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Lack of metaraminol biotransformation by rabbit tissues *in vitro*

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METARAMINOL (1-*m*-hydroxy norephedrine) has been shown to possess significant clinical utility in the treatment of acute hypotension of diverse etiolgy.[1, 2] In addition to its well-established usefulness as a therapeutic agent, metaraminol has recently received widespread attention as an experimental tool in autonomic pharmacology as a result of its ability to deplete tissue catecholamine stores.[3, 4] Although it has been demonstrated that small amounts of unchanged metaraminol remain bound in tissues for one to two weeks after injection[4, 5] no reports have appeared on the biotransformation of the drug *in vivo* or *in vitro*. The present comunication describes studies of the metabolism of metaraminol *in vitro*.

Tissues were removed from adult male New Zealand rabbits and homogenized at 0°–5° in a Potter-Elvehjem-type homogenizer. Hepatic mitochondria, as a source of monoamine oxidase (MAO), were prepared from liver homogenates by the procedure of Schneider.[6] Amphetamine deaminase, an enzyme system known to oxidatively deaminate several sympathomimetic amines possessing α-methyl groups, was prepared from liver homogenates according to Axelrod.[7] Hepatic microsomal glucuronyl transferase was prepared by the method of Hsia *et al.*[8] All incubations were carried out in a Dubnoff metabolic shaking apparatus at 37° under air.

In MAO experiments, the incubation mixture was that described by Zile and Lardy.[9] Table 1 presents data showing that, although tyramine was metabolized at a brisk rate by MAO, no disappearance of metaraminol was observed. To examine the possibility that MAO action on metaraminol might produce a metabolite with extraction characteristics and extinction properties similar to those of metaraminol, paper chromatography was utilized. Extracts of incubation mixtures were spotted on strips of Whatman 1 paper and developed by the ascending technic for 19 hr in three solvent systems: (1) n-butanol: acetic acid:water (12:3:5); (2) isopropanol:ammonia:water (8:1:1); (3) isobutanol saturated with 0·1 N HCl. The chromatograms were air dried, and spots were visualized by spraying with Gibbs reagent followed by borate buffer (pH 9·2). In all three systems single spots were observed having the same R_f values as authentic metaraminol, i.e. 0·66, 0·81, and 0·42 respectively.

Axelrod[7] showed amphetamine deaminase to be capable of deaminating several phenyl isopropyl amine derivatives. Since metaraminol is a doubly hydroxylated phenyl isopropyl amine, it was reasoned that it might serve as a substrate for the enzyme. Table 2 presents data demonstrating that no metabolism of metaraminol occurred in the presence of amphetamine deaminase. Experiments in which the amounts of enzyme and cofactors present in the incubation medium were increased by 3 to 4-fold demonstrated that, although amphetamine metabolism was enhanced by about 4 to 5-fold no disappearance of metaraminol could be detected.

TABLE 1. METABOLISM OF TYRAMINE AND OF METARAMINOL BY RABBIT LIVER MONOAMINE OXIDASE

Incubation time (min)	Tyramine (μmoles metabolized)	Metaraminol
5	0·90	0
10	1·35	0
20	2·10	0
30	3·30	0
45	4·10	0
60		0

Mitochondria equivalent to 500 mg liver were incubated with 150 μmoles potassium phosphate buffer (pH 7·5), 20 μmoles neutralized KCN, 6 μmoles substrate (tyramine or metaraminol), and H_2O to make 3 ml. After various periods of incubation, aliquots were removed from incubation mixtures and assayed for residual tyramine[14] or metaraminol.[15]

TABLE 2. METABOLISM OF AMPHETAMINE AND OF METARAMINOL BY A RABBIT LIVER SUPERNATANT FRACTION

Incubation time (min)	Amphetamine (μmoles metabolized)	Metaraminol
15	0·07	0·00
30	0·15	0·00
60	0·18	0·00

Supernatant fraction (9,000 *g*) equivalent to 135 mg wet weight liver was incubated for various times with 5 μmoles nicotinamide, 0·1 μmole NADP, 5 μmoles $MgCl_2$, 0·6 μmole substrate 20 μmoles glucose-6-phosphate, 0·5 ml phosphate buffer (0·2 M, pH 7·4), and distilled water to make 4 ml. Incubation was at 37° under air. At the conclusion of the incubation period, aliquots of the reaction mixtures were removed and assayed for remaining substrate.[15, 16]

In studying the enzymatic deamination of amphetamine Axelrod[7] characterized the reaction products as ammonia and phenyl acetone. If metaraminol were to undergo metabolism by this system, the analogous reaction products would be ammonia and 1-hydroxyl-1 (*m*-hydroxy phenyl) acetone. Control experiments showed that the latter compound did not interfere with the metaraminol assay used in this study. Hence, our failure to demonstrate metaraminol metabolism by this system cannot be related to the generation of such a metabolite.

It is well established that many organic compounds possessing phenolic or alcoholic hydroxyl groups are conjugated with glucuronic acid both *in vivo* and *in vitro*. It has been shown that a hepatic microsomal enzyme, glucuronyl transferase, catalyzes glucuronide formation with the nucleotide uridine diphosphate glucuronic acid (UDPGA) functioning as glucuronide donor.[10] Because it possesses a phenolic hydroxyl, metaraminol was considered a likely candidate for glucuronide formation. Table 3 presents data demonstrating that no measurable metaraminol conjugation occurred in a system which readily formed *o*-amino phenol glucuronide.

TABLE 3. ASSAY OF RABBIT LIVER GLUCURONYL TRANSFERASE ACTIVITY EMPLOYING *o*-AMINO PHENOL AND METARAMINOL AS SUBSTRATES

Incubation time (min)	*o*-Amino phenol $E_{560m\mu}$*	Metaraminol $E_{660m\mu}$†
0	0·00	0·645
15	0·093	0·645
30	0·170	0·645

* Change in E represents product (*o*-amino phenol glucuronide) formation.

† Change in E represents substrate disappearance. Microsomes equivalent to 100 mg wet weight liver were incubated with 1 μmole recrystallized *o*-amino phenol (or 0·5 μmole metaraminol), 2·0 μmoles ascorbic acid, 1·0 μmole UDPGA, 25 μmoles Tris buffer (pH 7·65) containing 0·004 M $MgCl_2$ and 0·008 M nicotinamide in a total volume of 3 ml. After various periods of incubation, aliquots were withdrawn and assayed for *o*-amino phenol glucuronide[17] or residual metaraminol.[15]

The chromogenic reagent (Gibbs) employed in the metaraminol assays in this study is known to react with compounds bearing free phenolic hydroxyl groups, resulting in the production of colored addition products.[11] We reasoned on this basis that a phenolic glucuronide formed from metaraminol would not couple with Gibbs reagent and would thus be manifest as a disappearance of substrate during the incubation. Experimental corroboration for this reasoning comes from the work of Porteous, Garton, and Williams[12, 13] who found that phenol glucuronide, in contrast to free phenol, does not form a colored addition product with Gibbs reagent.

To study the possibility of metaraminol metabolism in whole homogenates of rabbit organs, liver, kidney, and lung were homogenized in 0·1 M phosphate buffer (pH 7·0) and incubated with metaraminol for 60 min. The incubation media were fortified with nicotinamide (5 μmoles), NADP (0·1 μmole), $MgCl_2$ (5 μmoles), and glucose-6-phosphate (20 μmoles). In none of the systems could disappearance of metaraminol be demonstrated. Paper chromatographic analysis of extracts of the incubation media in three solvent systems (see above) revealed single spots with R_f values similar to that of authentic metaraminol.

The present study indicates that metaraminol is resistant to biotransformation by hepatic enzymes known to metabolize structurally related sompounds. Similarly, fortified whole homogenates of rabbit kidney, lung, and liver were found incapable of metabolizing the drug. These observations suggest that enzymatic inactivation does not account for termination of the biologic effects of metaraminol in the intact animal and lend indirect support to the view that tissue metaraminol uptake contributes appreciably to terminating its biologic effects.[5]

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Inhibition of endogenous catecholamine biosynthesis by 3-iodo-L-tyrosine

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THE enzyme, tyrosine hydroxylase, which catalyzes the initial step in the formation of norepinephrine from the dietary precursor tyrosine, has been recently isolated and partially purified from the adrenal medulla.[1] Several aromatic L-amino acids were found to be effective inhibitors of tyrosine hydroxylase *in vitro*.[1] The inhibition *in vivo* of tyrosine hydroxylase by α-methyltyrosine has been described.[2] It was recently shown that 3-iodo-L-tyrosine is a potent inhibitor of tyrosine hydroxylase *in vitro*,[3,4] and that the inhibition is of a competitive nature. The present study shows that 3-iodo-L-tyrosine does lower endogenous levels of catecholamines in various organs. The decrease in the catecholamine levels is primarily due to the inhibition of catecholamine synthesis at the tyrosine hydroxylase stage.

Rats were treated at various time intervals with 3-iodo-L-tyrosine; solutions for injections were prepared by dissolving the substance with 0·1 N HCl. The pH was then readjusted to 4·5 to 5·5 by addition of 0·1 N NaOH immediately before i.p. injection. The animals were killed at various time intervals and the tissues removed rapidly and immediately analyzed or stored in the freezer. The hearts, brains, and salivary glands were homogenized in cold 0·4 N perchloric acid. After centrifugation, the supernatant fluid was adjusted to pH 5 with K_2CO_3, and the precipitated potassium perchlorate was removed by filtration. The catecholamines were adsorbed on alumina and then eluted with 0·2 N acetic acid. Norepinephrine and dopamine were determined by fluorometric method.[5, 6] The adrenal glands were homogenized in 3% of trichloroacetic acid and the excess of the trichloroacetic acid removed by extraction with ether. The aqueous extract was adjusted to pH 5·5, and the total catecholamines were determined fluormetrically.[5]

The data in Table 1 show that 4 hr after the administration of 200 mg 3-iodo-L-tyrosine/kg the levels of catecholamines were decreased in all analyzed organs. Brain catecholamines were reduced to a greater extent than the catecholamines in the heart or salivary glands. The total catecholamines in the adrenal glands were only slightly reduced. After repeated administration of 3-iodo-L-tyrosine, the levels of the catecholamines in the brain were again reduced to a greater extent than in the other analyzed organs, but the difference was less apparent than after single-dose administration. It is conceivable that the pronounced reduction of catecholamines in the brain after a single administration of 3-iodo-L-tyrosine is due to the rapid turnover of the catecholamines in the central nervous system. The minimal changes in the adrenal catecholamine levels may also be due to the slow turnover of the catecholamines in these organs. The concentration of the inhibitor in different tissues may also affect the degree of the inhibition.

In separate experiments we investigated whether 3-iodo-L-tyrosine induces release of norepinephrine-^{3}H or prevents its uptake by the heart. It is evident from the data presented in Table 2 that a single dose of 200 mg 3-iodo-L-tyrosine/kg produces no significant release of norepinephrine-^{3}H from the heart. Table 2 also shows that repeated administration of 3-iodo-L-tyrosine slightly prevents the uptake in the heart of exogenous norepinephrine-^{3}H. Since the endogenous levels of norepinephrine in the heart of animals treated with 3-iodo-L-tyrosine are reduced to a much greater extent than are the exogenous norepinephrine-^{3}H levels (Table 2), it can be concluded that the decrease in the catecholamine levels is mainly due to the inhibition of catecholamine biosynthesis.