in this species.

Amine oxidase is an enzyme that has never been isolated pure, and we can therefore at present not decide whether the differences in the relative rates of oxidation are explained by species differences in the pattern of substrate specificity or by the presence, in variable amounts, of closely related distinct enzymes.

At present, the physiological significance of amine oxidase is still under discussion, but the available evidence can best be understood if we assume that it is a catalyst which is of importance in the metabolism of 5-hydroxytryptamine as well as of the sympathomimetic amines. This does not exclude other metabolic pathways for both types of amine. A conjugation of the phenolic hydroxyl group in 5-hydroxytryptamine may occur. An oxidative reaction of the type



is also possible.

These considerations are of particular interest in connexion with our findings on the enzymic oxidation of bufotenine. *N*-Dimethylamino compounds are known to be oxidized more slowly than the corresponding primary amines, and it is therefore not surprising to find a much reduced rate of oxidation of this compound. In Erspamer's (1946) experiments some evidence pointed in favour of amine oxidase as the catalyst of the inactivation of bufotenine, but the possibility remains that oxidation by amine oxidase is not the only enzymic reaction involved.

SUMMARY

1. The rates of oxidation of tyramine, tryptamine and 5-hydroxytryptamine in homogenates of liver and kidney of the guinea-pig, cat and pig have been measured.

2. 5-Hydroxytryptamine was oxidized by all preparations. The rate of its oxidation was high in the guinea-pig; it was lower in the pig, and in the cat the rate was very low.

3. 5-Hydroxytryptamine was also oxidized by homogenates from *Octopus vulgaris*, *Eusepia officinalis* and *Loligo forbesii*.

4. Bufotenine was very slowly oxidized by cat and guinea-pig liver.

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