Antihypertensive Metabolites of α-Methyldopa

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SUMMARY α-Methyldopa acts through a metabolite in the central nervous system. Of the three metabolites most prominently considered as potential mediators of α-methyldopa hypotension — α-methyldopamine, α-methylnorepinephrine (MNE), and α-methylepinephrine (ME) — ME is the most potent depressor agent following intravenous infusion into rats, following injection into the lateral ventricle, and following injection into the solitary tract nucleus (NTS). The depressor effect of ME in the NTS is attenuated as effectively by timolol as by yohimbine, while the combination of both timolol and yohimbine completely abolishes the pharmacological activity of ME in the NTS. ME is approximately eightfold more potent than MNE in the NTS and also has a greater susceptibility to timolol attenuation. (Hypertension 6 (Suppl II): II-45-II-50, 1984)

KEY WORDS • methyldopa • methylepinephrine • methylnorepinephrine • methylldopamine • CNS • blood pressure

THERE is a consensus that α-methyldopa exerts its antihypertensive effect through the actions of a metabolite within the central nervous system. There is less agreement about the identity of the principal active metabolite and the precise site of its action. Central dopa decarboxylase inhibition interferes with the depressor effect of α-methyldopa.1-3 This suggests that α-methyldopamine or one of its metabolites is critical for drug effect.

α-Methyldopamine is readily converted to α-methylnorepinephrine (MNE) in catecholaminergic neurons. For this reason, much attention has been focused on α-methyldopamine and MNE as potential mediators of the antihypertensive effect of α-methyldopa. When administered into the lateral cerebral ventricle of rats and cats, both α-methyldopamine and MNE lower blood pressure.4

Inhibition of dopamine-β-hydroxylase by FLA 63 (bis-4-methyl-1-homo-piperazinylthiocarbonyl disulfide) prevents the depressor effect of α-methyldopa.5-6 Furthermore, centrally active α-adrenergic blocking agents attenuate the hypotensive response of α-methyldopa more effectively than does the dopamine antagonist haloperidol.1-4, 6-8 These observations support a role for a more distal metabolite than α-methyldopamine mediating α-methyldopa action through an action on α-receptors.

Inasmuch as norepinephrine (NE) has long been appreciated as a neurotransmitter in the central nervous system, it was natural that MNE would be proposed as a central “false” transmitter that accumulates and displaces the endogenous neurotransmitter from critical sites. The hypothesis that MNE was the critical α-methyldopa metabolite seemed even firmer with recognition that MNE binds more readily than NE to α2-adrenergic receptors in some tissues, while binding to α1-adrenergic receptors less readily than NE.9

Not all data, however, supported this hypothesis. Following a single dose of α-methyldopa, the time course of brain MNE accumulation did not correlate well with the rate of development of hypotension.10 Freed and co-workers used deuterium-labelled α-methyldopa in the rat hypothalamus to estimate turnover rates of metabolites following a 24-hour infusion of nondeuterated α-methyldopa.11 Blood pressure reduction did not correlate with either MNE concentration or turnover. Although there was a correlation with α-methyldopamine concentration, the above data with dopamine-β-hydroxylase inhibition cast doubt on the importance of α-methyldopamine as the key metabolite.

The identification of epinephrine-containing neurons in brain stem sites previously implicated in blood pressure regulation12-15 requires that consideration be given to the hypothesis that the action of α-methyldopa might involve these neurons. Several interactions can be envisaged. If epinephrine-containing neurons were
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adrenergic blockade. Using perfused rat mesenteric arteries, Malik demonstrated that although ME inhibit-
stimulation, and (3) the immediate depressor effect of the agent could be retained by the heart and released on
methyldopa administration, (2) after ME infusion, the ME was synthesized in the adrenal gland following
infused ME could be attenuated and reversed by /3-
methyladrenaline, oxyephedrine, and methylcobefrin,

2. There have been few previous studies of this com-
paring the pharmacology of the metabolite ME (Figure
functionally depressor in some sites (e.g., the solitary tract nucleus, NTS), then the synthesis of an epineph-
nergic analog such as /3-methylpinephrine (ME) could lead to a reduction in blood pressure if it were more
termediate, required less epinephrine input would
catecholamines competed better for presumed /3-binding sites than did any of the three 
metabolites.

*Ranking in order of potency in competing for tritiated dihydroalprenolol was (-)jer-thyro-ME > (-)jer-thyro-MNE > (-)NE. Thus ME appeared to be the most potent of the compounds tested in binding putative /3-adrenergic re-
cisor. Such a situation is depicted in Figure 1C. The role of E-containing neurons in blood pressure control is considered elsewhere in this volume.16

Because of these considerations, we have investi-
the metabolism of the metabolite ME (Figure
2). There have been few previous studies of this com-
MNE appeared to compete better for presumed /3-binding sites than did NE and ME, which, in turn, were more potent than /3-methyladrenaline.

Ranking in order of potency in competing for triti-
ed NE production could also lead to blood pressure reduction if it were not attended by a more than compensatory quantity of an active "false" neurotransmitter. Such a situation is depicted in Figure 1C. The role of E-containing neu-
pressure in the hypotensive activity of /3-methyladrenaline (MD). If /3-methylpinephrine (ME) were rapidly synthesized in epinephrine-containing neurons, its potency at epinephrine recep-
tors mediating depressor responses might be such as to effect a drop in blood pressure (B). If, however, epinephrine levels were depleted by the /3-methyladrenaline, and /3-methylpinephrine syn-
thesis was inadequate to replace the stimulation that epinephrine had been providing to an epinephrine receptor that mediated vasoconstriction, reduced effective epinephrine input would occur (C). It should be noted that the mechanism depicted in (B) presupposes that the neurotransmitter elicits vasodepression while that in (C) presupposes that the neurotransmitter elicits vasocostriction.

3. The studies of Muscholl indicated that: (1) /3-
trityrated prazosin, tritiated clonidine, and tritiated dihydroalprenolol. Rat forebrain was the tissue chosen for study because the sought adrenergic subtypes had been shown to be present here by previous investigators.24 The methodology and results of our investigations have been presented elsewhere in detail.25

Ranking in order of potency in competing with prazosin was (—)E > (—)NE > (—)erythro-MNE > (—)jer-thyro-ME > (—)jer-thyro-MNE > (—)NE. Thus both endogenous catecholamines competed better for presumed /3-binding sites than did any of the three /3-methyladrenaline metabolites.

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cisor. It has been postulated that the /3-adrenergic blocking agents in forebrain are predominantly of the /3 subtype, whereas those on human lymphocytes are predominantly /3. We therefore assessed competition of these compounds for lymphocyte /3-adrenergic blocking agents with 125-iodinated-hydroxyisoproterenol as radioligand. Here ME was decidedly more effective than E, NE, and MNE in competing for putative /3 sites.25

The binding characteristics of MNE and ME vis-
aggregating responses to nerve stimulation, it did not inhibit the effect of NE infusion.22

In order to study ME further, it was necessary to consider the implications of its two asymmetric carbon atoms, which yield four isomeric forms. Erythro-ME already had been separated from three-ME by earlier investigators who noted that the former was considerably more active. We resolved racemic erythro-ME into its (+) and (—) isomers by fractional crystalliza-

We next studied the capacities of the enantiomers and racemic modifications of /3-methyladrenaline, MNE, and ME to bind with adrenergic receptor sites of the /3, /3, and /3 subtypes. This was done using radioligand binding competition with the following respective compounds: tritiated prazosin, tritiated clonidine, and tritiated dihydroalprenolol. Rat forebrain was the tissue chosen for study because the sought adrenergic subtypes had been shown to be present here by previous investigators.24 The methodology and results of our investigations have been presented elsewhere in detail.25

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The binding characteristics of MNE and ME vis-
vis clonidine binding suggested that the former might be more active at this site (although, of course, that effect might be agonism or antagonism). We therefore investigated a system previously well characterized as being mediated by /3-adrenergic receptor sites: the human platelet.

Human platelets were isolated from normal vol-
teurs by differential centrifugation; aggregation (nephelometry) and binding potency (using tritiated yohimbine) were assessed. There was excellent agreement between these parameters; MNE was perhaps slightly more potent than ME in causing aggregation
and in competing for yohimbine binding sites, but the endogenous catecholamines clearly were more efficacious than either of these metabolites. We therefore would conclude that, if the central adrenergic blocking agents mediating hypotension were indistinguishable functionally from the platelet α₂-adrenergic receptor sites, then MNE would be equal to ME or slightly more potent in inducing a fall in blood pressure.

Before directly examining this question, we studied the cardiovascular effect of peripherally administered ME in rats. Over the dosage range 0.5 to 2.0 μg, ME as a bolus lowered mean arterial blood pressure by more than 25 mm Hg within 15 seconds (Figure 3). In contrast, the same dosage of MNE elicited a pressor response only. Heart rate responses tended to be opposite in direction to the absolute changes in blood pressure, consistent with baroreceptor reflex buffering.

Administration of ME, MNE, and E into the lateral cerebral ventricle (unilaterally) of the anesthetized rat elicited disparate effects. ME was the most potent depressor agent throughout the 5 to 20 μg dosage range during the first 20 minutes. The peak depressor response of ME was at 2 minutes, but the effect persisted beyond 40 minutes with all but the smallest dose. In striking contrast, the initial effect of MNE during the first 5 minutes after administration was pressor, followed by a prolonged but only modest depressor response. ME was by far the more potent depressor agent, although the decline in heart rate was less marked with ME than with MNE.

We next turned to the nucleus of the solitary tract as a site of drug administration. As in the intracerebroventricular drug studies, the racemates of erythro-ME and erythro-ME were compared with (−)E. In Figure 4, it can be seen that ME is a potent depressor agent in the NTS over the 0.3 to 10.0 nmol dosage range. The effect was already near maximal at 2 minutes and was still present at 20 minutes. In contrast MNE was far less potent at all dosages and at all times tested. ME appeared to be 5 to 10 times more potent than MNE. Epinephrine seemed intermediate in potency but resembled ME more than MNE. It is noteworthy that the heart rate changes with both ME and MNE were more modest than those with E.

These data presented us with an interesting paradox. Two agents (ME and MNE) with roughly comparable effects in a well-characterized α₂-adrenergic-receptor system (the human platelet) had markedly different effects on blood pressure; ME was severalfold more potent in eliciting hypotension. Several explanations are possible in such a situation: (1) Differential diffusion to critical areas involved in cardiovascular regulation may be responsible. (2) There could be differential uptake and/or metabolism of the two compounds. (3) Real differences in pharmacological profile may be present.

Of these possibilities, the third seemed to be the most likely, and yet what could be the basis for such a difference in potency, in view of the roughly equivalent potency in the platelet α₂-adrenergic-blocking-agent system? One explanation could be that in the case of ME a non-α₂-mechanism might contribute to the depressor effect in the NTS. To test the hypothesis that central β-adrenergic receptors somehow might be involved in α-methyldopa hypotension, we investigated the capacities of α₂-blockade and β-blockade to...
Figure 3. The effect of intravenous catecholamine on blood pressure and heart rate in Sprague-Dawley rats. Epinephrine (E) yields an initial pressor response that resolves within 2 minutes. α-Methylnorepinephrine (α-MNE) yields a mild pressor response following intravenous administration. With α-methylenephrine (α-ME), however, the initial response is depressor and no pressor response appears at any point. Heart rate changes are essentially the inverse of the blood pressure changes. Left panel: n = 10, ▲, (±)-α-MNE; n = 6, ■, (-) E; n = 6, ■, (±)-α-ME. Right panel: n = 10, ▲, (±)-α-MNE; n = 6, ■, (-) E; n = 6, ■, (±)-α-ME.

attenuate the effects of NTS injections of E, MNE, and ME. Based on our studies of its central pharmacology,26, 27 we selected yohimbine to achieve relatively selective α₁-blockade.25, 26 Results of these studies are shown in Figure 5. It can be seen that α₁-blockade (yohimbine) attenuated the depressor response to all catecholamines. Our radioligand-binding data had suggested that the potency of α-methyldopa metabolites at β-adrenergic receptors might be important in the action of these compounds.25 Furthermore, β-adrenergic-receptor (timolol) blockade was also efficacious against the catecholamines. These results suggest that β-adrenergic receptors play a role in mediating the depressor responses of catecholamines injected into the NTS and that ME has the capacity to stimulate beta adrenergic receptors. Other investigators have also identified β-adrenergic receptors in the NTS.28

That ME is the most potent depressor metabolite is suggestive of its importance in mediating the hypotensive effect of α-methyldopa; however, it also must be present in critical vasomotor nuclei in sufficient concentrations to exert this effect. ME has been identified in rat hypothalamus in concentrations of 4.8 ng/g wet weight,28 yet it appears unlikely that ME is primarily synthesized by the enzyme phenyl-ethanolamine-N-methyltransferase.29-31 Presumably its synthesis occurs through the action of a still poorly characterized and possibly "nonspecific" methyltransferase.32 The modest quantity of ME heretofore detected following α-methyldopa administration would need to be highly localized to be a major contributor to the antihypertensive effect of α-methyldopa.

Conclusion

These studies describe the pharmacologic effects of metabolites of α-methyldopa in one site known to be involved in the central regulation of arterial pressure. They do not, however, permit any inferences regarding the mechanism of action of α-methyldopa. Other issues relevant to that mechanism include the extent to which sites and pathways other than those injected may mediate the central adrenergic regulation of peripheral resistance; the type of receptors that would mediate any action of those other sites; and the extent to which ME is actually biosynthesized, stored, and released by the neurons of interest.

Indeed, following the administration of α-methyldopa,
dopa, the concentrations of ME in the forebrain, the hypothalamus, and brain stem of the rat are all below 10 ng/g. Certainly these amounts are far less than a stoichiometric replacement of the E depletion. This could represent a low level of biosynthesis due to poor substrate affinity for the major phenylethanolamine-n-methyltransferase in brain, or it could reflect diminished neuronal storage. Thus, the hypothetical mechanism depicted in Figure 1C has attractions that may outweigh those of the mechanism depicted in Figure 1B. Clearly more research is required to address the importance of methyldopa metabolites in the E neurons of the brain.

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