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FORMATION OF α-METHYLNOREPINEPHRINE AS A METABOLITE OF METARAMINOL IN GUINEA PIGS

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Abstract—Metaraminol was metabolized to α-methylnorepinephrine by homogenates from guinea pig liver *in vitro*, and α-methylnorepinephrine was found in tissues of guinea pigs treated with metaraminol *in vivo*. The conversion of metaraminol to α-methylnorepinephrine with rat liver homogenates *in vitro* was low, and no α-methylnorepinephrine was found in metaraminol-treated rats *in vivo*. α-Methylnorepinephrine concentrations were measured in liver, heart, spleen, kidney and lung of guinea pigs treated with metaraminol. The ratio of tissue concentrations of α-methylnorepinephrine/metaraminol varied from near 0 in the adrenal glands to greater than 1 in liver at early times, though α-methylnorepinephrine disappeared from liver at a slightly faster rate than did metaraminol, possibly due to the ability of α-methylnorepinephrine to be metabolized by O-methylation. Iprindole pretreatment reduced the concentration of α-methylnorepinephrine in heart and liver of metaraminol-treated rats but did not alter the depletion of norepinephrine in these tissues. The possibility that α-methylnorepinephrine is involved in some of the pharmacological effects of metaraminol in guinea pigs is raised.

Metaraminol, (−)-1-(*m*-hydroxyphenyl)-2-amino-1-propanol, is a marketed sympathomimetic pressor drug and has been used extensively as a pharmacological tool because of its ability to deplete tissue catecholamines. Metaraminol differs from norepinephrine structurally in having an α-methyl substituent and lacking a *p*-hydroxyl group. Because of these structural differences, metaraminol is not metabolized by monoamine oxidase (EC 1.4.3.4, amine:oxygen oxidoreductase [deaminating] [flavin-containing]) or by catechol *O*-methyl transferase (EC 2.1.1.6, *S*-adenosyl-L-methionine:catechol *O*-methyltransferase), the two enzymes responsible for the metabolic degradation of norepinephrine. In 1965, Maitre and Staehelin [1] reported that metaraminol was metabolized in the guinea pig by *p*-hydroxylation to form α-methylnorepinephrine, though many investigators have considered metaraminol as a compound that does not undergo metabolism [2–6]. Maitre and Staehelin used paper chromatography to separate α-methylnorepinephrine from norepinephrine followed by bioassay based on pressor responses in pithed rats [1]. Here we are describing studies using high performance liquid chromatography with electochemical detection that confirm the presence of α-methylnorepinephrine in tissues of guinea pigs but not in rats after metaraminol administration. The *in vitro* conversion of metaraminol to α-methylnorepinephrine by guinea pig liver is also reported.

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METHODS

Male Wistar rats, weighing 130–150 g, were obtained from the Laboratory Supply Co., Indianapolis, IN. Male guinea pigs of the Hartley strain, weighing 190–225 g, were obtained from the Murphy Breeding Laboratories, Plainfield, IN.

Metaraminol bitartrate (Winthrop Laboratories, New York, NY) was injected in an aqueous solution. Animals were decapitated, and tissues were quickly excised and frozen on dry ice. The tissues were stored frozen at −15° for 1–7 days prior to analysis.

Metaraminol was extracted from tissues, separated by high performance liquid chromatography, reacted with *o*-phthalaldehyde, and determined by spectrofluorometric detection using an Aminalyzer (Aminco, Silver Spring, MD) as described previously [7]. Catecholamines (norepinephrine and α-methylnorepinephrine) were measured by high performance liquid chromatography with electrochemical detection (HPLC-EC) [8]. Catecholamines were extracted from tissue homogenates and adsorbed onto alumina. Eluates from the alumina were applied to a Vydac 10 μ SCX column. The mobile phase for chromatography contained 0.1 M Na_2HPO_4, 0.05 M citric acid, and 10% methanol. The electrochemical detector consisting of a Ag–AgCl reference electrode, a carbon paste working electrode, and a LC-2A controller was purchased from Bioanalytical Systems (Lafayette, IN).

The conversion of metaraminol and related compounds to catecholamines was examined in liver microsomes obtained from both rats and guinea pigs. A radioenzymatic method of product quantitation was used in these experiments which were part of a broader study of phenolic compounds capable of

being converted to catechols. The microsomes were prepared from livers of fasted, untreated animals. The livers were homogenized in 4 vol. of cold 0.15 M KCl. The microsomes were prepared by recentrifuging the 10,000 *g* supernatant fraction at 100,000 *g* for 45 min. The microsomal pellet was resuspended in cold 0.05 M phosphate buffer, pH 7.4, at the original volume. Two ml of the resuspended microsomes was added to 0.1 ml of 0.1 M magnesium chloride, 0.6 ml of phosphate buffer (0.5 M, pH 7.4), 0.1 ml of 1 mM NADPH, and 0.1 ml of metaraminol (0.1 mM). The incubations were performed for 30 min at 37°. An aliquot (0.3 ml) was removed from the incubation solution at various time periods and frozen on dry ice for subsequent analysis. Levels of α-methylnorepinephrine were determined by a radioenzymatic method. Actual concentrations were determined by extrapolation from a standard curve prepared using microsomal suspensions to which various concentrations of the catechol were added. The standard curves for these studies were linear from 25 to 1000 ng/ml. An aliquot of the microsomal suspension (0.3 ml) with or without added α-methylnorepinephrine was added to 20 μl of 70% perchloric acid to precipitate the protein. An aliquot of the supernatant fluid (50 μl) was combined with 50 μl of 0.1 N perchloric acid and 50 μl of a cofactor solution containing 0.3 mg of dithioerythritol, 3 μl of 0.1 M magnesium chloride solution, 35 μl of 2 M Tris–HCl (pH 9.6), 6 μl of catechol-*O*-methyltransferase prepared from rat liver, and 1.75 μCi of tritiated [^{3}H]-*S*-adenosylmethionine (New England Nuclear, Boston, MA; 8–12 Ci/mmole) in 6 μl of H_2O. The final pH was 8.2. The solution was incubated for 30 min at 37°. At the end of the incubation the reaction was stopped by the addition of 0.5 ml of borate buffer (0.5 M, pH 10) to the 150-μl sample in a centrifuge tube. The solution was vortexed and 5 ml of ethyl acetate was added and vortexed again. The organic layer was drawn off after centrifugation and washed with 0.5 ml of borate buffer. Following centrifugation, the organic layer was extracted with 0.5 ml of 0.1 N HCl. The HCl layer was washed with 2 ml of ethyl acetate by vortexing and centrifugation. The pH was adjusted with 0.5 ml of borate buffer to pH 10. The aqueous solution was extracted with 3 ml of ethyl acetate which was then transferred to a counting vial and assayed for radioactivity.

RESULTS

Figure 1 shows recorder tracings from HPLC-EC analysis of tissues from control rats and guinea pigs and from animals treated with metaraminol. Reference standards of norepinephrine and α-methylnorepinephrine were readily separated (Fig. 1a). The large peak corresponding to norepinephrine in the heart from an untreated guinea pig (Fig. 1b) was markedly reduced after metaraminol treatment (Fig. 1c), and a new peak matching the retention time of α-methylnorepinephrine appeared. The peak corresponding to norepinephrine in the heart from an untreated rat (Fig. 1d) was similarly reduced after metaraminol treatment, but no α-methylnorepinephrine was found (Fig. 1e). Metaraminol is not detected by this analytical procedure since the material applied to the HPLC column was an alumina eluate, and metaraminol (not a catechol) is not adsorbed onto alumina. Analysis of liver extracts from a metaraminol-treated guinea pig gave results similar to those obtained with heart extracts (Fig. 1c), but the α-methylnorepinephrine peak was much larger than the norepinephrine peak (not shown).

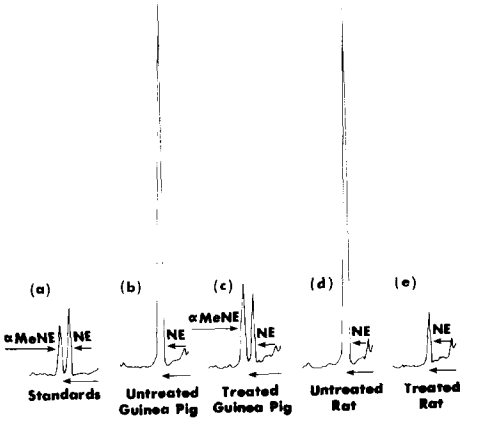


Fig. 1. Typical HPLC-EC tracings showing the presence of α-methylnorepinephrine in guinea pig but not rat tissues after metaraminol injection. Key: (a) Segment showing the separation of authentic samples of α-methylnorepinephrine and norepinephrine [the peak at left is α-methylnorepinephrine, whose average retention time (514 sec) is longer than that for norepinephrine (442 sec) (peak at right)]; (b) segment showing the presence of norepinephrine in heart from an untreated guinea pig; (c) segment showing the reduced concentration of norepinephrine (peak at right) and the presence of α-methylnorepinephrine (peak at left) in heart from a metaraminol-treated guinea pig; (d) segment showing the presence of norepinephrine in heart from an untreated rat; and (e) segment showing the reduced concentration of norepinephrine but no α-methylnorepinephrine in heart from a metaraminol-treated rat. These tracings are representative of those for hearts of all animals (see Table 1), and are qualitatively similar to those for livers as well. Metaraminol-treated animals received metaraminol bitartrate (5 mg/kg, s.c.) 4 hr before they were killed.

Quantitative data on norepinephrine and α-methylnorepinephrine as well as metaraminol concentrations in guinea pig and rat tissues are shown in Table 1. In guinea pig heart, norepinephrine concentration was markedly reduced after metaraminol treatment, and both metaraminol and α-methylnorepinephrine were present in concentrations higher than that of the remaining norepinephrine. Norepinephrine concentration was lower in guinea pig liver than in heart and was also substantially lowered after metaraminol treatment. Metaraminol was present in liver at a concentration less than one-fifth that in heart, whereas the concentration of α-methylnorepinephrine in liver was almost as great as in heart. In the rat, no α-methylnorepinephrine was detected in heart or liver, although metaraminol was present at concentrations slightly lower than in guinea pig tissues and norepinephrine was markedly depleted as in guinea pigs.

The levels of metaraminol and α-methylnorepinephrine in guinea pig tissues at various times after

Table 1. Effect of metaraminol treatment on amine concentrations in rats and guinea pigs*

	Amine concentration (ng/g)		
	Norepinephrine	Metaraminol	α-Methylnorepinephrine
Guinea pig-heart			
Control	1610 ± 105		
Metaraminol-treated	122 ± 22	1953 ± 188	241 ± 49
Guinea pig-liver			
Control	244 ± 21		
Metaraminol-treated	33 ± 3	345 ± 62	206 ± 24
Rat-heart			
Control	665 ± 28		
Metaraminol-treated	45 ± 8	977 ± 69	ND†
Rat-liver			
Control	42 ± 1		
Metaraminol-treated	2 ± 2	240 ±21	ND

* Metaraminol bitartrate (5 mg/kg, s.c.) was injected in rats and guinea pigs. Norepinephrine, metaraminol and α-methylnorepinephrine concentrations were measured 4 hr later. Values are means ± S.E.M. for five animals per group.

† ND = not detectable.

after i.p. injection of metaraminol are shown in Fig. 2. In heart, α-methylnorepinephrine levels were lower than those of metaraminol at all times; the rates of disappearance of the two amines were similar. In liver, α-methylnorepinephrine levels were at least as high as those of metaraminol at the shortest time point studied in this experiment (8 hr), but α-methylnorepinephrine disappeared slightly more rapidly than metaraminol and was lower at all subsequent times than metaraminol.

The tissue distribution of α-methylnorepinephrine and metaraminol in guinea pigs at an early time point (4 hr) is shown in Fig. 3. Metaraminol levels were very high in adrenal gland and spleen. In other tissues, the concentration of metaraminol varied in the order: heart > epididymal fat > kidney > liver > lung > muscle > brain. All tissues except brain had metaraminol concentrations higher than those present in blood. α-Methylnorepinephrine concentration was highest in liver; the concentration of this metabolite varied among tissues in the order: liver > heart > spleen > kidney > lung, being undetectable in the other tissues and in blood. Thus, the ratio of α-methylnorepinephrine/metaraminol concentration varied widely, being higher than 1 in this experiment only in the case of the liver.

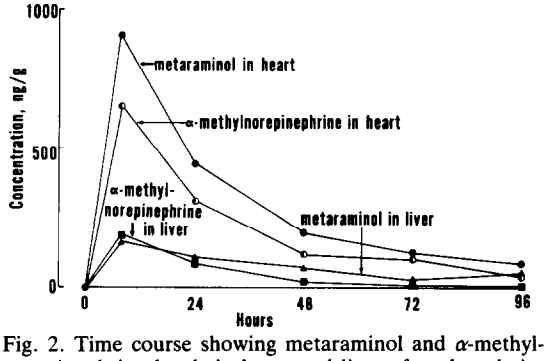


Fig. 2. Time course showing metaraminol and α-methylnorepinephrine levels in heart and liver after the administration of metaraminol (4.7 mg/kg, i.p.) to guinea pigs.

The conversion of metaraminol to α-ethylnorepinephrine *in vitro* was demonstrated with liver microsomes from both rat and guinea pig livers (Fig. 4). Microsomes from guinea pig liver were much more active than microsomes from rat liver in catalyzing this reaction. The specificity of this conversion was examined by comparing three other compounds: *p*-hydroxynorephedrine (the structural isomer of metaraminol having the hydroxyl group in the para position), *m*-hydroxyamphetamine (metaraminol without the β-hydroxyl group), and *p*-hydroxyamphetamine (*p*-hydroxyephedrine without the β-hydroxy group) (Table 2). The microsomes from

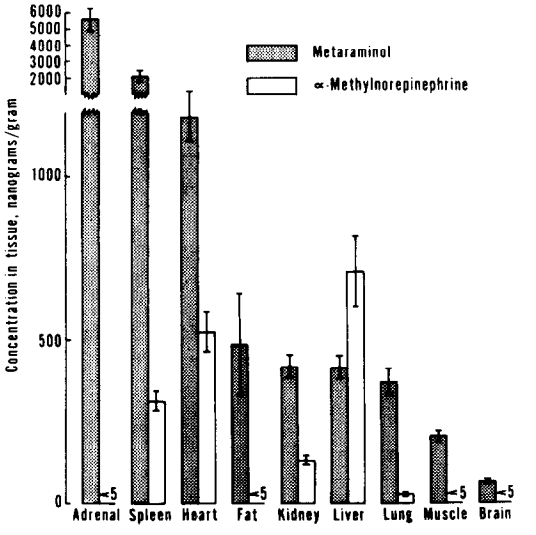


Fig. 3. Concentration of metaraminol and α-methylnorepinephrine in guinea pig tissues 4 hr after the injection of metaraminol bitartrate (4.7 mg/kg, i.p.). Mean values ± S.E. for five guinea pigs per group are shown. α-Methylnorepinephrine was not detectable in adrenal, fat, muscle, brain or blood. Blood levels of metaraminol were 72 ± 8 ng/ml.

guinea pig liver formed catechols from *m*-hydroxyamphetamine at a rate even higher than with metaraminol, whereas the *p*-hydroxy compounds were converted to catechols at slower rates. Catechol formation with rat hepatic microsomes occurred much more slowly with all compounds.

Since guinea pig liver has a much higher density of noradrenergic innervation than does rat liver (R. W. Fuller, S. Y. Felten, K. W. Perry, H. D. Snoddy and D. L. Felten, studies to be published), the possibility was considered that noradrenergic nerve fibers contributed to the hydroxylation of metaraminol by microsomal preparations from guinea pig liver. To test that possibility, guinea pigs were treated with 6-hydroxydopamine to chemically denervate the liver. Table 3 shows that, although norepinephrine concentration in liver was reduced to 30 per cent of the control by this treatment, there was no reduction of metaraminol conversion to α-methylnorepinephrine *in vitro* by the microsomal fraction.

The effect of pretreatment with iprindole, a compound known to inhibit the *p*-hydroxylation of amphetamine [9] and some related compounds [10] in rats, on α-methylnorepinephrine and metaraminol levels in guinea pig tissues is shown in Table 4. Iprindole pretreatment reduced by at least half the concentration of α-methylnorepinephrine found in liver and heart without changing significantly the concentration of metaraminol. The depletion of norepinephrine in liver and heart after metaraminol injection was not altered by iprindole pretreatment (Table 5), suggesting that this depletion after metaraminol injection was not dependent on α-methylnorepinephrine formation.

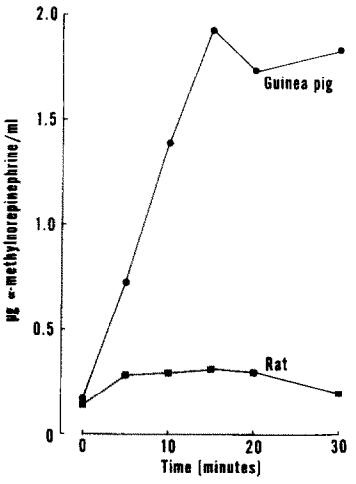
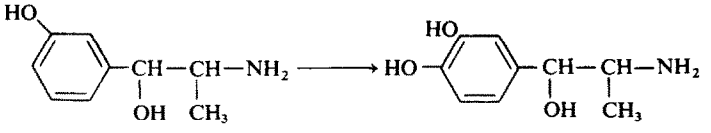


Fig. 4. *In vitro* formation of α-methylnorepinephrine from metaraminol by guinea pig liver homogenates but not by rat liver homogenates.

DISCUSSION

Our findings confirm the report of Maitre and Staehelin [1] that metaraminol is metabolized in the guinea pig by *p*-hydroxylation to form α-methylnorepinephrine as shown below.



The *in vitro* experiments indicate that the guinea pig preferentially oxidizes *m*-hydroxylated compounds to catechols and has a much greater capacity for this reaction than does the rat. Gram and Wright [2, 3] had earlier shown that metaraminol was not metabolized by the rabbit. Thus, the conversion of metaraminol to α-methylnorepinephrine may be significant only in the guinea pig and not in other animal species. Of particular interest would be whether this metabolic transformation occurs in man, since metaraminol is used clinically as a drug. We have not determined quantitatively the extent to which metaraminol is converted to α-methylnorepinephrine in the guinea pig, though levels of the metabolite were approximately the same as those of metaraminol itself in liver, suggesting that the metabolism is significant quantitatively.

Table 2. Catecholamine formation by liver microsomes *in vitro*

Substrate	α-Methylnorepinephrine or α-Methyldopamine (μg formed/ml) Time (min) 0	5	10	15	20	30
Guinea pig						
p-Hydroxynorephedrine	0.04	0.20	0.19	0.15	0.25	0.26
m-Hydroxyamphetamine	0.10	1.32	2.64	3.15	2.58	3.72
p-Hydroxyamphetamine	0.12	0.52	0.74	1.09	1.16	1.51
Rat						
p-Hydroxynorephedrine	0.23	0.27	0.28	0.34	0.26	0.18
m-Hydroxyamphetamine	0.09	0.27	0.35	0.29	0.33	0.16
p-Hydroxyamphetamine	0.12	0.36	0.44	0.36	0.33	0.17

Table 3. Effect of 6-hydroxydopamine pretreatment on the norepinephrine concentration of guinea pig liver and on the conversion of metaraminol to α-methylnorepinephrine by guinea pig liver microsomes*

Dose of 6-hydroxydopamine	Norepinephrine (μg/g liver)	α-Methylnorepinephrine formation *in vitro* (μg/ml)
0	0.97 ± 0.09	1.71 ± 0.42
100	0.29 ± 0.08 ($P < 0.05$)	2.13 ± 0.14 (NS)†

* 6-Hydroxydopamine hydrobromide (100 mg/kg, i.p.) was injected 1 week before guinea pigs were killed. Mean values ± S.E. for three animals per group are shown.

† NS = not significant.

Table 4. Effect of iprindole pretreatment on α-methylnorepinephrine formation from metaraminol in guinea pigs*

	α-Methylnorepinephrine (ng/g)		Metaraminol (ng/g)	
	Liver	Heart	Liver	Heart
Metaraminol alone	276 ± 36	1332 ± 157	325 ± 84	1256 ± 82
Metaraminol + iprindole	105 ± 15† (−62%)	642 ± 57† (−52%)	229 ± 38	1313 ± 86

* Metaraminol bitartrate (4.7 mg/kg, i.p.) was injected 4 hr before guinea pigs were killed and 10 min after iprindole hydrochloride (10 mg/kg, i.p.). Values are means ± S.E.M. for five guinea pigs per group.

† $P < 0.05$.

Controversy had arisen earlier concerning the stoichiometry of the displacement by metaraminol of norepinephrine in the heart. Shore *et al.* [11] found essentially a mole-for-mole replacement of norepinephrine with metaraminol in the heart after relatively low doses of metaraminol were given to rats. Udenfriend and Zaltzman-Nirenberg [12], on the other hand, found that in the guinea pig the amount of metaraminol present was less than the amount of norepinephrine missing from the heart. Our results suggest that one would not expect the same mole-for-mole relationship in the guinea pig, since presumably the α-methylnorepinephrine formed as a metabolite or metaraminol is stored in intraneuronal granules that previously contained norepinephrine. Thus, the total of metaraminol plus α-methylnorepinephrine might be expected to equal the amount of missing norepinephrine. Since α-methylnorepinephrine and metaraminol are present in guinea pig heart at approximately equal concentrations after the i.p. injection of metaraminol (Table 4, Figs 3 and 4), the fact that Udenfriend and Zaltzman-Nirenberg [12] found metaraminol levels to be only about half of the amount of missing norepinephrine at 24–48 hr after metaraminol injection is consistent with the possibility that the discrepancy might be accounted for by α-methylnorepinephrine.

Table 5. Effect of iprindole pretreatment on norepinephrine depletion by metaraminol in guinea pigs*

Treatment group	Norepinephrine concentration (ng/g) Liver	Heart
Control	244 ± 22	2233 ± 10
Metaraminol	27 ± 6† (−89%)	222 ± 52† (−90%)
Metaraminol + iprindole	21 ± 5† (−91%)	213 ± 53† (−90%)

* Metaraminol bitartrate (4.7 mg/kg, i.p.) was injected 4 hr before guinea pigs were killed and 10 min after iprindole hydrochloride (10 mg/kg, i.p.). Mean values ± S.E. for five guinea pigs per group are shown.

† $P < 0.01$.

The conversion of metaraminol to α-methylnorepinephrine was shown to be catalyzed by guinea pig liver homogenates *in vitro*, and other tissues were not tested for their ability to catalyze the conversion. The fact that α-methylnorepinephrine levels were as high or higher than metaraminol levels in liver but not in any other tissue of the guinea pig *in vivo* is compatible with the idea that the liver is the major if not the only site of this metabolic transformation. Thus metaraminol, which is not metabolized by catechol *O*-methyltransferase, is converted to a metabolite that can be *O*-methylated by this enzyme. The extent to which α-methylnorepinephrine, formed from metaraminol, is metabolized by *O*-methylation *in vivo* remains to be determined, but the slightly faster disappearance of α-methylnorepinephrine than of metaraminol in guinea pig liver

(Fig. 3) might be explained by the fact that the former compound can be metabolized by *O*-methylation.

α-Methylnorepinephrine has been identified as a metabolite of α-methyldopa [13, 14]. α-Methylnorepinephrine has been measured in various tissues after both acute and chronic treatment with α-methyldopa and has been suggested to mediate the antihypertensive actions of α-methyldopa [15, 16]. The role of α-methylnorepinephrine in the antihypertensive activity of metaraminol is not clear. The studies reported herein suggest that this catechol product may be of importance in the guinea pig while being of less significance in the rat.

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