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ANTIHYPERTENSIVE EFFECTS OF ALPHA-METHYLATED CATECHOLAMINE ANALOGUES IN THE RAT*

BY

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Various mechanisms of action have been discussed in connection with the antihypertensive effect of alpha-methyl-DOPA (α-M-DOPA) (Muscholl, 1966). The currently accepted hypothesis states that the effect of this compound is due to its metabolites, alpha-methyl-dopamine (α-M-DA) and alpha-methyl-noradrenaline (α-M-NA), which accumulate in the catecholamine stores. The major emphasis is placed on the effects of α-M-NA, which supposedly acts as a "false neurotransmitter" (Day & Rand, 1964). Alpha-methyl-meta-tyrosine (α-MMT), which has a metabolic fate similar to that of α-M-DOPA, has recently been shown to lower blood pressure in man (Horwitz & Sjoerdsma, 1964; Holtmeier, Klein-Wisenberg & Marongiu, 1966). In the case of α-MMT, the corresponding "false transmitter" would be metaraminol, which in itself possesses antihypertensive properties in man after prolonged oral application (Crout, Johnston, Webb & Shore, 1965). A further alpha-methylated amino acid, alpha-methyl-tyrosine (α-MT), depletes catecholamine stores (Spector, Sjoerdsma & Udenfriend, 1965). In the guinea-pig this substance is in part metabolized to α-M-DA and α-M-NA (Maître, 1965), but it has been suggested that α-MT acts principally by inhibition of noradrenaline (NA) synthesis rather than by a "false transmitter" mechanism (Spector *et al.*, 1965).

The "false transmitter" hypothesis for the hypotensive effect of these amines has been based on results obtained under a variety of experimental conditions and in various animal species. Therefore, the validity of this hypothesis has been difficult to assess. Assuming that this theory is valid, one should expect that the efficacy of the antihypertensive agent depends on the "falseness" of the "false transmitter." Thus, an inverse proportionality should exist between antihypertensive activity of the precursor and sympathomimetic potency of the "false transmitter."

The following studies were made in the rat: (a) comparison of the antihypertensive effects of α-M-DOPA, α-M-NA, α-MMT, metaraminol, and α-MT, (b) comparison of the effects of prolonged treatment with these compounds on the catecholamine stores in the same animals, (c) comparison of the pressor and positive inotropic effects of NA, α-M-NA, and metaraminol in the conscious rat and the isolated rat heart, (d) comparison

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of the pressor effect of NA, α-M-NA, and metaraminol in urethane-anaesthetized rats pretreated with α-M-DOPA, α-M-NA, α-MMT or metaraminol, (e) investigation of possible impairment of sympathetic function after prolonged treatment with these compounds.

METHODS

Antihypertensive effects in renal hypertensive rats

Under ether anaesthesia the left renal arteries of male rats of 120–140 g body weight were clamped with silver clips (lumen of 0.2 mm). At intervals of one week systolic blood pressures were measured in light ether anaesthesia by the plethysmographic method of Wilson & Byrom (1939). After stable blood pressure levels above 170 mm Hg were reached, the animals were divided into groups with mean blood pressure values of 178–209 mm Hg. During a treatment period of 11 days the following substances and doses were administered:

D,L-alpha-methyl-DOPA (α-M-DOPA): 30, 100 and 300 mg/kg/day orally and 10, 30 and 100 mg/kg/day subcutaneously. L-alpha-methyl-noradrenaline hydrochloride (α-M-NA) (Corbasil, Hoechst): 10 mg/kg/day orally and two daily subcutaneous injections of 0.1, 0.3 and 1 mg/kg. Higher doses or administration of 2 mg/kg/day subcutaneously in a single injection daily were toxic. D,L-alpha-methyl-meta-tyrosine (α-MMT): 3, 10, and 30 mg/kg/day orally and 2, 10, 20, and 100 mg/kg/day subcutaneously. L-metaraminol-bitartrate (metaraminol) (Aramine, MSD): 1, 3, and 10 mg/kg/day orally and 0.1, 0.3, 1 and 2 mg/kg/day subcutaneously. D,L-alpha-methyl-tyrosine (α-MT): 100 and 300 mg/kg/day orally. α-MT was given as a suspension in acacia in a volume of 2 ml./kg per os. All other substances were administered in aqueous solutions in a volume of 1 ml./kg subcutaneously or 2 ml./kg per os. Fresh solutions and suspensions were prepared before each administration.

On the 3rd, 7th and 11th day of treatment, blood pressure was measured 2 hr after the daily administration. On the 4th and 8th day of treatment, blood pressure was measured immediately before the daily administration; that is, 16 hr after the last α-M-NA administration or 24 hr after the last administration of all other substances.

Measurement of the catecholamine content in heart and brain

Selected groups of hypertensive rats in the determination of antihypertensive activity were killed by decapitation. Tissue catecholamine content was determined 24 hr after the last administration of α-M-DOPA or α-M-NA and 2.5 to 3 hr after the last administration of α-MMT, metaraminol or α-MT.

(*a*) *Extraction of catecholamines.* Hearts and brains were homogenized, extracted twice with 10% trichloro-acetic acid and centrifuged. Catecholamines of the combined supernatants were adsorbed on to acid-washed alumina (Woelm, Akt.Stufe I) at pH 8.4, washed with de-ionized and twice glass-distilled water, and then eluted with 0.25 N HCl. The eluates were centrifuged for 10 min at 30,000 *g* and 0° C (Anton & Sayre, 1962) and stored at −18° C until estimation or chromatography was performed. The organs of two or three rats were pooled for each extract. The recoveries of NA and of α-M-NA added to tissue homogenates averaged 88% (82–102%) and 91% (85–104%), respectively. No corrections for incomplete recovery have been made.

(*b*) *Separation of catecholamines.* Some eluates obtained from animals which had been given α-M-DOPA or α-M-NA were chromatographed (Maître, 1965) in order to separate the catecholamines, which have different fluorescent or biological activities.

(*c*) *Estimation of catecholamines.* The NA content of all eluates was estimated according to the physico-chemical method of von Euler & Lishajko (1959) except that 10 N NaOH was used rather than 5 N NaOH. Fluorescence of the trihydroxyindoles was measured in a Farrand spectrofluorometer. Each extract was estimated by duplicate analysis. A known amount of NA was added to an aliquot part of each extract. The eluates of organs from animals treated with

α-M-DOPA or with α-M-NA were also estimated on the blood pressure of the pithed rat. Since α-M-NA gives a very weak fluorescence (about 4%) as compared with that of NA and since both amines are equally active on the blood pressure of the pithed rat, it is possible to differentiate between NA and α-M-NA in the same extract by using both assay procedures (Muscholl & Maître, 1963).

The eluates from the chromatogram strips were lyophylized and their amine content was estimated according to the methods described above.

Comparison of the cardiovascular activities of NA, α-M-NA and metaraminol

(*a*) *Pressor activities in acute experiments.* In conscious rats blood pressure was measured in the abdominal aorta with a Statham pressure transducer. Test substances were injected through a cannula implanted in a jugular vein. Arterial cannulas were implanted under pentobarbitone-Na anaesthesia (50 mg/kg intraperitoneally) according to the method of Weeks & Jones (1960), 3–5 days before the experiment. At the same time, saline-filled, stoppered polyethylene tubing of 0.1 mm internal diameter was inserted into a jugular vein and led subcutaneously to the nape of the neck. During the experiment the animals were placed in plexiglas restraining cages, and the arterial cannulas were filled with physiological saline solution containing heparin.

The test substances and doses used were:

L-noradrenaline hydrochloride: 1, 2.2 and 5 μg/kg. L-alpha-methyl-noradrenaline hydrochloride: 5, 11, and 56 μg/kg. L-metaraminol bitartrate: 25, 56 and 123 μg/kg.

All substances were injected in a volume of 1 ml. physiological saline solution/kg body weight. The order of injection of the three substances was randomized. Nine values were obtained for each dose of each substance.

(*b*) *Positive inotropic activities in the isolated rat heart.* The hearts of normotensive rats of 250–410 g body weight were removed under urethane anaesthesia (1.5 g/kg intraperitoneally). They were perfused according to the method of Langendorff with a pressure of 60 cm H_2O at 36° C with Locke solution containing 0.01 g/l. ascorbic acid and saturated with a mixture of 5% CO_2 and 95% O_2.

The force of contraction of the spontaneously beating heart was measured with a force displacement transducer (Grass Model FT .03) attached to the tip of the heart. An initial load of 0.2–0.5 g was applied. After an initial control period of 20 min, the hearts were perfused for 10 min with Locke solution containing the test substance.

The following substances and concentrations were used:

D,L-noradrenaline hydrochloride: 1×10^{-9}, 3×10^{-9} and 1×10^{-8} g/ml. L-alpha-methyl-noradrenaline hydrochloride: 1×10^{-9}, 3×10^{-9} and 1×10^{-8} g/ml. L-metaraminol bitartrate: 1×10^{-7}, 3×10^{-7} and 1×10^{-6} g/ml.

Five hearts were perfused with each concentration of each substance.

(*c*) *Pressor responses after pretreatment with α-M-DOPA, α-M-NA, α-MMT and metaraminol.* The treatment schedule for a period of 10 days was as follows:

D,L-alpha-methyl-DOPA: 300 mg/kg/day per os. L-alpha-methyl-noradrenaline hydrochloride: 1 mg/kg subcutaneously twice daily. D,L-alpha-methyl-meta-tyrosine: 30 mg/kg/day per os. L-metaraminol bitartrate: 1 mg/kg/day subcutaneously.

Sixteen hours after the last injection of α-M-NA or 24 hr after the injection of the other three substances a dose-response relationship was established in the anaesthetized rat. Male rats of 180–250 g body weight were anaesthetized with urethane (1.7 g/kg subcutaneously), and blood pressure was measured in a carotid artery with a mercury manometer. Test substances were injected into a cannulated jugular vein.

In the rats pretreated with α-M-DOPA or α-M-NA, the pressor response to NA or α-M-NA was determined. In the rats pretreated with α-MMT or metaraminol the response to NA or metaraminol was established. For each pressor substance and pretreatment a group of 9–11 rats was used. The pressor effects of NA and the " false neurotransmitters " were compared with the pressor effects in saline-pretreated control animals.

Influence on the pressor response to eserine

In the urethane-anaesthetized rat eserine produces a prolonged increase in blood pressure. According to Lešić & Varagić (1961) this pressor effect is the result of stimulation of central sympathetic centres; they found that reduction of central sympathetic tone or impairment of sympathetic transmission results in partial or complete suppression of the pressor response to eserine.

Normal male rats of 180–250 g body weight were treated for 10 days with the same substances and doses as in the above investigation of the antihypertensive activity.

Sixteen hours after the last α-M-NA injection, or 24 hr after injection of the other substances, the pressor response to eserine was tested. Blood-pressure measurement was performed as described above. The test dose of eserine salicylate was 150 μg/kg intravenously. For evaluation of the eserine response the maximal increases in blood pressure were measured.

The statistical methods of Snedecor (1946), Lord (1947), Hogben (1964), and Warner (1964) were used.

RESULTS

Antihypertensive effects in renal hypertensive rats

α-M-DOPA given orally or subcutaneously reduced blood pressure. Oral doses approximately three times as high as the subcutaneous doses were required to produce a similar antihypertensive effect (Fig. 1). After oral administration the fall in blood

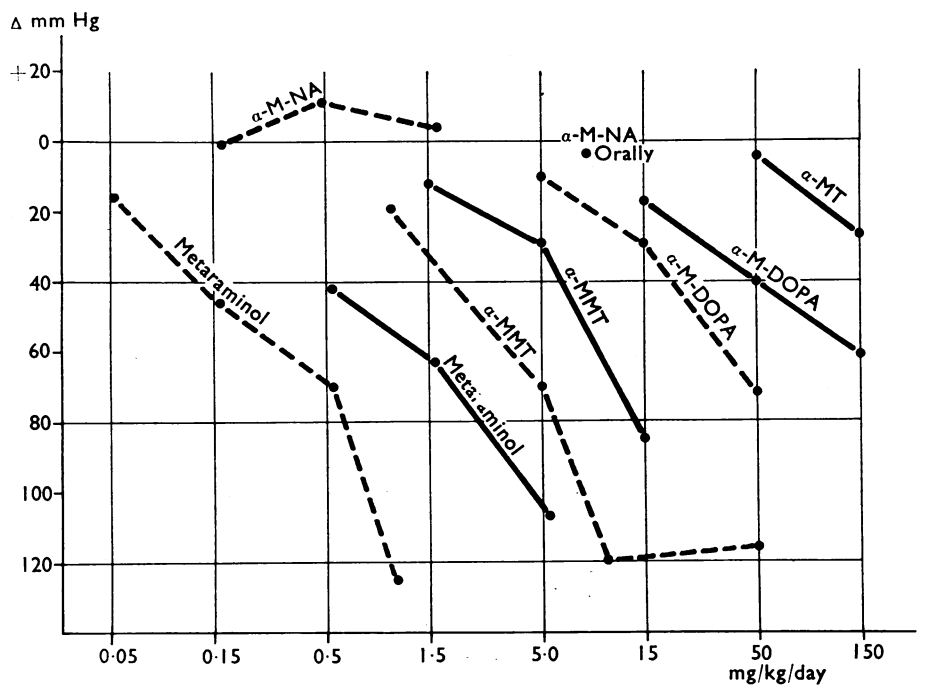

Fig. 1. Antihypertensive effects of metaraminol, α-methyl-meta-tyrosine (α-MMT), α-methyl-noradrenaline (α-M-NA), α-methyl-DOPA (α-M-DOPA) and α-methyl-tyrosine (α-MT) in the renal hypertensive rat. —=after oral administration; — — —=after subcutaneous administration. Blood pressure was measured 2 hr after the administration on the 11th day of treatment. The ordinate represents the mean difference in blood pressure from initial control values. In order to permit comparison of the effects of the various substances, the abscissa gives the dose on the basis of L-isomer of the base or amino acid on a log scale.

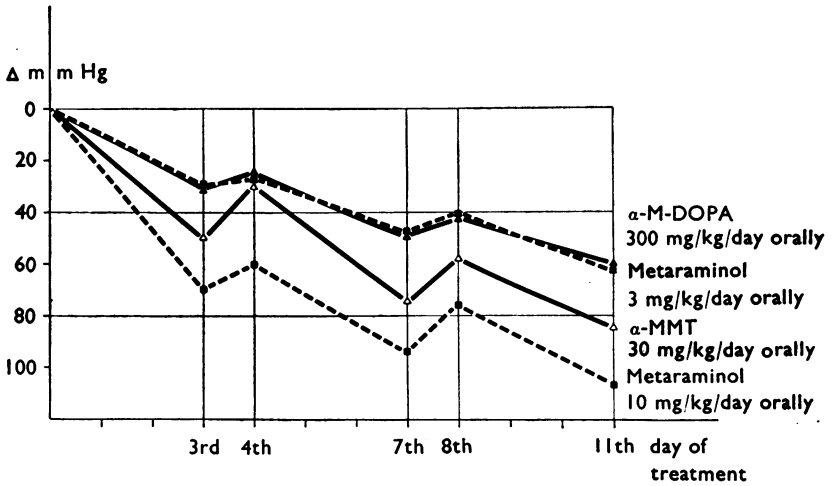

Fig. 2. Antihypertensive effects during 11 days' treatment with metaraminol, α-methyl-meta-tyrosine (α-MMT) or α-methyl-DOPA (α-M-DOPA) in the renal hypertensive rat. Blood pressure was measured 2 hr after the daily administration on the 3rd, 7th and 11th day of treatment and before the daily administration on the 4th and 8th day of treatment. Only the doses and substances which produced a decrease in blood pressure of more than 50 mm Hg on the 11th day of treatment are given. The ordinate represents the mean decrease in blood pressure from initial control values; the abscissa the day of treatment.

pressure was more definite on the 11th than on the 3rd day of treatment (Fig. 2). After treatment with 100 mg/kg subcutaneously, the antihypertensive effect was more pronounced 2 hr after the injection on the seventh day of treatment than 22 hr later (Table 1).

α-M-NA had no effect on blood pressure after doses up to 10 mg/kg orally once daily or 0.3 mg/kg subcutaneously twice daily. After the maximal tolerated dose, 1 mg/kg subcutaneously twice daily, an antihypertensive effect was seen on the 4th and 8th days of treatment, when blood pressure was measured 16 hr after the last injection. When blood pressure was measured 2 hr after the last injection, values were in the same range as the initial control values (Table 1).

The fall in blood pressure produced by this dose of α-M-NA may be the result of damage to the myocardium. Macroscopically visible focal necroses of the myocardium were seen when the animals were sacrificed. No such changes were seen in the other treatment groups.

α-MMT showed an antihypertensive effect qualitatively similar to that of α-M-DOPA. Here also the subcutaneous route of administration was approximately three times more effective than the oral route. After oral as well as subcutaneous administration, α-MMT was approximately 10 times more active than α-M-DOPA (Fig. 1 and Table 1). The antihypertensive effect was more pronounced 2 hr after injection than 22 hr later (measured on the 3rd to 4th and 7th to 8th day of treatment, Table 1). The differences between the blood pressure values measured on the 3rd and 4th and on the 7th and 8th days of treatment were greater than the corresponding differences seen after treatment with other substances, and were particularly pronounced after subcutaneous administration (Table 1).

TABLE 1

ANTIHYPERTENSIVE EFFECTS OF α-METHYL-DOPA (α-M-DOPA), α-METHYL-NORADRENALINE (α-M-NA), α-METHYL-META-TYROSINE (α-MMT), METARAMINOL AND α-METHYL-TYROSINE (α-MT) IN THE RENAL HYPERTENSIVE RAT

Mean blood pressure values in mm Hg and standard errors ($\bar{x} \pm s\bar{x}$) are given. The blood pressure levels on the 11th day of treatment are compared with the initial control values. For α-M-NA the values on the 8th day of treatment are compared with the control values. * Significantly different at $P<0.05$; ** at $P<0.01$; *** at $P<0.001$. n=number of animals in each group

Treatment (mg/kg/day)	n	Control values	Day of treatment: 3rd 2 hr after last inj.	4th before daily inj.	7th 2 hr after last inj.	8th before daily inj.	11th 2 hr after daily inj.
α-M-DOPA							
30	6	193± 8	186±10	184±10	181±12	182±10	176±10
Orally 100	10	204± 7	179± 6	186± 7	165± 4	181± 9	164± 8**
300	17	199± 4	168± 4	174± 5	150± 4	157± 5	138± 4***
10	6	193± 2	185± 4	193± 4	183± 2	184± 3	183± 5
s.c. 30	6	200± 8	188± 8	183±10	177±10	172±10	171±10*
100	8	198± 8	130± 5	150± 6	131± 7	158± 4	126± 6***
α-M-NA							
Orally 10	6	198± 5	199±12	184± 7	192± 6	190± 3	195± 6
0·2	6	203± 7	203± 5	202± 5	196± 5	197± 6	202± 6
s.c. 0·6	12	204± 5	204± 6	190± 5	206± 6	200± 5	215± 6
2·0	6	203± 7	187±10	160± 7	193±10	165±10**	207±10
α-MMT							
3	6	200± 9	190±10	192±10	188±11	187± 9	188±11
Orally 10	6	200± 8	191±11	195±11	183±11	168±14	171±14
30	6	198± 3	148± 3	168± 6	123± 7	140± 6	113± 9***
2	4	200± 2	186±11	201± 8	193± 6	196± 6	181± 8*
10	6	202±10	188±10	177±10	149±14	158±14	132±17***
20	4	209±13	136±11	148±16	86± 2	144±17	89± 6***
100	4	206± 7	131±18	183± 7	105±16	169±24	90±10***
Metaraminol							
1	6	172± 2	158± 6	153± 4	131± 3	133± 6	130± 3***
Orally 3	6	208± 7	178± 6	181± 8	160± 8	167±10	145±11***
10	6	209± 8	139± 7	149± 9	115±13	133± 9	102± 7***
0·1	5	197±11	186±12	181±12	177±10	185±11	181±13
s.c. 0·3	6	192±13	177± 7	170±11	156±14	161±16	146±13*
1·0	6	193±10	157±11	151± 8	140± 9	141±10	123± 6***
2·0	3	203±15	113± 7	90± 7	88±15	118± 7	78± 5***
α-MT							
100	4	180± 6	169± 5	161± 8	166± 6	159± 8	176±11
Orally 300	6	178± 4	155± 4	157± 6	147± 5	138± 6	151± 9*

Metaraminol was the most active antihypertensive agent among the compounds tested. On the 11th day of treatment it was approximately 10 times more effective than α-MMT and approximately 100 times more active than α-M-DOPA (Fig. 1). As with the other agents tested, the antihypertensive effect increased with the duration of treatment (Fig. 2). The subcutaneous route of administration was approximately three times more effective than the oral route.

α-MT reduced blood pressure only slightly after 300 mg/kg/day orally (Fig. 1, Table 1).

Catecholamine content in heart and brain

α-M-DOPA. In the heart, after a course of treatment lasting 11 days, a dose-dependent NA depletion ranging from 60 to 81% was found. An average α-M-NA content of 1.3 μg/g wet heart tissue was determined after treatment with α-M-DOPA 300 mg/kg/day orally. This amount corresponded to 150–200% of the total cardiac catecholamine content in normal animals (Fig. 3 A). Approximately the same degree of catecholamine depletion was found in the brain as in the heart. Likewise, after 300 mg/kg/day α-M-DOPA orally, the total catecholamine content was found to be enhanced to the same extent as in the heart.

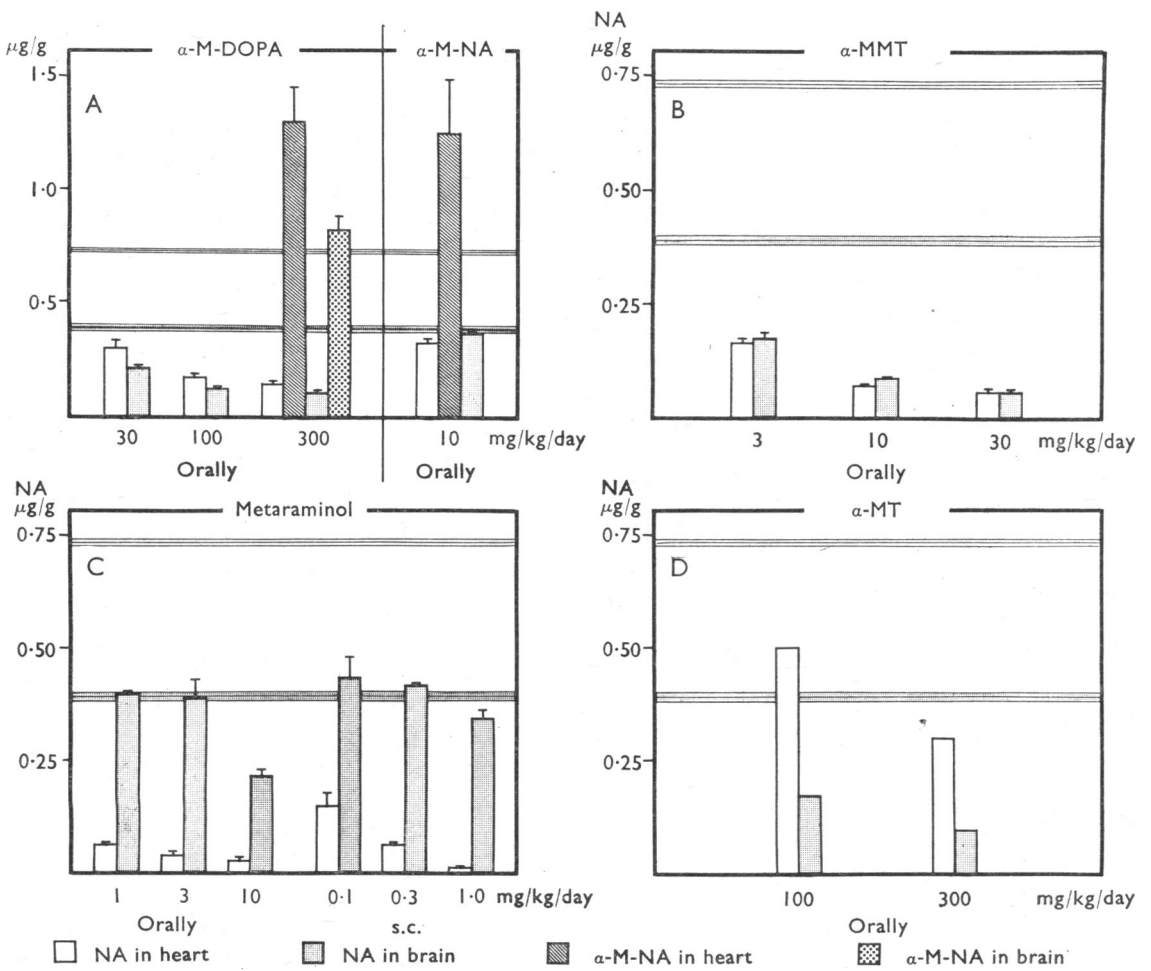

Fig. 3. Noradrenaline (NA) and α-methyl-noradrenaline concentrations in the heart and brain of renal hypertensive rats after 11 days' treatment with α-methyl-DOPA (α-M-DOPA) or α-methyl-noradrenaline (α-M-NA) (A); α-methyl-meta-tyrosine (α-MMT) (B); metaraminol (C) or α-methyl-tyrosine (α-MT) (D). Values are means for pooled extracts from 2–3 animals each. Two extracts were taken from each α-MT treatment group and 3–5 extracts from all other groups. The cross bars represent standard errors. Concentrations in control animals are given as corresponding horizontal lines.

α-M-NA. In the heart 10 mg/kg/day α-M-NA orally reduced the NA content by 55%. As after treatment with α-M-DOPA, total catecholamines corresponded to 150–200% of the normal catecholamine content due to the incorporation of 1.25 μg/g α-M-NA. No change in brain NA-content was found. The level of α-M-NA in the brain was less than 0.03 μg/g (Fig. 3 A).

α-MMT. A dose-dependent noradrenaline depletion was found in the heart and brain. A maximal depletion of approximately 90% was produced in the heart by 10 or 30 mg/kg/day orally. This corresponds to the depletion found after 10 mg/kg/day subcutaneously. In the brain the catecholamine depletion was somewhat less pronounced than in the heart, and reached a maximum of 86% after 30 mg/kg/day orally (Fig. 3 B).

Metaraminol. 1 mg/kg/day orally produced the same degree of catecholamine depletion in the heart as 10 or 30 mg/kg/day α-MMT orally. A maximal depletion of 95% was reached after the two highest doses of metaraminol. After subcutaneous injection of 0.1, 0.3 or 1.0 mg/kg/day of metaraminol the NA content of the heart was reduced by 80% to more than 95%. In the brain no reduction in NA content was found after doses of 1 or 3 mg/kg/day orally and 0.1 or 0.3 mg/kg/day subcutaneously. However, after 10 mg/kg/day orally or 1 mg/kg/day subcutaneously a slight but significant, reduction of the brain content was observed (Fig. 3 C).

After 4 days' treatment with 0.3 or 1.0 mg/kg/day subcutaneously the NA content was reduced to very low levels (Table 2). Although the difference between the NA content in the heart after 4 or 11 days' treatment was found to be statistically significant, the accuracy of the measured NA values is uncertain at these very low concentrations.

TABLE 2

NORADRENALINE CONTENT OF HEART AND BRAIN OF RENAL HYPERTENSIVE RATS AFTER TREATMENT WITH METARAMINOL FOR 4 OR 11 DAYS

Values given are means and standard errors ($\bar{x} \pm s_{\bar{x}}$). n=number of extracts each from 2–3 animals

Treatment		n	Noradrenaline μg/g wet tissue	
			Heart	Brain
0·9% NaCl solution		5	0·790±0·04	0·410±0·013
Metaraminol				
0·3 mg/kg/day; s.c.	4 days	3	0·071±0·003	0·440±0·014
	11 days	3	0·061±0·004	0·415±0·005
Metaraminol				
1·0 mg/kg/day; s.c.	4 days	3	0·041±0·003	0·397±0·041
	11 days	3	0·011±0·004	0·343±0·017

α-MT. In these experiments the NA content was estimated only fluorometrically. The values are expressed as NA without correction for the presence of other catecholamines which might be formed after α-MT administration.

α-MT produced a dose-dependent reduction of the NA content in heart and brain. The extent of depletion, however, was considerably less pronounced than that produced by α-M-DOPA or α-MMT. In contrast to the other α-amino acids α-MT produced a more pronounced NA depletion in the brain than in the heart. In the heart an average depletion of 32% and 59% was found after 100 mg/kg/day orally and 300 mg/kg/day orally respectively. In the brain the depletion was 56% and 75% respectively (Fig. 3 D).

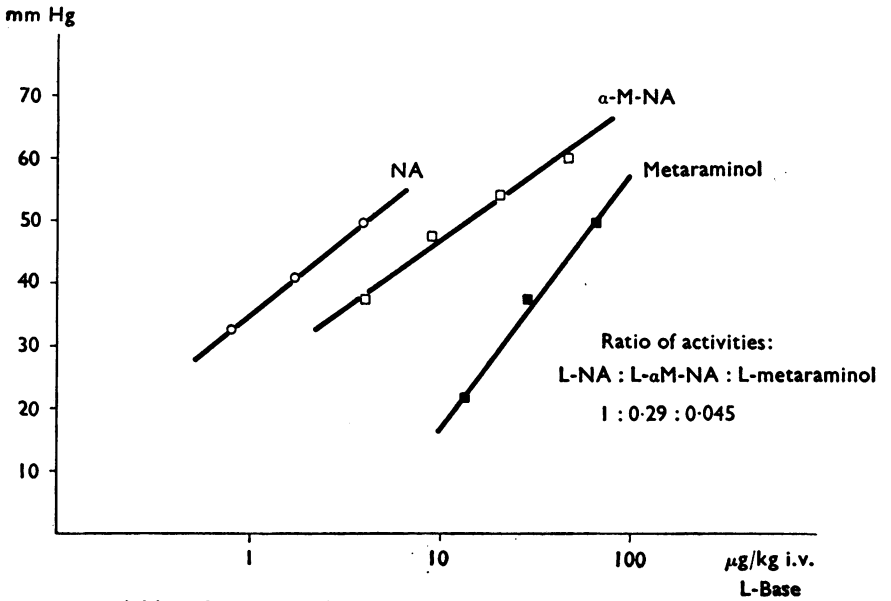

Fig. 4. Pressor activities of noradrenaline (NA), α-methyl-noradrenaline (α-M-NA) and metaraminol in the unanaesthetized rat. The regression lines were established on the basis of 3–4 doses per substance and nine values for each dose. The ordinate represents the maximal increase in blood pressure; the abscissa the log dose of L-isomer base on a log scale.

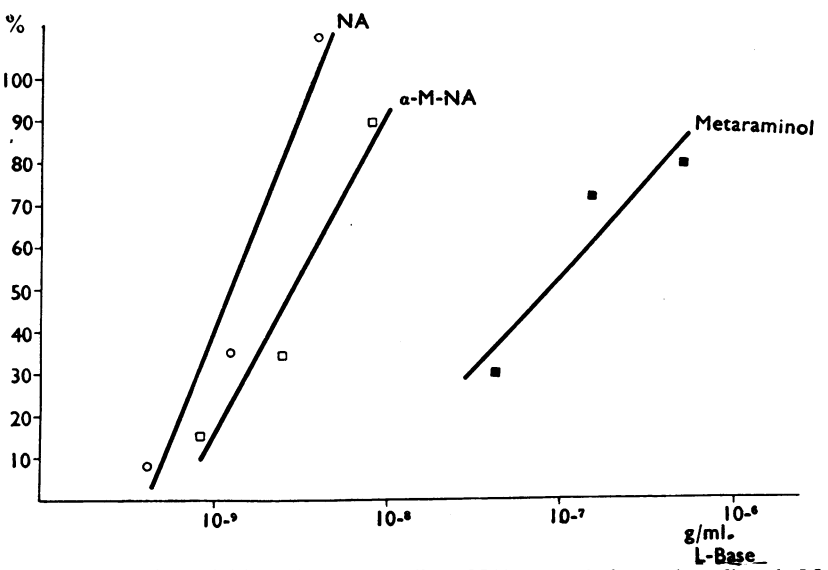

Fig. 5. Positive inotropic activities of noradrenaline (NA), α-methyl-noradrenaline (α-M-NA) and metaraminol in the isolated rat heart (Langendorff method). The regression lines were established on the basis of three concentrations per substance and five values for each concentration. The ordinate represents the increase in contractile force reached 10 min after beginning of the perfusion with the test substance expressed as % of the control values measured before perfusion with each test substance. The abscissa gives the concentration on the basis of L-isomer and base on a log scale.

Comparison of the cardiovascular activities of NA, α-M-NA and metaraminol

(a) Pressor activities in acute experiments. In the conscious rat the relative pressor activities of NA, α-M-NA and metaraminol injected intravenously were estimated from the dose-response regression lines (Fig. 4). The relationship of NA : α-M-NA : metaraminol was 1 : 0.29 : 0.045.

(b) Positive inotropic activities. On the isolated rat heart the relationship of NA : α-M-NA : metaraminol was 1 : 0.33 : 0.010 (Fig. 5). Heart rate was not influenced by the concentrations used, with the exception of the intermediate NA concentration, which reduced heart rate by 10 ± 2%.

(c) Pressor responses after pretreatment with α-M-DOPA, α-M-NA, α-MMT and metaraminol. In the urethane-anaesthetized rat the relative pressor activities of NA, α-M-NA and metaraminol, estimated from the dose-response regression lines, approximated to 1 : 0.29 : 0.11.

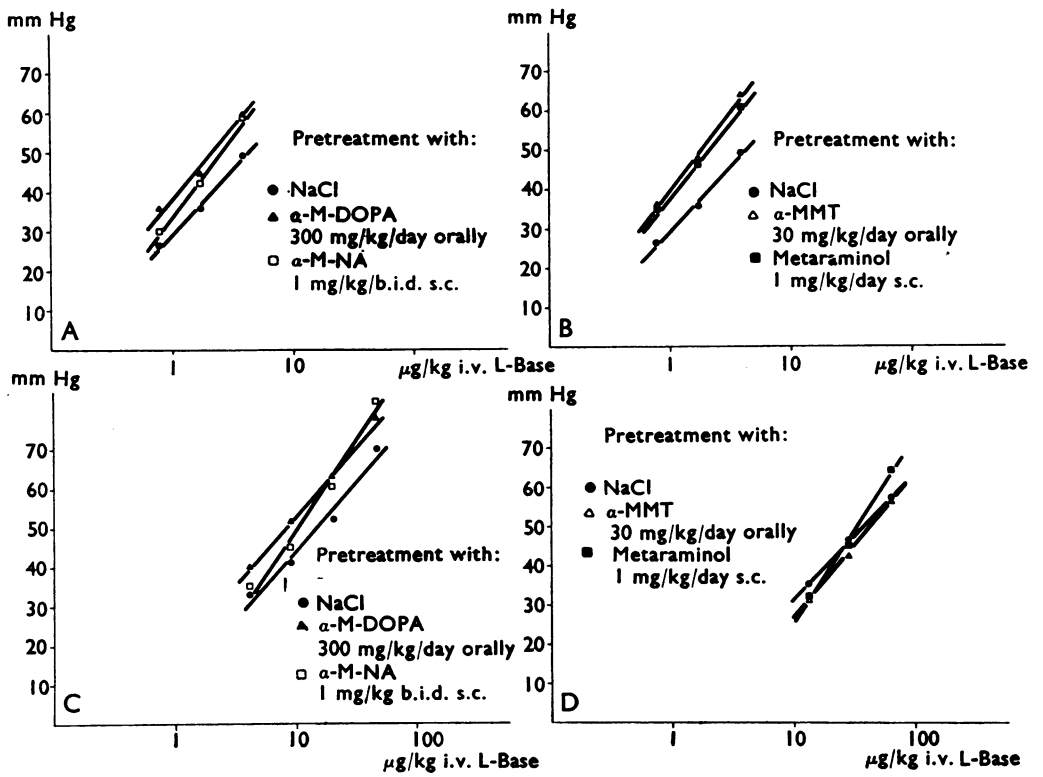

Fig. 6. A and B: pressor activity of noradrenaline in urethane-anaesthetized rats pretreated for 10 days with α-methyl-DOPA (α-M-DOPA) or α-methyl-noradrenaline (α-M-NA) (A), α-methyl-meta-tyrosine (α-MMT) or metaraminol (B). Fig. 6, C and D: pressor activity of α-methyl-noradrenaline in rats pretreated for 10 days with α-methyl-DOPA or α-methyl-noradrenaline (C) and pressor activity of metaraminol in rats pretreated for 10 days with α-methyl-meta-tyrosine or metaraminol (D). Regression lines were established on the basis of three doses per substance. The ordinates represent the maximal increases in blood pressure. The abscissae give the doses of the test substances expressed as L-isomer base on a log scale.

Pretreatment with α-M-DOPA, α-M-NA, α-MMT or metaraminol potentiated the pressor response to NA significantly (Fig. 6 A and B). In all cases $P<0.05$.

The response to α-M-NA was potentiated by pretreatment with α-M-DOPA or α-M-NA to approximately the same extent as NA ($P<0.05$) (Fig. 6 C).

After pretreatment with α-MMT or metaraminol, however, the pressor response to metraraminol remained unchanged (Fig. 6 A).

Pressor responses to eserine

After pretreatment with α-M-DOPA for 10 days the pressor response to eserine was reduced ($P<0.01$). Pretreatment with α-M-NA, α-MMT or metaraminol had no effect on the pressor response to eserine (Table 3).

TABLE 3

INFLUENCE OF 10 DAYS' PRETREATMENT WITH α-METHYL-DOPA (α-M-DOPA), α-METHYL-NORADRENALINE (α-M-NA), α-METHYL-META-TYROSINE (α-MMT) OR METARAMINOL ON THE PRESSOR RESPONSE TO ESERINE IN THE URETHANE-ANAESTHETIZED RAT

Values given are the mean increases in blood pressure and standard errors ($\bar{x} \pm s_{\bar{x}}$). n=number of experiments. **=significantly different from control values at $P<0.01$

Pre-treatment (mg/kg/day)	n	Pressor response to 150 μg/kg eserine salicylate i.v. (mm Hg)
0·9% NaCl solution	15	41·5±3·0
α-M-DOPA 300 orally	10	26·5±4·9**
α-M-NA 2 s.c.	11	41·8±4·8
α-MMT 30 orally	10	44·6±6·2
Metaraminol 1 s.c.	8	35·4±2·5

DISCUSSION

All substances showed antihypertensive activity. α-M-NA, however, was only active after the highest subcutaneous dose (1 mg/kg b.i.d.) and only 16 hr after injection. At this time the hypotensive effect corresponded to that of 100 mg/kg α-M-DOPA daily subcutaneously. The order of increasing hypotensive activity of the other substances 2 or 24 hr after oral or subcutaneous application was: α-MT (only given orally), α-M-DOPA, α-MMT and metaraminol. The relative efficacy of these substances was approximately: 0.002, 0.01 and 0.1 to 1.

In the case of α-MMT and, to a lesser extent, α-M-DOPA the blood-pressure values measured 2 hr after application were lower than those measured 24 hr after application. After treatment with the only effective dose of α-M-NA, on the other hand, the values measured 2 hr after application were higher than those measured 16 hr after application. No such differences were seen in the blood-pressure values after treatment with metaraminol or α-MT.

The higher blood-pressure values measured 2 hr after injection of α-M-NA may be the result of a relatively short-lasting increase in blood pressure, superimposed on a long-lasting decrease in blood pressure. No such differences were seen in the blood-pressure values measured 2 and 24 hr after administration of metaraminol. This may be explained by the fact that, in the rat, metaraminol is a much less potent pressor agent than α-M-NA.

The differences in the blood-pressure values measured 2 hr and 24 hr after administration of α-M-DOPA and α-MMT are more difficult to interpret. Blood pressure values

were not measured before administration of the test substances on the 3rd and 7th days of treatment; but assuming that the blood-pressure levels before administration on the 3rd and 4th and on the 7th and 8th day of treatment were in the same range, the administration on the 3rd and 7th days of treatment produced an additional decrease in blood pressure. According to the "false transmitter" hypothesis, α-M-DOPA (Day & Rand, 1964) and α-MMT (Shore, Alpers & Busfield, 1966) are the precursors of the active substances, α-M-NA and metaraminol. If this is the case, it is surprising that the precursor, α-MMT, should produce a more pronounced transient effect than the active agent, metaraminol.

It has been suggested (Farmer, 1965) that α-M-DA may play a role in the antihypertensive effect of α-M-DOPA. The formation and accumulation of α-M-DA in the brain has been shown to be very rapid, while after 24 hr, only small amounts are still present (Carlsson & Lindqvist, 1962). The presence of this metabolite may explain the more pronounced antihypertensive effect of α-M-DOPA 2 hr after administration. Marked but transient impairment of sympathetic nerve function has been found after injection of α-M-DOPA in acute experiments, while chronic treatment was less effective (Sugarman, Margolius, Bariso & Gaffney, 1965). Furthermore, after blockade of dopamine β-hydroxylase, when only α-M-DA and not α-M-NA can be formed, a-M-DOPA has been found to inhibit contractions of the nictitating membrane and of the spleen produced by electrical stimulation (Thoenen, Haefely, Gey & Hürlimann, 1966a). A similar factor may be involved in the action of α-MMT.

In contrast to findings in man (Sjoerdsma, Engelman, Spector & Udenfriend, 1965) and in the normotensive cat, rat and guinea-pig (Spector *et al.*, 1965), α-MT reduced blood pressure in the present investigation. The intensity of the antihypertensive effect was less pronounced than that of α-M-DOPA.

No correlation was found, between the degree of NA depletion and the intensity of the antihypertensive effect. Metaraminol produced a similar decrease in myocardial NA content on the 4th and on the 11th day of treatment, while the effect on blood pressure increased during this time. An 80% depletion of myocardial NA was found after administration of a dose of α-MMT which did not influence blood pressure, but a dose of α-M-DOPA which produced an 80% NA depletion reduced blood pressure markedly.

In the groups which received the highest oral and subcutaneous doses of metaraminol, a decrease in brain NA content was found. This is not necessarily a direct effect of metaraminol, but may be due to the same mechanism postulated for guanethidine—that is, that the fall in blood pressure produced by prolonged administration of guanethidine may cause reflex stimulation of the central nervous system and indirect release of brain catecholamines (Sanan & Vogt, 1962).

After treatment with α-M-DOPA, the total catecholamine content of the brain and the heart was higher than normal, and α-M-NA produced a similar increase in the total catecholamine content of the myocardium. This increase in the total catecholamine content may be due to a higher affinity of α-M-NA for the tissue stores (Lindmar & Muscholl, 1965) as well as to the fact that α-M-NA is not metabolized by MAO (Blaschko, Richter & Schlossmann, 1937). Apparently α-MT depletes tissue NA stores by inhibition of NA synthesis rather than by a displacement mechanism (Spector *et al.*, 1965).

The relative sympathomimetic activities of NA, α-M-NA and metaraminol measured in normal animals may not apply to pretreated animals, as the activities of the " false transmitters " as well as remaining NA may be altered by pretreatment with the " false transmitters " or their precursors. Thus potentiation of the " false " and the " normal " transmitter would tend to decrease the hypotensive effect.

In rats pretreated with α-M-DOPA, α-MMT, α-M-NA or metaraminol, the pressor effect of NA was potentiated. This may be the result of inhibition of NA uptake (Hess, 1962; Iversen, 1964; Ross & Renyi, 1964; Dengler, 1964). The pressor effect of α-M-NA was enhanced to the same extent as that of NA after pretreatment with α-M-DOPA or α-M-NA, but the pressor effect of metaraminol was not influenced by pretreatment with α-MMT or metaraminol. The absence of metaraminol potentiation after pretreatment with α-MMT or metaraminol may enhance the hypotensive effects of these agents.

If the antihypertensive properties of α-M-DOPA, α-MMT and metaraminol are the results of displacement of NA by a " false transmitter," it should be possible to demonstrate inhibition of sympathetic function.

In the urethane-anaesthetized rat, eserine produces a paradoxical increase in blood pressure. This effect, according to the investigation of Lešić & Varagić (1961), is the result of activation of sympathetic centres in the central nervous system, and can be inhibited by agents which impair sympathetic stimulation or transmission. Inhibition of the pressor response to eserine was found only in the α-M-DOPA pretreated animals. In the case of α-MMT the response to eserine may be influenced by the central stimulating properties of this substance (van Rossum, 1963; Carlton, 1963; Carlton & Furgiuele, 1965); α-M-DOPA, on the other hand, produces central inhibition (Sjoerdsma, 1963).

Some experimental evidence for an impairment of sympathetic transmission after treatment with α-M-DOPA or α-MMT has been found (for references see Farmer, 1965; Muscholl, 1966; Haefely, Hürlimann & Thoenen, 1966). After treatment with α-MT, inhibition of the carotid sinus occlusion reflex or sympathetic transmission has been found (Bhagat & Shein, 1965; Thoenen, Haefely, Gey & Hürlimann, 1966b). Various investigators, however, have not found inhibition of sympathetic function or release of NA in animals treated with α-M-DOPA or α-MMT (for references see Muscholl, 1966; Davies, 1966).

In the present investigation α-M-DOPA and α-M-NA produced similar accumulation of α-M-NA in peripheral catecholamine stores. The antihypertensive effects differed, which does not support the hypothesis that α-M-NA is the active agent. It cannot be decided whether the difference in the extent of NA depletion after treatment with α-M-DOPA or α-M-NA is great enough to explain the difference in the effect of these substances on blood pressure. α-MMT and metaraminol, on the other hand, produced similar effects on peripheral catecholamine stores and on blood pressure. Thus, it would seem possible that metaraminol is the active agent.

SUMMARY

1. In renal hypertensive rats prolonged treatment with alpha-methyl-DOPA (α-M-DOPA), alpha-methyl-meta-tyrosine (α-MMT), alpha-methyl-tyrosine (α-MT)

or metaraminol produced dose-dependent decreases in blood pressure. Alpha-methyl-noradrenaline (α-M-NA) was active only after a toxic dose.

2. All substances produced dose-dependent depletion of myocardial catecholamines. The same order of relative activities was found in regard to NA depletion and antihypertensive effect, but no equivalence was found between the degree of catecholamine depletion and the intensity of the hypotensive effect. After pretreatment with α-M-DOPA or α-M-NA, the total catecholamine content of the tissue stores exceeded the normal noradrenaline (NA) content. Only the amino acids depleted brain catecholamines markedly.

3. In the normotensive, unanaesthetized rat metaraminol showed less pressor activity and in the isolated rat heart less positive inotropic activity than α-M-NA or NA. The relative pressor activities of these substances were unchanged after prolonged pretreatment with α-M-DOPA, α-MMT, α-N-NA or metaraminol in the normotensive, anaesthetized rat. The pressor effect of NA was potentiated after prolonged pretreatment with all substances studied, and the pressor effect of α-M-NA was potentiated after pretreatment with α-M-DOPA or α-M-NA. The pressor effect of metaraminol, however, was not influenced by pretreatment with α-MMT or metaraminol.

4. Inhibition of the pressor response to eserine, which may be due to impairment of sympathetic function, was found only after pretreatment with α-M-DOPA.

5. The results of the present study suggest that the effects of α-MMT and its metabolite are similar, while different effects are produced by α-M-DOPA and its metabolite, α-M-NA.

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