AUTONOMIC NERVOUS SYSTEM: NEWER MECHANISMS OF ADRENERGIC BLOCKADE[1,2]

By E. Muscholl
Department of Pharmacology, University of Mainz, Mainz, Germany

In this review an attempt will be made to present recent developments in the field of adrenergic blockade concerning α-methyl amino acids such as α-methylDOPA (methylDOPA), α-methyl-*m*-tyrosine (α-MMT), and α-methyl-*p*-tyrosine (α-MPT). Some pharmacological effects of these compounds have been known for several years but intensive research into their mode of action was started only when Oates et al. discovered that methylDOPA was a valuable antihypertensive agent (1). For the sake of conciseness, this review does not include adrenolytic drugs or reserpine. Furthermore, adrenergic neuron blocking agents are not dealt with since this subject has been reviewed comprehensively by Boura & Green (2). The evidence for an interference with sympathetic transmission by methylDOPA is mainly indirect and principally based on clinical experience with hypertensive patients. Pharmacological experiments revealed much slighter effects on adrenergic transmission than with the adrenergic neuron blocking agents or with reserpine. Despite several years of concentrated research, the mode of action of methylDOPA has not been established. However, the conversion of this drug into α-methyl amines, which in many respects behave like the natural transmitter norepinephrine (NE), has aroused great interest. Many findings concerning the mechanism of action of methylDOPA have been corroborated by experiments with close chemical congeners of this drug. These will therefore be included in the following discussion.

Antihypertensive Action of α-Methyl Amino Acids

In 1954 Sourkes (3) found that methylDOPA is a competitive inhibitor of the decarboxylation of DOPA to DOPAmine *in vitro*. MethylDOPA inhibits amino acid decarboxylase *in vivo* both in animals (4, 5, 6) and in man (1, 7, 8). Although decarboxylation of DOPA is an established step in the biosynthesis of NE (9), inhibition of decarboxylase activity by a drug was not necessarily expected to decrease the NE content of sympathetic neurons (10), since this process is very efficient and not the

[1] The survey of the literature pertaining to this review was concluded in May 1965.

[2] The following abbreviations will be used: NE (norepinephrine); DOPA (3,4-dihydroxyphenylalanine); MethylDOPA (α-methyl-3,4-dihydroxyphenylalanine); α-MMT (α-methyl-meta-tyrosine); α-MPT (α-methyl-para-tyrosine); α-MNE (α-methyl norepinephrine); α-MEpi (α-methyl epinephrine); DMPP (dimethylphenyl-piperazine).

rate limiting step in NE synthesis (6, 11). Nevertheless, methylDOPA was found to be an effective antihypertensive drug [Oates et al. (1)], and this was confirmed by many clinical studies (8, 12–16).

MethylDOPA is less potent than ganglion-blocking drugs or guanethidine but more potent than reserpine or thiazide diuretics (12, 13). Sympathetic blockade by methylDOPA is indicated by symptoms such as bradycardia and postural hypotension (8, 12, 13, 14, 16), by reduction in overshoot of the blood pressure after the Valsalva maneuver (13), and by a decreased cardiovascular response to exercise (17) or psychogenic stimuli (18). Decrease in blood pressure of hypertensive subjects is caused mainly by reduction of peripheral vascular resistance, whereas changes in cardiac output are generally minor (19). The drowsiness experienced by many patients, especially in the first week of treatment, suggests that methylDOPA has a central action. However, in spite of continued drug therapy, the drowsiness wears off, whereas the hypotension persists (12, 13, 16).

The hypotensive action of methylDOPA is not easily demonstrable in animal experiments. However, either large doses administered to conscious dogs (20, 21) or repeated doses given to renal hypertensive rats (21, 22) have been shown to lower mean arterial blood pressure.

Horwitz & Sjoerdsma found recently that the noncatechol compound α-MMT decreased blood pressure in hypertensive patients (23). Oral administration of up to 8.0 g per day was not effective, probably because intestinal absorption of the drug was insufficient. However, intravenous infusions of 0.5 or 1.0 g of D,L-α-MMT decreased the blood pressure of hypertensive subjects both in recumbent and in standing positions. These patients were selected because of their known responsiveness to equivalent doses of methylDOPA. Chronic administration of subeffective doses of methylDOPA enhanced the blood pressure reduction produced by α-MMT. Confirmatory evidence came recently from studies using renal hypertensive rats which responded to repeated subcutaneous doses of 20 mg/kg α-MMT with a marked decrease in blood pressure (24).

Significance of Decarboxylase Inhibition

The following experiments provide evidence that inhibition of DOPA decarboxylase is not the cause of the antihypertensive action of methylDOPA. Administration of decarboxylase inhibitors more potent than methylDOPA such as α-methyl-DOPA-hydrazine (25) or 4-bromo-3-hydroxybenzyloxyamine (26), did not lower the blood pressure of hypertensive patients (10, 26). Moreover, the urinary excretion of 3-methoxy-4-hydroxymandelic acid was not found to have decreased in patients successfully treated with methylDOPA (10, 15, 27, 28) as one would expect if NE synthesis was inhibited.

MethylDOPA depletes NE stores throughout the body, as discussed

in the following section. A detailed study by Hess et al. (6) revealed that depletion of NE by methylDOPA or α-MMT was not the result of impaired biosynthesis of NE. Both amino acids are decarboxylase inhibitors *in vitro* and *in vivo,* and both lowered the NE levels in hearts and brains of guinea pigs for several days. There was also a depletion of brain serotonin and DOPAmine, but the concentrations of these amines returned to normal much faster than the NE concentrations. The inhibition of decarboxylase activity in guinea pig tissues after α-MMT and the tissue levels of methylDOPA known to inhibit this enzyme paralleled the loss of serotonin and DOPAmine from the brain. However, decarboxylase activity was back to normal and methylDOPA had disappeared from the body long before the NE concentrations had recovered. It was suggested that decarboxylase inhibition may partly account for the decrease in serotonin and DOPAmine levels, but this could not explain the long-lasting depletion of NE. Inhibition of β-hydroxylase activity as another possible cause of NE depletion was ruled out by the observation that α-MMT inhibited this enzyme *in vitro* only in concentrations far too high to be attained *in vivo*. In animals pretreated with α-MMT, the heart NE concentration was increased severalfold either by short-acting monoamine oxidase inhibitors or by an infusion of DOPAmine. These effects were observed at the time when decarboxylase activity was maximally affected. Thus, biosynthesis of NE can occur during this period.

Dissociation of enzyme-inhibiting and NE-depleting activity was also demonstrated by Porter et al. (29). In mice, the potency of drugs inhibiting DOPA decarboxylase increased in this order: α-MMT, methylDOPA, and α-methyl-2,3-DOPA. However, as far as depletion of heart and brain NE was concerned, α-MMT
2,3-DOPA was quite ineffective. Furthermore, Levine & Sjoerdsma (26) found no depletion of heart and brain NE in guinea pigs following administration of the potent decarboxylase inhibitors 4-bromo-3-hydroxybenzyloxyamine (NSD 1055), N-methyl-N-(2-hydroxybenzyl) hydrazine (NSD 1039), and N-methyl-N-(3-hydroxybenzyl) hydrazine (NSD 1034).

Finally, the serotonin-lowering effect of methylDOPA, first observed by Smith (30), is likewise not wholly mediated through inhibition of decarboxylase. Brodie et al. (31) found that the increase in brain serotonin (and DOPAmine) produced by inhibition of monoamine oxidase persisted after pretreatment of the mice with NSD 1034. According to Drain et al. (32) brain serotonin levels in mice were unaltered following administration of the potent decarboxylase inhibitors 3-hydroxybenzyloxyamine (NSD 1024) and NSD 1034, but they were decreased by the comparatively weak inhibitor, methylDOPA. It may be added, however, that in the rat brain methylDOPA possibly interferes with serotonin synthesis since the concentrations of both serotonin and its degradation product 5-hydroxyindoleacetic acid are decreased (33); for further discussion of this point see (34).

Mechanism of Amine Depletion

Depletion of tissue amines by α-methyl amino acids is now well documented. Only those amino acids which are decarboxylated to the corresponding amines are capable of depleting endogenous NE by a process of displacement.

Depletion caused by amine metabolites.—Goldberg et al. reported that in the dog doses of 200 and 500 mg/kg D,L-methylDOPA decreased the NE concentration of the auricles and the serotonin concentration of the caudate nucleus (20). In the same species 100 mg/kg L-methylDOPA or D,L-α-MMT administered once daily for two days lowered the concentrations of catechol amines in brain stem, heart, and spleen by about 50 per cent, whereas the amines of the adrenal gland were not affected (35). Hess et al. (6) suggested that the NE depletion they had observed following methylDOPA and α-MMT (see above) was caused by the decarboxylation products of the α-methyl amino acids. From the work of Lovenberg et al. (36) it became evident that methylDOPA and α-MMT were not only decarboxylase inhibitors but also substrates of this enzyme although the turnover rate is much lower than that of DOPA. Supportive evidence came from the studies of Porter et al. (29) who determined the power of α-methyl amino acids and the corresponding amines to deplete tissue amines in the mouse. On a weight base, α-methyl DOPAmine was more potent than methylDOPA, and α-methyl-*m*-tyramine was more potent than α-MMT in depleting heart NE. This observation was confirmed in the guinea pig (37) and in the rat (38). Furthermore, it was shown that the β-hydroxylated product of α-methyl-*m*-tyramine, metaraminol, was even more active in decreasing heart NE than the parent amine. The doses of α-MMT, α-methyl-*m*-tyramine, and metaraminol, causing a 50 per cent depletion of heart NE, were in the rat 30, 2.0, and 0.08 mg/kg intravenously and in the guinea pig 5.0, 0.8, and 0.15 mg/kg intraperitoneally (37, 38).

Another important finding was the observation that only the L-isomers of methylDOPA and α-MMT produced NE depletion whereas the D-isomers were ineffective (6, 29). This is easily explained by the substrate specificity of the amino acid decarboxylase for L-isomers as observed with DOPA (9), and with α-methyl amino acids both *in vitro* (36) and *in vivo* (8, 10). The latter group of investigators demonstrated also that the blood pressure of hypertensive patients was decreased only by the L-isomer but not by the D-isomer of methylDOPA.

There are several reports which indicate that the α-methyl amino acids must be decarboxylated in order to exert a hypotensive or a NE-depleting effect, thus strengthening the idea of their indirect action. According to Davis et al. (39) the decarboxylase inhibitor NSD 1039 (for chemical name see p. 109) abolished the hypotensive effect of methylDOPA in the renal hypertensive rat. Gessa et al. (38) blocked the NE-depleting effect

of α-MMT in the rat heart and brain by pretreatment of the animals with the decarboxylase inhibitors, NSD 1024, 1034, and 1055 (for chemical names see p. 109). Udenfriend & Zaltzman-Nirenberg (37) used α-methyl-DOPA-hydrazine (25) as a decarboxylase inhibitor. Depending on the dose administered, this agent inhibited the depletion of heart NE in guinea pigs caused by α-MMT up to 90 per cent. At high dose levels α-methyl-DOPA-hydrazine itself lowered the concentration of NE in the heart but, in the calculation of inhibition of depletion, allowance was made for this effect. Levine & Sjoerdsma (26) found that NSD 1055 administered one hour before methylDOPA blocked its heart NE-depleting action in the guinea pig. In contrast, injection of α-methyl DOPAmine was effective in lowering the heart NE concentration independent of NSD 1055 pretreatment. Therefore, NSD 1055 blocked the NE-depleting effect of methylDOPA by inhibiting its decarboxylation rather than by some other mechanism. The same authors showed that α-ethyl DOPA was an inhibitor of decarboxylase but not a substrate for the enzyme. Although administered α-ethyl DOPAmine depleted the guinea pig heart of its NE, α-ethyl DOPA did not lower NE concentrations because it was not decarboxylated *in vivo*. This confirms the idea that α-substituted amino acids are dependent upon decarboxylation for their NE-depleting actions.

It should be noted, however, that inhibition of decarboxylase is not the only point of attack of agents like NSD 1055 or 1034. These have been shown to inhibit DOPAmine-β-hydroxylase as well (see p. 109) and interference with the amine-depleting effect of α-methyl amino acids could result in part from prevention of their conversion to β-hydroxy compounds.

Displacement of norepinephrine.—Further insight into the mechanism of amine depletion was achieved by following up the metabolites of the α-methyl amino acids. The α-methyl amines concerned are good substrates of DOPAmine-β-hydroxylase *in vitro* as discovered by Creveling et al. (11, 40). Evidence for *in vivo* decarboxylation of α-methyl amino acids and subsequent side-chain hydroxylation resulting in displacement of endogenous amines by the newly formed amines was provided by Carlsson & Lindqvist (41). After intraperitoneal injection of 400 mg/kg methylDOPA into mice, the brain concentrations of serotonin and DOPAmine decreased by about 50 per cent within 3 to 6 hours and then rose again to normal values within 24 hours. MethylDOPA could be detected in the brain only for 12 hours. NE was depleted more markedly and for a longer period than DOPAmine. With fluorimetric analysis and by paper chromatography, α-methyl DOPAmine was detected in the brain; the concentrations ranged from 0.5 to 1.0 μg/g between 0.5 and 6 hours after the drug, and they decreased to about 0.2 μg/g in the course of 24 hours. In addition, α-methyl norepinephrine (α-MNE) was detected by paper chromatography 24 hours after a single dose of methylDOPA. After

repeated injections of methylDOPA which entirely depleted the mouse brain of its NE content, the amount of α-MNE found was of the same order of magnitude as the normal NE concentration. Likewise, after four intravenous doses of 200 mg/kg methylDOPA were administered to rabbits, practically all NE disappeared from the brain stem and α-MNE was detected in amounts corresponding to the missing NE. Furthermore, 24 hours after an injection of 400 mg/kg α-MMT into mice, the brain NE had disappeared almost completely and there was chromatographic evidence for the storage of metaraminol which apparently was derived from α-MMT undergoing decarboxylation and β-hydroxylation. These findings suggest that α-MNE, which remains in the brain longer than α-methyl DOPAmine, is responsible for the prolonged decrease of the NE concentration.

Porter & Titus (42) found labeled α-methyl DOPAmine in the brain one hour after injection of 2-^{14}C-methylDOPA into mice and one rat. Urinary excretion of α-methyl DOPAmine after administration of methylDOPA to hypertensive subjects was reported by Sjoerdsma et al. (10) and Buhs et al. (28). The latter authors failed to observe a decrease in blood pressure in three patients who received intramuscular doses of 25 and 50 mg α-methyl DOPAmine. Since the amounts of α-methyl DOPAmine recovered in the urine exceeded those which appeared in the urine when patients were successfully treated with methylDOPA, Buhs et al. concluded that methylDOPA itself rather than its decarboxylation product was the hypotensive agent in these patients. However, injected and biosynthetically formed α-methyl DOPAmine may differ in metabolism and pharmacological effects in such a way as to make observations of this kind very difficult to interpret.

The displacement hypothesis presented by Carlsson & Lindqvist has been questioned by Gessa et al. (38) and by Udenfriend & Zaltzman-Nirenberg (37). These authors did not detect α-methyl tyramines in quantities which balanced the loss of NE 24 hours after administration of α-MMT. In contrast, the findings pointed to the possibility that far less than stoichiometric amounts of the α-methyl amines are required to bring about and maintain depletion of NE. These conclusions were derived from experiments showing negligible concentrations of α-methyl-*m*-tyramine and metaraminol, estimated together and expressed as "α-methyl-*m*-tyramines," in the guinea pig heart 24 hours after 75 mg/kg α-MMT which caused depletion of practically all the NE. Therefore, a prolonged impairment of NE binding sites similar to that produced by reserpine was assumed to occur after α-MMT.

However, the displacement hypothesis was confirmed by a number of authors using methylDOPA in their experiments. Maître & Staehelin (43) injected rats and guinea pigs intraperitoneally with single or repeated doses of 20 to 800 mg/kg of the racemic compound. There was no decrease in pressor activity of heart extracts purified by adsorption onto

alumina and assayed on the blood pressure of the pithed rat. On this test preparation NE and α-MNE are equally effective. If the catechol amines contained in the heart extracts of guinea pigs were separated by paper chromatography and assayed individually, the NE concentration was found to be decreased by methylDOPA. However, the missing NE was replaced in the heart by an equipressor and therefore approximately equimolar amount of α-MNE. Maximum levels of α-MNE, accounting for more than 70 per cent of the pressor activity of the extracts, were observed 16 hours after methylDOPA. As early as one hour after methylDOPA, small amounts of α-MNE were detected, and as late as 144 hours after methylDOPA 30 per cent of the pressor activity was due to α-MNE. Muscholl & Maître (44) administered four doses of 100 mg/kg D,L-methylDOPA intravenously to rabbits in the course of two days and estimated the catechol amine content in the right ventricle by a differential assay procedure utilizing the potent pressor response and the weak fluorescence exerted by α-MNE compared with NE. The results were also checked by individual estimation of the amines after separating them by paper chromatography. NE was depleted from the myocardium which, at the same time, had accumulated concentrations of α-MNE exceeding the amounts of NE lost. Likewise, Schümann & Grobecker (45), using a fluorimetric method, observed an accumulation of α-MNE which exceeded the NE loss from guinea pig heart and brain after 100 mg/kg L-methylDOPA twice daily for eight days. In another study by Lindmar & Muscholl (46), rabbits were injected intravenously with four doses of 50 mg/kg L-methylDOPA within 30 hours. The animals were killed 18 hours after the last dose. The NE concentrations in auricles and right and left ventricles decreased by 38 to 60 per cent, and equimolar amounts of α-MNE were found in the tissues.

There is also a displacement of NE by metaraminol if α-MMT is administered to rats and rabbits [Anden & Magnusson (34, 47)]. Intraperitoneal injection of 400 mg/kg D,L-α-MMT into rats decreased the NE concentration of heart and brain stem by about 80 per cent in the course of 6 to 12 hours. After 24 hours, the NE concentration rose again, reaching the normal level five days after administration of α-MMT. The NE was replaced almost stoichiometrically by metaraminol 36 or more hours after α-MMT. At the earlier intervals, significant concentrations of α-methyl-*m*-tyramine were detected in heart and brain, and only then was there an apparent deficit of metaraminol compared to the amount of NE lost from the tissues. Likewise, accumulation of metaraminol concentrations corresponding to the loss of NE was observed in the guinea pig heart 12 to 24 hours after intraperitoneal injection of 0.15 and 0.5 mg/kg *l*-metaraminol. The discrepancy regarding the stoichiometry of NE depletion by α-MMT between these results and those already mentioned (37, 38) was explained by Anden (34) by different extraction procedures for tissues utilized by both groups of workers. Using the method of Gessa

et al. (38) Anden found "hardly any metaraminol," as he expressed it, in rat heart and brain after a high dose of α-MMT. Large volumes of a strong acid have to be used in order to extract all the metaraminol, which seems to be fixed to the tissues more firmly than the other known monoamines (34). In experiments which failed to demonstrate stoichiometric replacement, the tissues were homogenized in a small volume of 0.02 or 0.1 *N* hydrochloric acid and amines were extracted into butanol (37, 38). It is noteworthy that in all communications demonstrating replacement of NE by α-MNE after methylDOPA, large volumes of either perchloric acid (41) or trichloroacetic acid (43, 44, 46) have been used.

A detailed study by Shore et al. (48) presented further evidence for a stoichiometric exchange of metaraminol and NE. Rats received 1 mg/kg *l*-metaraminol intraperitoneally and were killed after 18 to 90 hours. At all times the molar concentration of NE depleted from the heart corresponded well to the molar concentration of metaraminol present. The half-life of NE repletion paralleled that of metaraminol disappearance. The following results suggest strongly that metaraminol replaces NE at its physiological binding sites in the sympathetic nerve ending. In immunosympathectomized rats (49) Shore et al. observed a marked inhibition of metaraminol uptake by the heart. Furthermore, metaraminol accumulated by the normal rat heart could be released by drugs known to deplete endogenous NE from the heart such as reserpine, guanethidine, and tyramine. Interestingly enough, metaraminol was also depleted by NE. In addition, drugs known to inhibit NE uptake or binding such as imipramine, reserpine, and guanethidine inhibited the accumulation of metaraminol by the rat heart after an intraperitoneal injection. Finally, optical specificity of metaraminol binding was demonstrated. Following injection into rats, *l*-metaraminol was retained by the heart for more than 24 hours. In contrast, injection of *d*-metaraminol produced high levels in the heart only for a short time. Then there was a rapid decline in *d*-metaraminol concentration until no amine could be detected after three hours. The lack of binding of the *d*-isomer was reflected in its inability to deplete NE.

Amine metabolites derived from ring-hydroxylation and N-methylation. —Up to this point we have only been dealing with α-MNE derived from methylDOPA and metaraminol derived from α-MMT. However, Maître & Staehelin (50) showed recently that α-MNE was present in the guinea pig heart 16 hours after intraperitoneal injection of α-MMT (100 mg/kg) or *l*-metaraminol (10 mg/kg). Paper chromatography and electrophoresis as well as fluorimetric and biological assays were used in order to identify α-MNE which, after administration of metaraminol, exceeded the amount of NE remaining in the heart by the factor 1.5. Thus, *p*-hydroxylation of metaraminol can occur to an appreciable extent *in vivo*. Enzymatic *p*-hydroxylation of *m*-tyramine, *m*-norsynephrine, and *m*-synephrine by rabbit liver microsomes was demonstrated by Axelrod (51) who also observed *m*-hydroxylation of the corresponding *p*-hydroxy com-

pounds. Finally, Maître (52) detected α-MNE in heart and brain of guinea pigs injected with α-methyl-*p*-tyrosine thus proving that *m*-hydroxylation of the amino acid or of one of its amine metabolites occurs *in vivo* (further discussion p. 124). It appears, therefore, that for a stoichiometric analysis of NE displacement after α-MMT and metaraminol, the catechol as well as the monophenol derivatives have to be considered. An apparent deficit of the sum of metaraminol plus α-methyl-*m*-tyramine compared to the amount of NE lost from the rat or guinea pig heart between 12 and 24 hours following α-MMT or metaraminol as observed by Anden (34) may be explained by the simultaneous, but hitherto unnoticed, occurrence of α-MNE in the myocardium.

Another metabolite of methylDOPA which only recently was detected is α-methyl epinephrine (α-MEpi). It was assumed that N-methylation of α-MNE may occur in an organ capable of N-methylating NE at a high rate. Actually, small amounts of α-MEpi were found in adrenal glands of rabbits treated with reserpine and subsequently with repeated doses of methylDOPA (53, 54). There was a constant relationship between α-methyl amines and NE newly synthetized and stored in the adrenals. The α-MNE represented about 10 per cent, and the α-MEpi about 1 per cent of the amount of NE present. In contrast to other known metabolites of α-methyl amino acids, α-MEpi lowers the blood pressure of various laboratory animals. It is not known whether this effect is responsible for, or contributes to, the hypotensive action of methylDOPA. Similar to other catechol amines, α-MEpi is removed from the circulation by uptake into different organs. In the rat heart and spleen, storage of α-MEpi was accompanied by depletion of an equimolar concentration of NE.

Absolute configuration of α-methyl amines.—Metaraminol and α-MNE contain two asymmetric carbon atoms, and therefore four optical isomers of these amines exist. The absolute configuration of the metabolic products of methylDOPA and α-MMT has not been determined. Considering the known stereospecificity of the synthesizing enzymes, it has been assumed that α-MNE and metaraminol formed biologically from the precursor amino acids consist of one isomer with the configuration 1 *R*:2 *S* (46, 55, 56). *R* and *S* are notations of absolute configuration according to the sequence rule by Cahn et al. (57). In his review article, Van Rossum (55) has quoted the evidence derived from rotation dispersion techniques which showed that levorotatory nordefrin (cobefrine) and metaraminol have the 1 *R*:2*S*-configuration (generally known as *erythro*-configuration). As a matter of fact, all investigators performing estimations of α-MNE isolated from biological material used nordefrin as a reference substance (43–46, 50, 53). Of the two enantiomorphs, the levorotatory one is biologically several hundred times more active than the dextrorotatory one (58, 59). The stoichiometric replacement of NE by α-MNE was ascertained by chemical methods (41, 43) which do not distinguish between the enantiomorphs, and by biological estimations (43, 44, 46) which do differentiate between the *l*-

and *d*-isomer. The bioassays were carried out using *l*-nordefrin as a reference substance. If the material formed in the body after administration of methyl-DOPA was the dextrorotatory or the racemic rather than the levorotatory α-MNE, the results of the biological estimations would not have precisely agreed with those of the chemical method. Hence, it is well established that α-MNE formed *in vivo* is levorotatory. Furthermore, it is very likely that metaraminol biosynthesized from α-MMT is levorotatory as well since it remains in the heart for several days (34, 48), as does the reference compound *l*-metaraminol (48), whereas the dextrorotatory isomer is lost from the heart within a few hours (48). However, there is no comparable evidence available to show which of the two diastereomeres (*erythro*- or *threo*-isomer) is formed in the organism, because the *threo*-isomers of nordefrin and metaraminol have not been used so far as reference compounds. Nevertheless, Muscholl obtained evidence that α-MEpi found in the adrenals after administration of methylDOPA behaved chemically and biologically in a way similar to 3,4-dihydroxyephedrine (*erythro*-configuration), but differed biologically from 3,4-dihydroxypseudoephedrine (*threo*-configuration). Since biological N-methylation of α-MNE is unlikely to alter the conformation of the parent molecule, this result strongly suggests that α-MNE formed in the organism consists of the levorotatory *erythro*-isomer, with the absolute configuration 1 *R*:2 *S*. Retrospectively, utilization by all authors mentioned of commercial *l*-nordefrin as a reference compound now seems justified.

Sympathetic Function and α-Methyl Amino Acids

The clinical evidence indicating a decrease in sympathetic tone after administration of methylDOPA was presented above. However, in experimental animals interference with sympathetic transmission, if it occurs at all, is less pronounced with methylDOPA than with adrenergic blocking drugs or with reserpine.

Stone et al. (35) compared the effects of repeated administration of methylDOPA, α-MMT, and the corresponding amines with those of reserpine, syrosingopine, and guanethidine in anesthetized dogs 16 to 24 hours after the last dose of these agents. Increases in heart rate and blood pressure by amphetamine and in blood pressure by phenylethylamine were antagonized by all drugs. In contrast, the pressor response caused by stimulation of the central end of the vagus nerve was blocked by reserpine, syrosingopine, and guanethidine but not altered by the α-methyl amino acids and amines. The NE concentrations of heart and spleen were decreased by reserpine by 71 and 85 per cent, respectively, and by methyl-DOPA and α-MMT by about 50 per cent. It was concluded that there are separate catechol amine stores upon which indirect sympathomimetic amines and nerve stimulation may act. Reserpine, syrosingopine, and guanethidine possibly deplete both kinds of stores, and therefore inhibited the response to both nerve stimulation and indirect sympathomimetic

amines. It was suggested that α-methyl amino acids deplete only the store necessary for the action of the indirect sympathomimetic amines and this may explain why sympathetic stimulation was still effective.

However, several authors report that administration of methylDOPA does not diminish the pressor response caused by tyramine. This agent is the prototype of indirectly acting sympathomimetic amines (60, 61) and is frequently used to test the ability of sympathetic nerve endings to release NE. No decrease in the pressor response to tyramine was observed in spinal cats after three to four doses of 100 mg/kg and in rats after 400 mg/kg every eight hours for two days (62). Tachycardia, following injection of tyramine into anesthetized mice, was unaltered 20 hours after 400 mg/kg methylDOPA but was blocked by 5 mg/kg reserpine. In these experiments both agents depleted heart NE to approximately the same extent (63). Surprisingly, there was an enhancement of the pressor response to tyramine in normotensive subjects treated for seven days (64) and in hypertensive patients treated daily for four to twenty weeks (65) with two to three g methylDOPA given orally. In both of these investigations administration of reserpine diminished the pressor response to tyramine as is known to occur in animals (60, 66) and in man (67). On the other hand, a significant decrease in the pressor response to tyramine was noted in the spinal cat after a cumulative dose of 400 mg/kg L-methylDOPA but not after a cumulative dose of 200 mg/kg (68). These findings suggest that only a narrow range of experimental conditions, dose regimens, or both, exists in the limits within which impairment of the tyramine response can be demonstrated.

The action of tyramine on the blood pressure has also been tested within two hours after terminating an infusion of methylDOPA (doses in the order of 100 to 200 mg/kg), but it was not found to be conspicuously altered in the dog (20, 62) or in the cat (69).

Apart from the work already discussed (35), there are a few papers concerned with the effect of methylDOPA on sympathetic transmission. Day & Rand (62) did not observe impairment of responses to sympathetic stimulation in the isolated rabbit ileum and isolated guinea pig vas deferens preparations if the organs were taken from animals treated daily for three to five days with 200 mg/kg. However, responses of the cat nictitating membrane to nerve stimulation frequencies of 0.5 to 100 per second were significantly lowered by treatment of the animals with 100 mg/kg methylDOPA for three to four days. Impairment of sympathetic transmission was most conspicuous at the low stimulation frequencies which correspond to the physiological discharge rate in sympathetic nerves (70). Only excessively high concentrations of methylDOPA (0.5 mg/ml) added to the organ bath decreased responses of the isolated rabbit ileum and guinea pig vas deferens to sympathetic nerve stimulation. Impairment of sympathetic transmission on the cat nictitating membrane by methylDOPA (up to 400 mg/kg) was not seen until several hours had elapsed. The apparent

failure to demonstrate instant effects on transmission is not surprising, since clinical experience has shown that hypotension after intravenous administration of methylDOPA does not occur until two to four hours have passed, and maximal responses are seen as late as four to eight hours after infusion (8). However, in conscious dogs Goldberg et al. (20) did not observe a decrease in heart rate, an alteration in response to tilting, or a relaxation of the nictitating membrane at the time when the blood pressure was significantly reduced after 100 to 200 mg/kg methylDOPA intravenously. Furthermore, in anesthetized dogs the increments in heart rate and contractile force produced by stimulation of presynaptic fibers of the stellate ganglion were not affected by methylDOPA (time of observation not mentioned). In another study, prolonged treatment of cats with methylDOPA (two days 150 mg/kg and one day 50 mg/kg intraperitoneally) did not alter the rise in heart rate following electrical stimulation of the stellate ganglion at 20 per second (68). Hearts of rabbits which had received four doses of 100 mg/kg methylDOPA within two days responded to 10 per second stimulation of the fibers leaving the right stellate ganglion with an increase in rate and force of contraction similar to that seen in control hearts (44). Since the stimulation frequencies in the two last-mentioned papers [frequency in (20) not indicated] were rather high, a possible inhibition of transmission produced by methylDOPA at physiological impulse rates may have escaped notice.

After administration of α-MMT, no pharmacological evidence of an impairment of sympathetic transmission has been obtained. The antihypertensive action discussed above may indicate, but is not necessarily proof of, transmission failure. As already mentioned, Stone et al. (35) did not notice an inhibition of the pressor response following stimulation of the central end of the vagus nerve by α-MMT. Anden & Magnusson (47) injected 400 mg/kg α-MMT intraperitoneally daily for two days and 0.2 mg/kg metaraminol intravenously four hours before the experiment into cats and rats. NE concentrations of heart, spleen, iris, and nictitating membrane decreased by about 95 per cent but there was no ptosis in the rats and no miosis or relaxation of the nictitating membrane in the cats. Furthermore, electrical stimulation of the cervical sympathetic trunk at frequencies of one to thirty per second was equally effective in contracting the nictitating membrane of treated and of control cats. Similarly, in cats and rats dilatation of the pupil and protrusion of the eye following sympathetic stimulation was unaltered by the treatment outlined above. Finally, blood pressure responses in adrenalectomized cats to splanchnic nerve stimulation, bilateral carotid occlusion, and doses of tyramine and carbachol (after atropine) were in the normal range. Hence it appears that adrenergic transmission still operates after doses of α-MMT and metaraminol which allow only a minute percentage of the original NE to be present in the organs.

Release of "False" Adrenergic Transmitters

The functional consequences of NE depletion by α-methyl amino acids may greatly differ from those observed after administration of reserpine, since there is not only a loss of transmitter as in the case of reserpine, but instead a replacement of part of the transmitter by a sympathomimetic amine. Carlsson & Lindqvist (41) suggested that α-methyl amines which had displaced NE in the brain could possibly take over its functions. Day & Rand compared the potencies of NE and α-MNE on various test preparations including the blood pressure of five different animal species (62). In all cases α-MNE was less effective than NE. Considering the biochemical findings of formation and storage of α-MNE in tissues, Day & Rand put forward the hypothesis that α-MNE is released as a false neurotransmitter which at the receptors exerts weaker actions than the natural transmitter NE, and thereby diminishes the effect of sympathetic nerve stimulation (62, 71). This mechanism was supposed to explain particularly the antihypertensive action of methylDOPA. Day & Rand regard the following findings as indirect evidence for their hypothesis. Administration of methylDOPA reduces, but does not abolish, the response of the nictitating membrane to nerve stimulation (see p. 117). In animals treated with reserpine, responses to sympathetic stimulation were abolished or reduced but, depending on the organ investigated, they could be partially restored by an infusion or by addition to the bath solution of methyl-DOPA, α-methyl DOPAmine, or α-MNE. These compounds behaved like DOPA, DOPAmine, and NE, tested under the same experimental conditions. Similarly, responses to tyramine, known to be reduced after reserpine (60), such as rise in blood pressure of cats and rats and contraction of the cat nictitating membrane, could be partially restored by methylDOPA or the corresponding amines. It was suggested that after giving α-MNE, or one of its precursors, the NE storage sites were replenished with α-MNE which thereupon acted as a sympathetic transmitter if released by nerve stimulation or tyramine.

In contrast to Day & Rand, Kroneberg & Stoepel did not observe restoration of the contraction of the nictitating membrane following nerve stimulation in cats pretreated with reserpine and infused with L-methyl-DOPA, although the responses of the blood pressure and the nictitating membrane to tyramine were clearly enhanced (69). Interestingly enough, there was no restoration of the tyramine response after infusion of D-methylDOPA. The onset of restoration was delayed following infusion of methylDOPA or α-methyl DOPAmine but restoration was noted immediately after infusion of α-MNE (69, 72). In close agreement with these experiments are results by Pettinger et al. (65) obtained on human subjects. Administration of reserpine decreased the pressor response to tyramine. The pressor action could be restored by methylDOPA and

α-methyl DOPAmine but this effect was delayed and its maximum was not seen until 90 minutes after the infusion. However, restoration of the tyramine response by α-MNE was observed immediately.

Day & Rand explained the apparent failure of methylDOPA to block sympathetic transmission to a major extent on the grounds that α-MNE incorporated into the amine store can serve in place of NE to mediate responses to sympathetic nerve impulses. On the other hand, NE depletion by methylDOPA is not very marked (see p. 110) and the NE remaining may be sufficiently concentrated to render sympathetic transmission possible. For instance, in animals pretreated with reserpine, sympathetic stimulation was still effective unless about 80 per cent of the NE was depleted (73), and responses to tyramine were only decreased to 50 per cent of the control value if 90 per cent of the NE was lost (74). Obviously, indirect evidence of this kind is too uncertain to clarify whether metabolites of methylDOPA are released in lieu of NE. A direct approach toward this question was made by Muscholl & Maître (44). Rabbits received four intravenous doses of methylDOPA and were killed 16 hours after the last injection. The hearts were isolated with the sympathetic supply from the right stellate ganglion intact and perfused according to the Langendorff technique. Stimulation of the postganglionic fibers or injection of the nicotinic drug, dimethylphenyl-piperazine (DMPP), increased rate and force of contraction of the heart and released a mixture of NE and α-MNE into the perfusion fluid. The proportion of NE to α-MNE in the perfusate (about 1:1) equaled the proportion found in the myocardium after termination of the experiment. The amounts of pressor catechol amines released into the perfusates by sympathetic stimulation or DMPP were in the same range as the amounts of NE released in control hearts by nerve stimulation and DMPP, respectively. It was concluded that the sensitivity of the adrenergic receptor coming in contact with the amine mixture determines whether α-MNE behaves like a "false" or like the physiological transmitter.

There is also evidence that sympathetic stimulation releases metaraminol previously incorporated into the amine store [Crout et al. (75)]. Cats were injected intravenously with *l*-metaraminol and their hearts isolated and perfused two hours or 17 to 20 hours later. At both time intervals endogenous NE estimated in the myocardium was partly replaced by metaraminol. Stimulation of the right sympathetic chain increased the force of cardiac contraction and released metaraminol into the perfusion fluid. A smaller but still significant release of metaraminol was observed following an injection of NE which, however, produced essentially the same increase in tension as nerve stimulation. The amount of metaraminol released by nerve stimulation and by NE was much greater in hearts studied at the early time period. Since the cardiac metaraminol concentration was nearly the same after both time intervals, it was suggested

that metaraminol taken up by the heart shifts with time from an "available" to a "less readily available" pool.

The finding of N-methylation of α-MNE *in vivo* to α-MEpi (53) prompted the investigation of uptake and release of the latter amine (53, 54). As is the case with other catechol amines, α-MEpi
the circulation by uptake into different organs such as heart, spleen, and adrenal glands. Rabbits were given an infusion of α-MEpi and the hearts were isolated and perfused. One hour after the infusion of α-MEpi into the intact animal the perfusate of the isolated heart contained amounts of 2.6 ng/min NE and 17 ng/min α-MEpi. Postganglionic sympathetic stimulation increased the amine output to 24 ng/min NE and 129 ng/min α-MEpi and raised the heart rate appreciably. A similar increase in heart rate elicited by electrical stimulation of the pacemaker region did not alter the amine output. DMPP stimulated the heart and released both NE and α-MEpi into the perfusate. In contrast, stimulation of the heart by tyramine was not accompanied by an increase in the NE output above the control value although α-MEpi was released at a rate similar to that attained after nerve stimulation or DMPP. Therefore, DMPP mimics the effect of nerve stimulation much more than tyramine does. The proportion of NE to α-MEpi in the perfusates during release by nerve stimulation or by the drugs was not conspicuously related to the proportion found in the hearts after termination of the perfusion experiments. Possibly, the α-MEpi had not mixed completely with the endogenous NE and was released at a relatively higher rate.

Further examples of "false" transmitters became known by the work of Kopin and associates (76, 77). According to several authors (78–81) tyramine can be β-hydroxylated both *in vitro* and *in vivo* to yield octopamine (norsynephrine). Side-chain hydroxylation and binding *in vivo* seems to be confined to sympathetic nerves (77, 81). Following infusion of ^{3}H-tyramine or α-methyl-^{3}H-tyramine into isolated cat spleen, stimulation of the splenic nerves released the β-hydroxylated derivatives, ^{3}H-octopamine and α-methyl-^{3}H-octopamine, into the perfusate (76). There was an increase in the output of ^{3}H-tyramine and α-methyl-^{3}H-tyramine as well but this appeared to be related to splenic contraction which extruded platelets loaded with the amines. The subcellular distribution of octopamine as studied by density gradient centrifugation resembled that of NE (82).

Various Peripheral Actions of α-Methyl Amino Acids and Amines

Metabolism.—After administration of methylDOPA, the main excretory product in urine was the drug itself (8, 28, 42, 83) or its mono-O-sulfate (28). Urinary excretion fell to small amounts after six to eight hours (8, 13, 83) and was virtually complete after 24 hours (8, 10). Small quantities of 3-O-methyl methylDOPA (28, 42, 83) and of its glucuronide

(83), trace amounts of α-methyl DOPAmine (8, 28, 42, 83) or 3-O-methyl-α-methyl DOPAmine (8, 42, 83) and its glucuronide (83) were also detected in urine. About ten hours after a dose of methylDOPA α-methyl normetanephrine probably derived from α-MNE appeared in the urine (27).

Effect on norepinephrine uptake.—MethylDOPA did not affect NE uptake by heart slices (84) or by blood platelets (85). However, NE uptake by heart and brain slices or by the perfused heart was strongly inhibited by α-methyl DOPAmine (84, 87), α-MNE (84, 86, 87), α-methyl-*m*-tyramine (87), metaraminol (86, 87), and α-methyl-*p*-tyramine (86, 87). The ATP-dependent NE uptake by adrenergic nerve granules was not altered by methylDOPA and α-MMT but was slightly inhibited by metaraminol and strongly inhibited by α-methyl-*m*-tyramine (88).

Effect of drugs on uptake and storage of α-methyl amines.—On the isolated rabbit heart α-MNE is removed from the perfusion fluid by intracellular uptake at the same rate as NE (46). The concentration ratios, amine taken up:amine present in perfusate, obtained with NE and α-MNE, were both about 30:1 (46). A number of drugs which block NE uptake were found to inhibit α-MNE uptake by this preparation as well, e.g., cocaine and sympathomimetic amines (89). Elevated extracellular concentrations of α-MNE resulting from uptake inhibition by cocaine may explain enhancement of responses to α-MNE observed after cocaine (58, 89, 90). In contrast, tetracaine neither caused supersensitivity toward α-MNE nor affected its uptake (89).

Reserpine or guanethidine depleted α-MNE stored in the rabbit heart after administration of methylDOPA (46). Yet α-MNE was more resistant to the action of both depleting agents than endogenous NE. Doses of reserpine and guanethidine which lowered, but did not completely block, the binding capacity of the amine stores caused a greater loss of NE than of α-MNE. Unlike NE, α-MNE is not metabolized by monoamine oxidase (91). If the fraction of free intracellular amines which are liable to be exposed to monoamine oxidase is increased by drugs impairing binding, then NE disappearance would exceed the loss of α-MNE by diffusion out of the cell. Balance experiments on the isolated heart which were designed to differentiate between uptake by the cell and storage within the cell (92) confirmed that in hearts of rabbits treated with reserpine, inhibition of monoamine oxidase raised the intracellular concentration of NE to the same level attained by α-MNE irrespective whether the enzyme was inhibited or not (46). The resistance of α-MNE against depletion by reserpine may perhaps explain the finding that cardiovascular actions of tyramine were not altered in cats whose NE stores were largely depleted by methylDOPA followed by a dose of reserpine (68); possibly the tyramine responses were mediated by the α-MNE remaining after such a treatment.

Metaraminol uptake and storage by the heart was inhibited by drugs

which have similar effects on NE uptake and storage such as reserpine, guanethidine, cocaine, and imipramine (48, 93).

Actions on effector organs.—Pressor actions of NE were not significantly altered by methylDOPA either in animals (35, 62, 69) or in men (13, 64). Depending on the function under examination, α-MNE is usually less effective than NE, especially as a vasoconstrictor agent in most animal species (62, 94, 95, 96), but not in the pithed rat (43, 44, 46). The cardiac-stimulating actions of NE and α-MNE do not differ markedly (95, 97). However, NE has a greater nictitating membrane-contracting activity than α-MNE (90, 95, 96) but a lower uterine (95, 96) or bronchiolar muscle (59) relaxing activity than α-MNE. In small doses α-MEpi is a potent vasodilator drug (53, 94, 98) whereas higher doses raise the blood pressure (53, 94). Vasodilatation by α-MEpi is increased by ergotamine (94), antagonized by pronethalol (53), and reversed by naphazoline (98). Metaraminol is much less effective than NE on various test organs (47, 97).

Inhibition of Transmitter-Synthesizing Enzymes

Although many drugs of different chemical structure, potency, and specificity are now known which inhibit DOPA decarboxylase both *in vitro* and *in vivo,* none of them is capable of completely blocking NE formation in the whole animal. Blockade of NE formation at the level of DOPA decarboxylase is unlikely to occur since this enzyme is present in large excess of the requirements for NE synthesis (11). The final step in NE formation, the β-hydroxylation of DOPAmine, has also been the aim of enzymatic blockade (11). Among benzyloxyamines and benzylhydrazines potent inhibitors of the purified enzyme were discovered (40, 99); however, they did not lower the NE concentration of the heart (100). Only one of these compounds, 4-bromo-3-hydroxybenzyloxyamine, decreased heart NE by less than 20 per cent. In another study (26), a somewhat lower dose did not deplete NE. Even chronic administration of benzyloxyamine for five days did not decrease NE levels (100). It is therefore likely that β-hydroxylation is not rate limiting in NE synthesis under physiological conditions. Nevertheless, inhibition of decarboxylase or of β-hydroxylase was shown to delay DOPAmine or NE synthesis if this was increased beyond the physiological rate by the administration of an excess of substrate (4) or by a compound rapidly depleting endogenous NE, such as oxypertine (100, 101), or by infusing DOPAmine into animals whose NE stores were previously depleted by metaraminol (100).

Only recently has it been demonstrated that hydroxylation of tyrosine to DOPA is the rate limiting step of the NE biosynthesis in the perfused guinea pig heart (102). Nagatsu et al. (103) isolated tyrosine hydroxylase and found several *in vitro* inhibitors of this enzyme. One of them, α-methyl-*p*-tyrosine (α-MPT), was further investigated *in vivo* by Spector et al. (104). After intraperitoneal doses of 80 mg/kg of the racemic

compound, the NE concentrations in heart, spleen, and brain stem of the guinea pig fell steadily, reached a minimum value at eight hours after α-MPT
no longer detectable in the tissues. Repeated doses administered at three hour intervals for 24 hours decreased NE to undetectable levels, but did not affect serotonin concentrations. The possibility that α-MPT depleted NE by a release mechanism similar to that exerted by α-MMT rather than by inhibition of NE synthesis was ruled out by the following observations. Inhibition of decarboxylase failed to block the action of α-MPT, and inhibition of monoamine oxidase did not increase the NE concentrations of several tissues although it increased NE levels after treatment of the animals with α-MMT. Furthermore, administration of α-MPT lowered the rate of incorporation of ^{14}C-tyrosine into DOPAmine, NE, and epinephrine but did not affect incorporation of ^{3}H-DOPA into tissue catechol amines. The ability of heart and spleen to increase their NE concentrations after intraperitoneal doses of NE was not affected by a previous dose of α-MPT.

The simultaneous incidence of severe NE depletion and unimpaired binding capacity for NE is unique. Other agents or procedures causing a NE depletion also block the capacity of tissues to store exogenous NE, e.g., reserpine (105), guanethidine (106), sympathetic postganglionic denervation (107), and immunosympathectomy (108). Therefore, treatment of experimental animals with α-MPT will probably be used in the future as another tool for studying the significance of adrenergic mechanisms in drug effects.

The pressor response to NE was diminished in rats and guinea pigs infused with α-MPT over a period of 24 hours and returned to normal within 48 hours. It was assumed that after α-MPT the rate of inactivation of circulating NE was increased because the binding mechanism was still intact and the stores, containing much less NE than in normal animals, had a greater capacity to accumulate the hormone (104). However, it was recently demonstrated that in the dog a dose of α-MPT which did not lower the NE content of the heart shifted the dose-response curve of administered NE to the right and decreased vasopressor responses to carotid occlusion and to tyramine (109). Perhaps α-MPT has some adrenolytic activity. There was miosis and relaxation of the cat nictitating membrane, but significant hypotensive effects were not seen in cats, rats, and guinea pigs (104). In accordance with these findings is the observation that daily oral doses of 100 or 300 mg/kg α-MPT administered for 11 days only slightly decreased the blood pressure of the renal hypertensive rat (24). One wonders, therefore, whether α-MPT has some other as yet unknown action which counteracts the sympathetic transmission failure supposedly resulting from an almost complete loss of NE. Such an action could be due to formation of "false" transmitters which take over part of the function of NE. In fact, formation of pressor amines derived from α-MPT was recently demonstrated by Maître (52).

In guinea pigs given a single oral dose of 300 mg/kg α-MPT, the heart NE fell in 16 hours by about 65 per cent and the brain NE by about 80 per cent. At the same time, α-MNE was detected in the heart at about 30 per cent of the remaining NE concentration and α-methyl DOPAmine and α-MNE were present in the brain, each at about 200 per cent of the remaining NE content. Thus, NE depletion produced by α-MPT may not only be a consequence of inhibition of NE synthesis but may be partly due to displacement of NE by catechol or phenolic metabolites of the amino acid. However, with the few experimental data available at present, the relative inefficiency of α-MPT as a blood pressure lowering agent compared with methylDOPA or α-MMT cannot be explained.

Conclusions

Undoubtedly, methylDOPA is a valuable antihypertensive drug in man but only a few pharmacological screening tests using laboratory animals reveal such an effect. The efforts to elucidate its mode of action have produced a wealth of information, especially biochemical. Many of the findings summarized suggest that, from the pharmacological point of view, α-MNE is perhaps the most important metabolite of methylDOPA and that its behavior is strikingly similar to NE in the following respects: route of biosynthesis, uptake and storage by sympathetic nerves, release by nerve impulses, and qualitative actions on effector organs. Thus, the specificity of various adrenergic mechanisms appears to be limited and this is reflected by the concept of α-methyl amino acids serving as antimetabolites of natural NE precursors. It was suggested that the partial inhibition of sympathetic transmission observed after methylDOPA is due to release of α-MNE which as a "false" transmitter is inferior to NE at the receptor sites. At first sight, this hypothesis is difficult to reconcile with the observation of unimpaired sympathetic transmission in animals whose NE stores were heavily depleted after treatment with α-MMT followed by metaraminol (47). Since metaraminol which replaces NE and which is released by nerve stimulation is even less effective than α-MNE, a sympathetic blockade more severe than the one occurring after methylDOPA should result from administration of α-MMT plus metaraminol. However, one important difference between the two "false" transmitters, α-MNE and metaraminol, is the much more pronounced ability of the latter amine to inhibit NE uptake (86). If metaraminol is released by nerve impulses concomitantly with NE, it may inhibit reuptake of NE by the nerve endings and thereby potentiate its effect on the receptors. It is known that drugs blocking NE uptake increase the transmitter output during sympathetic stimulation (110). Recently it was found that the urinary excretion of NE in stressed rats was not altered by pretreatment with α-MMT plus metaraminol (111). This indicates an unchanged rate of NE output into the circulation.

Considering the subject reviewed it should be borne in mind that a combined release of any "false" transmitter and NE will bring into play

various pharmacological actions differing from those of a mere NE release. Availability of one amine for release, its effect on the receptors, and its inactivation by the several mechanisms known are all likely to be affected by the presence of another sympathomimetic amine. Therefore, it will remain difficult to prove, or to disprove, the hypothesis that release of "false" neurotransmitters causes sympathetic transmission failure. Nevertheless, this idea proved to be a valuable working hypothesis and it may serve as a model not only for the pharmacological effects of α-methyl amines but for other drug actions as well.

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