Changes in Brain α-Adrenergic Receptors After α-Methyldopa Administration to Spontaneously Hypertensive Rats

CURT R. FREED, CHING H. WANG, AND DAVID C. U'PRICHARD

SUMMARY The hypotensive action of methyldopa has been linked to production of the metabolites methyldopamine and methylnorepinephrine in brain. We have studied the effect of long-term (72 hour) intravenous infusions of methyldopa to awake restrained spontaneously hypertensive rats and normotensive Wistar-Kyoto control animals to look for differences in hypotensive effect, differences in concentrations of natural and α-methylated catecholamines, and differences in α1 and α2-adrenergic receptor populations. Results described here indicate that hypertensive rats have a greater reduction in blood pressure and a larger increase in hypothalamic and brain stem methylnorepinephrine concentrations than do the normotensive animals. The methylnorepinephrine concentration reached a plateau value in hypothalamus in both strains while pons and medulla showed progressive, dose-related increases in concentration. These regional and strain differences in the metabolism of α-methyldopa suggest that the production of methylnorepinephrine in brain stem nuclei is most correlated with the hypotensive action of methyldopa. α1 Agonist binding (p-amino-clonidine) declined in both hypothalamus and brain stem, and the fall was greater in hypertensive than in normotensive rats. α1-Adrenergic receptor binding (prazosin) was increased, again more in hypertensive than in normotensive rats. The down regulation of α1-adrenergic receptors and the up regulation of α2-adrenergic receptors are compatible with increased α2-adrenergic agonist presynaptic inhibition of catecholamine release with resultant postsynaptic α2-adrenergic receptor supersensitivity. Spontaneously hypertensive rats showed greater methylnorepinephrine production, larger up regulation of α2-adrenergic receptors, and greater down regulation of α2-adrenergic receptors than did the normotensive animals; these changes may be physiological markers for the greater antisypathetic action of methyldopa in hypertensive animals. (Hypertension 6 (Suppl II): 11-34—11-39, 1984)

KEY WORDS  • spontaneously hypertensive rats • α-methyldopa • α1- and α2-adrenergic receptors • hypertension • sympathetic nervous system

METHYLDOPA is believed to lower blood pressure by being metabolized in brain to one or more α1-agonists. 1,3 Three metabolites—methyldopamine, methylnorepinephrine, and methylepinephrine—have been proposed as the important agonist, although methylnorepinephrine is considered by most to be the active metabolite. Regardless of which of these compounds is the critical one, the presence of excess α1 agonist in brain might well be expected to down regulate the number of α2-adrenergic receptors. This phenomenon has been observed by us and others. 6,7 If the mechanism for blood pressure reduction is related to α1 agonist formation, then dose-related reductions in blood pressure are likely to be associated with dose-related changes in α1 adrenergic receptor number. Both α1 and α2-adrenergic receptors have been described pre- and postsynaptically. 8,9 A simple model based on studies of peripheral tissues such as the isolated cat spleen suggests that stimulation of presynaptic α2-adrenergic receptors inhibits the release of transmitter. 10,11 The reduction in transmitter release leads to reduced stimulation of the postsynaptic α1-adrenergic receptor. On a long-term basis, this lowered stimulation leads to increases in α1-adrenergic receptor number. 8 Similar α1-adrenergic receptor supersensitivity has been produced with central sympathectomy using 6-hydroxydopamine or median forebrain bundle lesions. 12,13

From the Division of Clinical Pharmacology. University of Colorado School of Medicine. Denver. Colorado 80262 (Dr. Freed); Department of Pharmacology. Northwestern University School of Medicine. Chicago. Illinois 60611 (Drs. Wang and U'Prichard); Nova Pharmaceutical Corporation. Baltimore. Maryland 21224 (Dr. U'Prichard).

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Address for reprints: Curt R. Freed, M.D., University of Colorado Health Sciences Center: C237. Denver. Colorado 80262
Biochemical differences between spontaneously hypertensive rats (SHR) and normotensive Wistar Kyoto rats (WKY) have been shown for catecholamine synthetic enzymes, catecholamine concentrations, and a-adrenergic receptor populations. Hypertensive animals also have shown greater reductions in blood pressure to a given dose of an antihypertensive drug. Although the hypertensive animals have been shown to be different from controls in these neurochemical and pharmacological parameters, the neurochemical differences have not been correlated with differences in drug responsiveness.

We have studied the hypotensive response of WKY and SHR to long-term infusions of a-methyldopa. We have compared the concentrations of dopamine and norepinephrine and their a-methylated analogues in multiple brain regions of the animals and also measured changes in a1 and a2-adrenergic receptors in these brain areas to look for systematic differences in the adrenergic systems of the two strains that might explain the difference in pharmacological effect of methyldopa.

Methods

Male WKY and SHR, 12 to 16 weeks of age, received doses of methyldopa ranging from 0 to 20 mg/kg/hr together with the peripheral decarboxylase inhibitor carbidopa, 2.5 mg/kg/hr. Animals were awake in restraining cages and drug infusions were via the jugular vein. Mean arterial blood pressure was monitored with a cannula in the descending aorta as previously described.

After 72 hours of drug infusion, animals were sacrificed by decapitation and the brain hemisected. One half of the brain was further dissected and assayed for dopamine, norepinephrine, methyldopa, methylnorepinephrine, and methylnorepinephrine in the hypothalamus, pons, and medulla by high-performance liquid chromatography with electrochemical detection. Brain stem sections were defined by the boundaries of the fourth ventricle. The anterior brain stem (pons) was dissected from the rostral tip of the ventricle to the caudal tip of the fourth ventricle. The posterior brain stem section (medulla) extended from the cerebellar peduncle to the caudal tip of the fourth ventricle. The other half of the brain was divided into two blocks that corresponded to the thalamus-hypothalamus and pons-medulla regions; these regions were assayed with tritiated ligands p-aminoclonidine and rauwolscine respectively. Assays were performed with a single subsaturating concentration of ligand; however, previous studies in male Sprague-Dawley rats with competition curves over a full range of ligand concentrations showed that methyldopa treatment led to changes in adrenergic receptor number, not affinity. Table 1 shows the conditions used for the receptor assays.

Changes in blood pressure, catecholamine concentrations, and adrenergic receptor densities were compared for the two strains using a Student’s t-test for the baseline values and two-way analysis of variance (ANOVA) for the dose-related changes seen after methyldopa infusion.

Results

The hypertensive animals used in this study had mean blood pressures of 125 mm Hg; the control animals had blood pressure values of 100 mm Hg. Although the SHR had higher blood pressures at the time of arterial catheter placement (approximately 150 mm Hg), blood pressure dropped to the mean value 24 hours after the SHR were placed in the restraining cages. The WKY control animals did not show this change in mean blood pressure between the time of catheter placement and the beginning of the drug infusions. Hypertensive animals also had a greater fall in blood pressure after methyldopa infusion; however, as shown in Figure 1, the lowest blood pressure achieved

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<th>Table 1. Adrenergic Receptor Assay Conditions</th>
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*Membranes were preincubated for 30 minutes at 25 °C.
L = low-affinity state; H = high-affinity state; NE = norepinephrine.
for the two strains was similar at the maximum dose of methyldopa tested. The concentrations of methyldopa were the same in both strains at each dose tested and there were no regional differences in methyldopa concentrations (data not shown). Figure 2 presents the changes in catecholamine concentrations seen in the pons after increasing doses of methyldopa. There was a progressive depletion of norepinephrine and dopamine and dose-related increases in methyldopamine and methynorepinephrine. Furthermore, the increase in methynorepinephrine was greater in the hypertensive than in the normotensive animals when data were grouped and analyzed by two-way ANOVA. These results were similar to those seen in the medulla but contrast with those measured in the hypothalamus. As demonstrated in Figure 3, in the hypothalamus, methyldopa dose-related increases in methyldopamine and methynorepinephrine.

**Figure 2.** Catecholamine concentrations in the pons of normotensive rats (WKY) and spontaneously hypertensive rats (SHR) after 72-hour infusion of methyldopa. There is a dose-related increase in methynorepinephrine concentration in both rat strains that is significantly greater in the hypertensive rats (p < 0.02 by two-way ANOVA). Each point is the mean and SEM of at least five animals.

**Figure 3.** Catecholamine concentrations in the hypothalamus of normotensive rats (WKY) and spontaneously hypertensive rats (SHR) after methyldopa infusion. There is a significant difference in the control concentration of norepinephrine in the two strains (p < 0.03; SHR less than WKY by Students t-test). There is also a small but significantly higher concentration of methynorepinephrine in the hypertensive rats when all doses are taken into account with two-way ANOVA (p < 0.05). All points are the mean and SEM of at least five animals.
ynorepinephrine concentration reached a maximum at a methyldopa dose of 5 mg/kg/hour. The hypertensive rats did have a small but significantly greater concentration of methylnorepinephrine when all drug doses were taken into account by two-way ANOVA. Hypertensive animals not receiving methyldopa had a lower hypothalamic norepinephrine concentration than did controls.

The \( \alpha_1 \) and \( \alpha_2 \)-adrenergic receptor changes in the thalamus-hypothalamus and pons-medulla regions are shown in Figures 4 and 5. \( \alpha_2 \) Agonist binding determined by \( p \)-aminoclonidine was reduced significantly more in hypertensive rats, which suggests that the higher concentrations of methylnorepinephrine in the hypertensive rats may be linked to larger reductions in \( \alpha_2 \)-adrenergic receptor high-affinity states. Although the SHR showed apparently fewer \( \alpha_2 \)-adrenergic receptors in the high-affinity state before methyldopa, these differences were not significant. \( \alpha_1 \) Antagonist binding measured with rauwolscine showed no significant change in either tissue area (data not shown).

To demonstrate that the reduction in \( \alpha_2 \) agonist binding was not due to residual \( \alpha \)-methylnorepinephrine present after membrane washing, we determined \( ^{1}H \)-\( p \)-aminoclonidine binding to cerebral cortex membranes which had been preincubated for 30 min at 37\(^\circ\)C with 20 \( \mu \)M \( d \),\( l \)-\( \alpha \)-methylnorepinephrine. Instead of a decrease, this treatment led to a 25\% and 40\% increase in \( \alpha_2 \) agonist binding in two replications of the experiment.

Changes in \( \alpha_2 \)-adrenergic receptors measured by prazosin binding showed that SHR had significantly greater increases in \( \alpha_2 \)-adrenergic receptors in both the hypothalamus and the pons-medulla regions than did the normotensive controls.

**Discussion**

These experiments indicate that SHR have a greater hypotensive response to a given dose of \( \alpha \)-methyldopa than do normotensive animals. The hypertensive animals also produced more methylnorepinephrine and showed a greater change in \( \alpha_2 \)-adrenergic receptors in the hypothalamus and brain stem areas. An increase in binding of \( \alpha_2 \)-adrenergic receptors also was demonstrated, which was greater in SHR. The change in \( \alpha_2 \)-adrenergic receptors was a reduction in agonist bind-

![Figure 4](http://hyper.ahajournals.org/)

**Figure 4.** \( \alpha_2 \)-adrenergic receptor changes in hypothalamus-thalamus and pons-medulla measured by agonist binding. Hypertensive animals (SHR; closed circles) have reduced \( \alpha_2 \)-adrenergic receptors compared with control animals (WKY; open circles) \( p < 0.01 \), both regions by two-way ANOVA. Each point is the mean and SEM of four animals.

![Figure 5](http://hyper.ahajournals.org/)

**Figure 5.** \( \alpha_1 \)-adrenergic receptor changes in the hypothalamus-thalamus and pons-medulla regions measured by prazosin binding. Two-way analysis of variance shows a greater increase in \( \alpha_1 \)-adrenergic receptors in hypertensive rats (SHR; closed circles) than in normotensive rats (WKY; open circles). \( p < 0.01 \), both areas; \( n = \) four animals per experimental point.)
ing, not antagonist binding, which suggests that there was only a reduction in high-affinity states with no change in the total number of $\alpha_1$-adrenergic receptors. It is unlikely that differences in residual agonist concentrations led to observed changes as membranes were washed and preincubated before $\alpha_1$-adrenergic agonist receptor assays were performed. Furthermore, experiments using cerebral cortical membranes preincubated with $\alpha_2$-methylnorepinephrine showed an increase rather than a decrease in $\alpha_2$-adrenergic receptors. This somewhat surprising result has also been observed in the study of dopamine receptors. Striatal membranes preincubated with dopamine show an increase in $^3$H-dopamine binding.

The down regulation of $\alpha_1$ receptors (high-affinity state) was likely due to the presence of the $\alpha_1$ agonist methylnorepinephrine. The fact that SHR have a greater production of this compound and a greater change in this receptor population supports this concept. Changes in agonist binding alone have been reported in the past and so are expected in this setting. Cantor et al. have reported that $\alpha_1$-adrenergic receptor binding measured by WB-4101 is greater in hypothalamus of 16- to 20-week-old SHR compared with control animals and that there is no change in adrenergic receptor binding after treatment with clonidine. Although we did not see a difference in baseline hypothalamic $\alpha_1$-adrenergic receptor binding with prazosin as the ligand, we did see an increase in receptor numbers after methyldopa infusion. Differences in ligand and in antihypertensive agent may account for the different observations.

We do not believe that the receptor changes are the cause of the hypotensive response but that they are a marker for the presence of increased $\alpha_1$ agonist and decreased $\alpha_2$ agonist activity. Animals were infused for 72 hours to allow receptor changes to take place. Following a methyldopa infusion, blood pressure fell maximally over a few hours and remained reduced. Although we have not studied the time course of the receptor changes, we doubt that it would parallel the hypertensive changes. We cannot say whether receptor changes modulate the hypotensive action of methyldopa. Because there is down regulation of $\alpha_1$-receptor high-affinity states and up regulation of $\alpha_2$-adrenergic receptors, these changes would tend to reduce the hypotensive efficacy of an $\alpha_1$ agonist such as methylnorepinephrine. That methyldopa leads to persistent hypotension in humans and experimental animals means that these offsetting receptor changes are not large enough to negate the antihypertensive effect of the drug.

In the past we have argued that methylamphetamine, not methylnorepinephrine, may be the active metabolite of methyldopa, because in the hypothalamus of normotensive Sprague-Dawley rats we observed a dose-related increase in methylamphetamine production but only a constant concentration and turnover rate of methylnorepinephrine at all hypotensive doses of methyldopa. In the present experiments we have seen the same plateau in methylnorepinephrine concentra-

Another intriguing outcome of these experiments is the dose-related increase in $\alpha_1$-adrenergic receptors, which was greater in the SHR than in the normotensive animals. This increase in receptors implies that there may be a reduction in neurotransmitter overflow to postsynaptic $\alpha_2$-adrenergic receptors that is greater in hypertensive than in normotensive animals. It suggests that the model of $\alpha_1$ presynaptic inhibition of neurotransmitter release is applicable to the hypothalamus and brain stem. Our data indicate that the effect of methylnorepinephrine is to reduce binding to high-affinity states of the $\alpha_1$-receptor, and to up-regulate $\alpha_2$-adrenergic receptors. Because up regulation occurs in the setting of reduced impulse flow, we infer that the predominant action of methylnorepinephrine is the presynaptic stimulation of the $\alpha_2$-adrenergic receptor with resultant inhibition of transmitter release and subsequent postsynaptic $\alpha_2$-adrenergic receptor up regulation.

Because methyldopa leads to a reduction in sympathetic outflow from the brain and, as we have shown, since methyldopa appears to reduce adrenergic neurotransmitter flow as indicated by an increase in $\alpha_2$-adrenergic receptors, it is possible that this receptor increase is an index for the degree of antisympathetic effect of the drug. The fact that hypertensive animals had a greater fall in blood pressure and a greater increase in $\alpha_2$-adrenergic receptors supports this possibility. Of course, $\alpha_2$ agonist action at other sites such as presynaptic serotonin neurons also may play a role in reducing net sympathetic outflow.

We conclude that SHR differ from WKY in the degree to which they metabolize methyldopa to methylnorepinephrine in the hypothalamus and brain stem. They also show larger changes in $\alpha_1$ and $\alpha_2$-adrenergic receptors in response to this drug treatment. These exaggerated physiological responses may account for the greater hypotensive effect of $\alpha$-methyldopa observed in hypertensive animals.

Acknowledgments
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C R Freed, C H Wang and D C U'Prichard

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