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# SOME BIOCHEMICAL AND PHARMACOLOGICAL ACTIONS OF (—) ERYTHRO-META-(META-CHLORO-BENZYLOXY)-2-(1-AMINOETHYL)-BENZYL ALCOHOL
## A DERIVATIVE OF METARAMINOL*

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**Abstract**—(−)Erythro-meta-(meta-chlorobenzyloxy)-2-(1-aminoethyl)-benzyl alcohol, a meta-chlorobenzyl ether of metaraminol, administered to mice, rats and dogs, causes depletion of peripheral stores of norepinephrine and their partial replacement with metaraminol. Consequently, the ether depresses adrenergic nerve transmission in dogs and lowers blood pressure in renal hypertensive rats. However, acute pressor responses are either minimal (rats) or absent (dogs) following administration of the ether, due to the slow formation of metaraminol. Unlike α-methyl-*m*-tyrosine, the chlorobenzyl ether of metaraminol does not produce central nervous system effects. In the brain the amino acid is converted to metaraminol, while metaraminol as derived from the ether is absent or appears only in low concentration.

THE ABILITY of metaraminol to produce depletion of norepinephrine from the heart and other tissues is well known.[1,2] Metaraminol has also been shown to replace norepinephrine within the adrenergic neuron, and it can be liberated, presumably much in the same manner as the normal transmitter, by nerve activation.[3] This process has become known as "false" or substitute adrenergic transmission and was first invoked by Day and Rand[4,5] to explain the antihypertensive action of methyldopa. The amino acid is metabolized to α-methyldopamine and α-methylnorepinephrine, one or both of which may function as "false" or substitute transmitters within peripheral and central adrenergic neurons.

Metaraminol is for the most part a direct-acting sympathomimetic agent,[6] and it is considerably less potent as a pressor agent than norepinephrine.[7,8] Theoretically, metaraminol possesses the properties of a false transmitter of adrenergic impulses, and because of this it might be expected to have antihypertensive actions, especially under conditions of increased activity of the sympathetic nervous system.

Metaraminol and α-methyl-meta-tyrosine, a substance which upon metabolism yields metaraminol, have been studied for their ability to lower blood pressure in hypertensive subjects. Crout *et al.*[9] reported a lowering of blood pressure in a limited study of hypertensive patients given small doses of metaraminol orally for several days. α-Methyl-meta-tyrosine has also been reported to be effective in lowering pressure in hypertensive patients when administered intravenously, but not after oral administration.[10,11] However, the appearance of bizarre central nervous system

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symptoms following α-methyl-meta-tyrosine administration discouraged further trials. In renal hypertensive rats, metaraminol was reported by Brunner *et al.*[12] to be 10 times more effective than α-methyl-meta-tyrosine and one-hundred times more active than α-methyldopa in reducing pressure. Recently Albertson *et al.*[13] demonstrated an antihypertensive action of metaraminol in dogs. Metaraminol itself, while evidently active in lowering pressure under appropriate circumstances, has the potential disadvantage of producing pressor responses if given in too large amounts or if erratic absorption should occur. It seemed desirable, therefore, to develop derivatives of metaraminol which would be susceptible to metabolic conversion to the amine without causing acute cardiovascular effects and which would not penetrate into the central nervous system.

The meta-*O*-alkyl and *O*-benzyl ethers of metaraminol prepared by Saari *et al.*[14] appeared to meet these requirements. It was shown that certain of these derivatives are essentially devoid of sympathomimetic activity, yet are sufficiently converted to metaaminol to affect norepinephrine depletion. The site of metabolism of at least one of these derivatives appears to be the liver, in so far as SKF 525-A blocked the norepinephrine depleting activity. The purpose of this report is to describe the biochemical and pharmacological activity of one such derivative of metaraminol; namely, (−) erythro-meta-(meta-chlorobenzyloxy)-2-(1-aminoethyl)-benzyl alcohol.

## METHODS

*Biochemical determinations.* Norepinephrine and metaraminol concentrations were determined in tissues after grinding in a motor driven glass homogenizer with 9 vol. of 0·4 N perchloric acid. Aliquots of the extract were used for the determination of norepinephrine by the method described by Porter *et al.*,[15] following purification on alumina.[16] Other aliquots were used for the determination of metaraminol by the method of Shore and Alpers.[17]

Urinary excretion of metaraminol by female beagle dogs was determined in samples collected by catheterization at hourly intervals for 7 hr and for a total of 24 hr. The pH of a 3-ml sample of urine was adjusted to 9 by the addition of dilute (0·1 N) HCl or NaOH. One ml of 0·5 M borate buffer, pH 9, was added and the solution shaken with 25 ml of *n*-butanol–*n*-heptane (1 : 1, v/v). The extract was washed by shaking with 3 ml borate buffer, and then 20 ml was back extracted with 0·5 ml of 0·01 N HCl. Two hundred λ of the HCl extract were streaked on a 5 × 20 cm silica gel G TLC plate (250 μ thick) and developed with butanol–acetic acid–water (4 : 1 : 1) for 3 hr. The plate was air-dried and sprayed with pH 9 borate buffer, then with 1% *o*-phthalaldehyde, and after 7 min with 3 N HCl. Metaraminol appeared as a strongly fluorescent band, $R_F$ 0·75. Areas of unsprayed chromatograms centered at $R_F$ 0·75 were scraped off and eluted with 2 ml of 0·01 N HCl. One ml of the eluate was used for determination of metaraminol by the method of Shore and Alpers.[17]

*Effect on adrenergic transmission.* Groups of four beagle dogs of either sex were pretreated orally for 4 days with 5 mg/kg/day of the meta-chlorobenzyl ether derivative, or for 1 day with metaraminol (0·5 mg/kg); untreated animals served as controls. Sixteen to 20 hr after the last treatment, the animals were anesthetized with vinbarbital (50 mg/kg, i.v.) and artificially respired. Mean arterial pressure and heart rate were recorded. A monopolar electrode was placed on the postganglionic portion of the

cardioaccelerator nerve, and chronotropic responses were obtained to stimuli of 3 msec duration with increasing frequencies of 0·5 to 15·8 cycles/sec delivered over a 10-sec period. Following this procedure, pressor and chronotropic responses to increasing doses of tyramine (25–200 mcg/kg, i.v.) and norepinephrine (0·5–4·0 mcg/kg i.v.) were obtained. Injections were spaced at 5–10 min intervals to permit return of pressure and heart rate to control levels. At the termination of the experiment, the right atrium was removed, frozen and later analyzed for norepinephrine and metaraminol as described above.

*Effects on blood pressure.* Mongrel dogs were anesthetized with a mixture of barbital (25 mg/kg) and thiopental (10 mg/kg) i.v. and bilaterally vagotomized. Blood pressure and heart rate were recorded continuously for 6 hr. To insure a stable heart rate throughout the recording session, bretylium (2·5 mg/kg i.v.) was administered at the beginning of the experiment; 30 min later metaraminol (0·06, 0·125 or 0·25 mg/kg) or the meta-chlorobenzyl ether derivative (5–10 mg/kg) was administered intraduodenally. The number of animals receiving each treatment is indicated in Table 5.

Metaraminol and its meta-chlorobenzyl ether were tested in unanesthetized, renal hypertensive rats for effects on blood pressure. Male Camm–Sprague–Dawley rats were made hypertensive by removal of the right kidney and application of a clip to the left renal artery. Animals were then maintained until an established state of hypertension was present, at which time an indwelling cannula was implanted in the caudal artery. Pressures were continuously recorded throughout the duration of the experiment. Mean arterial pressure was calculated for 0, 0·5, 1, 2, 4, 7, 16 and 24 hr following treatment. Compounds were administered orally by intubation. Four animals were given 5 mg/kg of the meta-chlorobenzyl ether derivative of metaraminol, and four were given 2·0 mg/kg of metaraminol; six control animals received water orally.

*Behavioral effects in dogs.* Three beagle dogs were trained and tested, standing, in a modified Pavlov sling located in a ventilated isolation chamber. The "lever", a horizontal tube, was located just above the animal's head and at right angles to the body axis. The behavioral response recorded was raising the head approximately 5–6 cm, in a modified Sidman avoidance schedule which programmed an electric shock to the hind legs through ECG electrodes if a response was not made every 36 sec. Every response reset the timer, and responses emitted during a shock terminated it and started the next 36-sec avoidance period. The dogs were placed in the chamber and tested for 30 min every 2 hr. The meta-chlorobenzyl ether derivative of metaraminol (10 mg/kg) or α-methyl-meta-tyrosine (25 mg/kg) was administered orally after the control session. The dose of α-methyl-meta-tyrosine employed caused approximately the same depletion of peripheral stores of norepinephrine as was produced by the metachlorobenzyl ether of metaraminol.

*Materials.* The synthesis of (−) erythro-meta-(meta-chlorobenzyloxy)-2-(1-aminoethyl)-benzyl alcohol was described by Saari *et al.*[14] Both the methane sulfonate and sulfate salts were used. Other compounds employed, and their suppliers, were as follows: metaraminol [(−)erythro] bitartrate, Merck and Co., Rahway, New Jersey; tyramine hydrochloride and norepinephrine (−), K & K Laboratories, Plainview, New York; α-methyl-*m*-tyrosine (±), Regis Chemical Co., Chicago, Illinois. Base weights of compounds were used exclusively.

## RESULTS

*Effects on norepinephrine in the heart.* On a milligram per kilogram basis, the meta-chlorobenzyl ether derivative of metaraminol was about one-seventh as potent as metaraminol in depleting the hearts of mice of norepinephrine (norepinephrine determined 16 hr after oral administration of compounds). The $ED_{50}$ and 95 per cent confidence limits for metaraminol was 0·10 (0·08, 0·12) mg/kg and for the meta-chlorobenzyl ether 0·67 (0·49, 0·92) mg/kg. The slopes of the dose–response lines for the two compounds were identical (− 0·55 and − 0·54 respectively). In terms of metaraminol equivalents, the meta-chlorobenzyl ether derivative was about one-fourth as active as metaraminol.

Depletion of heart norepinephrine was more rapid after the administration of metaraminol than after the administration of its meta-chlorobenzyl ether (Table 1).

TABLE 1. TIME COURSE FOR DEPLETION OF NOREPINEPHRINE AND THE APPEARANCE OF METARAMINOL IN THE HEARTS OF MICE FOLLOWING ORAL ADMINISTRATION OF METARAMINOL AND ITS META-CHLOROBENZYL ETHER DERIVATIVE*

| | Amine concentration | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Norepinephrine (mcg/g) | | | | | Metaraminol (mcg/g) | | | | |
| Time (hr) | 0 | 1 | 4 | 8 | 16 | 0 | 1 | 4 | 8 | 16 |
| *Metaraminol 0·2 mg/kg* | | | | | | | | | | |
| | 0·52 | 0·28 | 0·06 | 0·06 | 0·19 | 0·00 | 0·28 | 0·31 | 0·36 | 0·26 |
| ± S.D. | 0·07 | 0·03 | 0·00 | 0·00 | 0·00 | — | 0·03 | 0·03 | 0·00 | 0·03 |
| *Meta-chlorobenzyl ether of metaraminol 1·5 mg/kg* | | | | | | | | | | |
| | 0·52 | 0·46 | 0·11 | 0·06 | 0·15 | 0·00 | 0·17 | 0·31 | 0·39 | 0·23 |
| ± S.D. | 0·07 | 0·02 | 0·01 | 0·03 | 0·01 | — | 0·01 | 0·02 | 0·03 | 0·08 |

* Three groups of five mice per treatment. Animals sacrificed at times indicated.

The two compounds were administered orally in amounts which had approximately equal effects when amines were measured in the tissues 16 hr later. Under these conditions, the maximal effect of the meta-chlorobenzyl ether occurred in about 8 hr.

The disappearance of norepinephrine was accompanied by the accumulation of metaraminol in the hearts of both the metaraminol and meta-chlorobenzyl ether-treated animals (Table 1). The replacement was not on a mole-for-mole basis. The slower onset of action of the meta-chlorobenzyl ether is consistent with the suggestion that it is metabolized to metaraminol, which is responsible for its ability to deplete norepinephrine (for other details, see Discussion).

In the rat heart the relationship of activities between metaraminol and the meta-chlorobenzyl ether derivative was similar to that observed in the mouse. The $ED_{50}$ for norepinephrine depletion by metaraminol, 16 hr after oral administration, was 0·12 (95 per cent C.L. 0·10, 0·16) mg/kg, and for the meta-chlorobenzyl ether derivative 0·94 (95 per cent C.L. 0·84, 1·04) mg/kg. In animals given the meta-chlorobenzyl ether derivative daily p.o. for 12 weeks, there was a dose dependent decrease in

TABLE 2. NOREPINEPHRINE AND METARAMINOL CONCENTRATION IN THE RAT HEART OF ANIMALS GIVEN META-CHLOROBENZYL ETHER DERIVATIVE OF METARAMINOL ORALLY FOR 12 WEEKS

| Dose (mg/kg/day) | Amine concentration in heart (mcg/g ± S.D.) | | |
|---|---|---|---|
| | Norepinephrine | Decrease (%) | Metaraminol |
| 0 | 0·65 ± 0·10 | — | 0 |
| 3 | 0·14 ± 0·04 | 79 | 0·27 ± 0·05 |
| 10 | 0·07 ± 0·01 | 90 | 0·31 ± 0·12 |
| 30 | 0·02 ± 0·00 | 97 | 0·38 ± 0·11 |

Pool of six groups of five animals per dose. Animals sacrificed 16 hr after last treatment.

norepinephrine. Again a stoichiometric relationship between the norepinephrine deficit and the concentration of metaraminol in the hearts was not obtained (Table 2).

In rats 1 and 16 hr after oral administration of 10 mg/kg of the meta-chlorobenzyl ether derivative, there was a 15 per cent reduction in total brain norepinephrine; and after 30 mg/kg the value was 20 per cent, and both values are significantly different from controls ($P < 0·05$); the concentration of metaraminol was 0·3 mcg/g for both dose levels. Norepinephrine was not reduced following an oral dose of 10 mg/kg of metaraminol, but approximately 0·1 mcg/g of the latter amine was present in the brains 16 hr after treatment.

In dogs, daily oral administration of the meta-chlorobenzyl ether derivative for 4 days (5 mg/kg/day) resulted in a 90 per cent reduction of heart norepinephrine, from 2·59 ± 1·26 to 0·24 ± 0·06 mcg/g, and the appearance of 1·01 ± 0·14 mcg/g of metaraminol (mean ± S.D.). After a single treatment of dogs with 0·5 mg/kg of metaraminol, heart norepinephrine was reduced 83 per cent, to 0·45 ± 0·38 mcg/g; the metaraminol concentration was 0·96 ± 0·31 mcg/g. (These animals were used in studies on adrenergic responses as described below.) Chronic administration of the

TABLE 3. NOREPINEPHRINE AND METARAMINOL CONCENTRATION IN ATRIA OF DOGS GIVEN THE META-CHLOROBENZYL ETHER DERIVATIVE OF METARAMINOL ORALLY FOR 13 WEEKS

| Dose (mg/kg/day) | Amine concentration in atria (mcg/g ± S.D.) | | |
|---|---|---|---|
| | Norepinephrine | Decrease % | Metaraminol |
| 0* | 4·61 ± 0·54 | — | 0 |
| 3† | 0·44 ± 0·13 | 90 | 1·22 ± 0·30 |
| 10† | 0·21 ± 0·10 | 95 | 1·49 ± 0·48 |
| 30† | 0·03 ± 0·02 | 99 | 1·81 ± 0·07 |

* Eight Animals.

† Four Animals per dose. Animals sacrificed 16 hr after last treatment.

meta-chlorobenzyl ether derivative for 13 weeks caused a dose dependent reduction in norepinephrine in the atria, with a concomitant increase in metaraminol (Table 3). Nearly maximal depletion was obtained in animals given 30 mg/kg/day; as in the other species, a stoichiometric relation between the norepinephrine deficit and the concentration of metaraminol was not obtained.

In the dog, further evidence was obtained for the conversion of the meta-chlorobenzyl ether derivative of metaraminol to the parent amine by the measurement of the urinary excretion of metaraminol following oral administration of the ether. In animals given metaraminol (1·0 mg/kg) this amine was excreted rapidly, especially in the first few hours. Fifty-seven per cent of the administered amine was accounted for in the urine in 24 hr (Table 4). Metaraminol was excreted slowly in animals given 10 mg/kg of the meta-chlorobenzyl ether derivative, and only 2–3 per cent of the administered dose was excreted as metaraminol in 24 hr.

TABLE 4. URINARY EXCRETION OF METARAMINOL BY DOGS AFTER ORAL ADMINISTRATION OF METARAMINOL OR THE META-CHLOROBENZYL ETHER DERIVATIVE

| | Cumulative excretion of metaraminol % of Dose ± S.D. | |
|---|---|---|
| Time (hr) | Metaraminol* (1·0 mg/kg) | meta-Chlorobenzyl ether† (10·0 mg/kg) |
| 1 | 09·49 ± 16·23 | 0·01 ± 0·01 |
| 2 | 13·27 ± 16·37 | 0·11 ± 0·08 |
| 3 | 23·22 ± 14·71 | 0·22 ± 0·10 |
| 4 | 31·79 ± 09·40 | 0·38 ± 0·10 |
| 5 | 38·18 ± 05·19 | 0·54 ± 0·14 |
| 6 | 41·69 ± 04·28 | 0·76 ± 0·24 |
| 7 | 44·09 ± 04·42 | 0·90 ± 0·30 |
| 24 | 56·53 ± 06·72 | 2·92 ± 0·83 |

* Mean of three animals.
† Mean of four animals.

In this study the concentration of amines in the atria was measured 24 hr after the administration of compounds. In the atria of animals given metaraminol, the concentration of norepinephrine was 0·38 ± 0·17 mcg/g (mean ± S.D.), and of metaraminol, 1·12 ± 0·09 mcg/g; in the atria of the meta-chlorobenzyl ether group the concentrations of norepinephrine and metaraminol were 0·52 ± 0·35 and 1·53 ± 0·13 mcg/g, respectively; the concentration of norepinephrine in the atria of normal dogs was 3·5 ± 1·15 mcg/g.

*Effect on adrenergic transmission.* In dogs pretreated orally with an 80–90 per cent depleting dose of metaraminol (0·5 mg/kg; 1 day) or the meta-chlorobenzyl ether derivative (5·0 mg/kg daily for 4 days), the heart rate responses to cardioaccelerator nerve stimulation were reduced (Fig. 1). In the same animals the chronotropic responses to tyramine were also reduced while those to norepinephrine were slightly enhanced. Thus, effective reduction of heart norepinephrine by either treatment (see above) is capable of reducing adrenergic transmission.

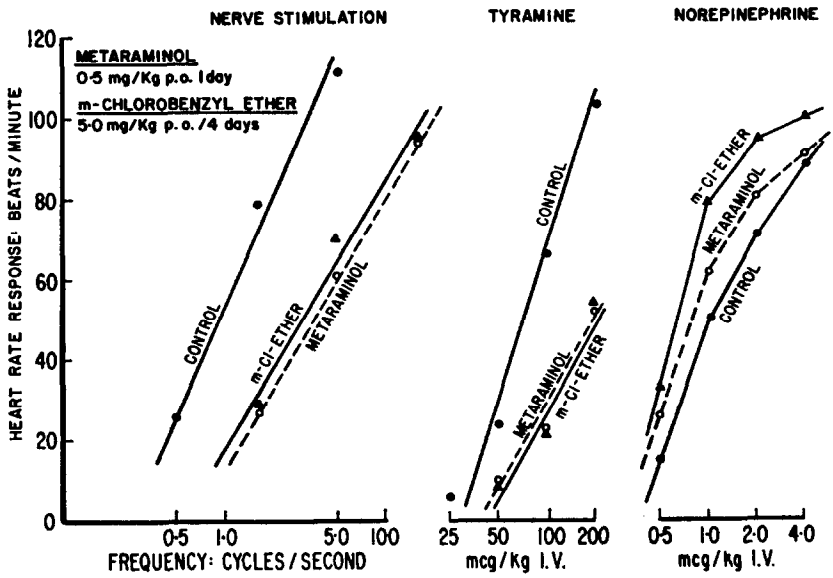


FIG. 1. Adrenergic responses of the dog heart following reduction of norepinephrine by metaraminol and the meta-chlorobenzyl ether derivative of metaraminol. (Amine concentrations are given in text.) (Mean of four dogs per treatment.)

*Acute cardiovascular actions.* The intraduodenal administration of the meta-chlorobenzyl ether derivative to anesthetized dogs in doses of 5 or 10 mg/kg did not produce a significant increase in blood pressure or heart rate over a 6-hr period. Metaraminol, on the other hand, caused a delayed (120 min) increase in pressure and rate at a dose of 0·125 mg/kg, and a more rapid (30–60 min) increase at 0·25 mg/kg

TABLE 5. EFFECT OF INTRADUODENAL ADMINISTRATION OF METARAMINOL AND THE META-CHLOROBENZYL ETHER DERIVATIVE ON BLOOD PRESSURE AND HEART RATE OF THE ANESTHETIZED DOG

| Compound (No. dogs/dose) | Dose (mg/kg i.d.) | | Time after administration (min) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Cont. | 15 | 30 | 60 | 120 | 180 | 240 | 300 | 360 |
| Saline control (12) | | bp | 148 | 143 | 137 | 128 | 121 | 120 | 119 | 116 | 132 |
| | | hr | 160 | 156 | 156 | 154 | 152 | 149 | 146 | 145 | 147 |
| Metaraminol (4) | 0·0625 | bp | 136 | 139 | 141 | 137 | 125 | 120 | 115 | 111 | 109 |
| | | hr | 144 | 146 | 147 | 149 | 149 | 145 | 141 | 135 | 134 |
| | 0·125 | bp | 134 | 129 | 129 | 135 | 149 | 155 | 156 | 146 | 138 |
| | | hr | 118 | 120 | 121 | 128 | 142 | 154 | 168 | 171 | 170 |
| | 0·25 | bp | 146 | 154 | 160 | 168 | 162 | 157 | 153 | 148 | 142 |
| | | hr | 160 | 162 | 171 | 198 | 218 | 206 | 197 | 192 | 189 |
| Meta-chlorobenzyl ether derivative (2) | 5·0 | bp | 154 | 144 | 134 | 124 | 129 | 122 | 107 | 98 | 92 |
| | | hr | 183 | 195 | 197 | 193 | 183 | 168 | 165 | 160 | 155 |
| | 10·0 | bp | 137 | 115 | 109 | 107 | 115 | 113 | 89 | 81 | 85 |
| | | hr | 142 | 144 | 147 | 151 | 156 | 157 | 150 | 147 | 145 |

i.d., intraduodenal route; b.p., mean arterial pressure (mm Hg); h.r., heart rate (beats/min).

(Table 5). It appears that the rate and degree of conversion of the meta-chlorobenzyl ether to metaraminol was insufficient for metaraminol activity to be revealed by changes in the parameters measured. In other studies, the intravenous administration of 0·005 mg/kg of metaraminol to an anesthetized dog produced an immediate increase in blood pressure and myocardial contractility, while under the same conditions doses of the meta-chlorobenzyl ether derivative 200 times greater than the dose of metaraminol had no effect during a 45-min observation period.

TABLE 6. ANTIHYPERTENSIVE ACTION OF THE META-CHLOROBENZYL ETHER DERIVATIVE OF METARAMINOL AND METARAMINOL IN RENAL HYPERTENSIVE RATS*

| | | Mean arterial pressure (mm Hg) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Time hr | | 0 | 0·5 | 1 | 2 | 4 | 7 | 16 | 24 |
| *Group I Placebo Control* ($n = 5$) | | | | | | | | | |
| Day 1 | Mean | 175 | 169 | 170 | 174 | 171 | 172 | 168 | 169 |
| | ± S.D. | 23 | 20 | 22 | 26 | 27 | 28 | 30 | 23 |
| Day 2 | Mean | — | 171 | 172 | 172 | 175 | 175 | 173 | 167 |
| | ± S.D. | | 28 | 31 | 30 | 25 | 28 | 30 | 28 |
| Day 3 | Mean | — | 170 | 164 | 169 | 172 | 172 | 172 | 171 |
| | ± S.D. | | 25 | 24 | 33 | 25 | 31 | 32 | 27 |
| *Group II Meta-chlorobenzyl ether derivative* (5·0 *mg/kg*) ($n = 4$) | | | | | | | | | |
| Day 1 | Mean | 192 | 209 | 218 | 197 | 188 | 176† | 169† | 164† |
| | ± S.D. | 7 | 18 | 13 | 9 | 4 | 7 | 5 | 18 |
| Day 2 | Mean | — | 188 | 198 | 194 | 176 | 165† | 152† | 154† |
| | ± S.D. | | 18 | 19 | 25 | 28 | 18 | 13 | 19 |
| Day 3 | Mean | — | 196 | 202 | 193 | 170 | 156 | 149† | 157† |
| | ± S.D. | | 4 | 11 | 22 | 16 | 20 | 15 | 20 |
| *Group III Metaraminol* (2·0 *mg/kg*) ($n = 4$) | | | | | | | | | |
| Day 1 | Mean | 162 | 171 | 175 | 177 | 172 | 152 | ‡ | 157 |
| | ± S.D. | 19 | 17 | 21 | 19 | 19 | 26 | | 11 |
| Day 2 | Mean | — | 177 | 177 | 177 | 185 | 149 | | 151 |
| | ± S.D. | | 11 | 13 | 23 | 33 | 20 | ‡ | 16 |
| Day 3 | Mean | — | 189 | 189 | 166 | 165 | 134† | | 141 |
| | ± S.D. | | 38 | 32 | 43 | 9 | 6 | ‡ | 17 |

* Compounds administered orally at 0 time Day 1 and after the 24-hr reading on subsequent days.
† Values differ significantly ($P < 0·05$) from corresponding pressure at 0 Time Day 1.
‡ Values not calculated for this period.

*Effect on blood pressure of unanesthetized renal hypertensive rats.* The meta-chlorobenzyl ether derivative of metaraminol produced an unequivocal and prolonged antihypertensive response in unanesthetized renal hypertensive rats (Table 6). Control animals (Group I) showed less than a 15 mmHg decrease in pressure at any time during the study. In animals given 5 mg/kg/day of the meta-chlorobenzyl ether derivative of metaraminol (Group II), mean arterial pressure was significantly lower than at 0 time of Day 1 during the 7–24 hr post-treatment period; there was no

cumulative effect during the 3-day study (Table 6). Metaraminol given to rats at a dose of 2 mg/kg produced an increase in pressure followed by a decrease below control values (Group III). The initial pressure in this group was lower, and statistically significant reduction in pressure occurred only on the third treatment day. The initial increase in pressure in animals given the meta-chlorobenzyl ether derivative of metaraminol presumably is related to a relatively rapid metabolism of the compound to metaraminol in this species.

*Behavioral effects in dogs.* The number of responses recorded over a 32-hr test period with animals given the meta-chlorobenzyl ether derivative of metaraminol were not significantly different from the number of responses recorded in control sessions (Table 7).

TABLE 7. BEHAVIORAL EFFECTS OF META-CHLOROBENZYL ETHER DERIVATIVE OF METARAMINOL AND α-METHYL-META-TYROSINE IN DOGS*

| | Number of responses recorded in 30 min | | |
|---|---|---|---|
| | | meta-Chlorobenzyl ether | |
| Time (hr) | Control placebo | of metaraminol (10 mg/kg p.o.) | α-Methyl-meta-tyrosine (25 mg/kg p.o.) |
| 0 | 533 | 485 | 383 |
| 2 | 509 | 466 | 1244 |
| 4 | 336 | 424 | 1551 |
| 6 | 506 | 392 | 1583 |
| 8 | 463 | 400 | 1147 |
| 10 | 499 | 391 | 544 |
| 12 | 394 | 350 | 884 |
| 14 | 401 | 374 | 373 |
| 16 | 398 | 459 | 372 |
| 18 | 401 | 372 | 623 |
| 20 | 493 | 397 | 338 |
| 22 | 390 | 331 | 435 |
| 24 | 388 | 424 | 443 |
| 26 | 367 | 431 | 667 |
| 28 | 423 | 376 | 470 |
| 30 | 336 | 346 | 414 |
| 32 | 397 | 331 | 461 |

* Three dogs tested on separate occasions using doses that produced approximately equivalent depletion of norepinephrine in peripheral adrenergic stores.

By contrast, administration of α-methyl-meta-tyrosine, which is also metabolized to metaraminol, produced stimulation (increased rates of responding). The effect was greatest 4–6 hr after treatment and was essentially absent by the 14th hr.

## DISCUSSION

The present study has shown that (−) erythro-meta-(meta-chlorobenzyloxy)-2-(1-aminoethyl)-benzyl alcohol, a meta-chlorobenzyl ether derivative of metaraminol, is effective in reducing the concentration of norepinephrine in the hearts of mice, rats and dogs. That this activity is due to its metabolic conversion to metaraminol, a

substance well known for its norepinephrine depleting activity,[1,18] is indicated by the fact that metaraminol accumulates in the heart of the several species studied.

As indicated in the study relating to the time course of depletion of norepinephrine in the mouse heart (Table 1), the concentration of metaraminol present, as determined by the relatively specific method of Shore and Alpers,[17] increased as the norepinephrine concentration decreased. Aside from a difference in potency, the only other difference between metaraminol and the meta-chlorobenzyl ether derivative appears to be that the onset of action of the latter compound is somewhat slower. Accumulation of metaraminol in the heart following administration of the meta-chlorobenzyl ether was also observed in the rat and dog; in the latter species metaraminol was eliminated in the urine.

The demonstration by Saari *et al.*[14] that SKF 525-A, a known inhibitor of certain metabolizing enzymes, blocked the norepinephrine depleting action of the meta-chlorobenzyl ether derivative is further evidence that metabolic conversion of the ether derivative is required for activity. This observation also suggests that the liver is the primary site of conversion since SKF 525-A inhibits the liver microsomal metabolizing enzyme system.

While the present data do not bear on the question whether the ether derivative can directly displace norepinephrine, this seems unlikely in view of the report of Saari *et al.*[14] that SKF 525-A inhibited its catecholamine depleting action almost completely. Hence, if the ether has direct depleting actions, it must be appreciably less potent than metaraminol.

The extent of the conversion of the meta-chlorobenzyl ether derivative of metaraminol to metaraminol was not measured in the present study. The data in mice and rats show that the ether derivative is about one-fourth to one-fifth as active on a molar basis as metaraminol. This suggests that at least 20–25 per cent of the ether is converted to metaraminol in these species. Only about 2–3 per cent of an oral dose of the ether derivative was recovered as metaraminol in the urine over a 24-hr period, whereas about 50 per cent of an oral dose of metaraminol was recovered. Thus, probably 6 per cent or more of the ether derivative was converted to metaraminol in the dog.

In the dog, the meta-chlorobenzyl ether derivative of metaraminol also appears to be relatively free of direct sympathomimetic actions. This was shown by Saari *et al.*[14] who found that doses of 5 or 10 mg/kg, given intravenously, were without effect on blood pressure of the dog. Metaraminol, on the other hand, is clearly active in doses of 0·05 mg/kg intravenously. In the present study, in which the meta-chlorobenzyl ether analog was given intraduodenally to the dog, no indication of direct cardiovascular activity was seen. These observations indicate that in this species the agent possesses little sympathomimetic activity *per se*, and, in addition, show that the rate of conversion to metaraminol is sufficiently slow to avoid appreciable cardiovascular actions. The meta-chlorobenzyl ether derivative differs from the simple alkyl ethers, such as the methyl and ethyl ethers, which are converted to metaraminol at a sufficently rapid rate to produce overt sympathomimetic actions in the dog.[14] In this species, the meta-chlorobenzyl ether derivative, like metaraminol, when administered in effective norepinephrine depleting doses, is capable of reducing adrenergic transmission. In unanesthetized renal hypertensive rats the meta-chlorobenzyl ether of metaraminol given orally produced a sustained hypotensive effect evident 7–24 hr

after treatment. This was preceded by an initial increase in pressure, an effect presumably related in this species to a more rapid metabolism to metaraminol; metaraminol *per se* produced a similar pattern of events.

As noted above, metaraminol derived from the meta-chlorobenzyl ether did not replace norepinephrine on a mole-for-mole basis. This was also the case found with metaraminol not only in these but in other investigations as well; a mole-for-mole displacement by metaraminol occurs only under particular circumstances of dose and time (for references see Porter *et al.*).[2] The mechanisms underlying the deficit when it occurs are not known.

It is of interest that metaraminol derived metabolically from its meta-chlorobenzyl ether did not produce marked catecholamine depletion in the brain. Relatively large doses of the meta-chlorobenzyl ether which in dogs clearly produced substantial reduction in peripheral norepinephrine levels in the heart did not cause behavioral changes; α-methyl-meta-tyrosine, on the other hand, clearly produced stimulation. Therefore, the ether derivative differs considerably from α-methyl-meta-tyrosine which also yields metaraminol on metabolism but which produced apparent central nervous system actions, as illustrated in these studies as well as studied in cats[7] and man.[10,11] The meta-chlorobenzyl ether derivative of metaraminol offers another means of introducing a "false" substitute transmitter in the sympathetic nervous system which appears to be restricted largely to peripheral structures. The compound may, therefore, be a helpful means of testing the utility of "false" adrenergic transmitters in hypertension and other clinical conditions.

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