Changes in Brain $\alpha$-Adrenergic Receptors After $\alpha$-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 $\alpha$-methylated catecholamines, and differences in $\alpha_1$ and $\alpha_2$-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 $\alpha$-methyldopa suggest that the production of methylnorepinephrine in brain stem nuclei is most correlated with the hypotensive action of methyldopa. $\alpha_2$ Agonist binding (p-aminoclonidine) declined in both hypothalamus and brain stem, and the fall was greater in hypertensive than in normotensive rats. $\alpha_1$-Adrenergic receptor binding (prazosin) was increased, again more in hypertensive than in normotensive rats. The down regulation of $\alpha_2$-adrenergic receptors and the up regulation of $\alpha_1$-adrenergic receptors are compatible with increased $\alpha_2$-adrenergic agonist presynaptic inhibition of catecholamine release with resultant postsynaptic $\alpha_2$-adrenergic receptor supersensitivity. Spontaneously hypertensive rats showed greater methylnorepinephrine production, larger up regulation of $\alpha_1$-adrenergic receptors, and greater down regulation of $\alpha_2$-adrenergic receptors than did the normotensive animals; these changes may be physiological markers for the greater antisympathetic action of methyldopa in hypertensive animals. (Hypertension 6 (Suppl II): 11-34—11-39, 1984)

Key Words • spontaneously hypertensive rats • $\alpha_1$- and $\alpha_2$-adrenergic receptors • hypertension • sympathetic nervous system

Methyldopa is believed to lower blood pressure by being metabolized in brain to one or more $\alpha_1$-agonists. 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 $\alpha_1$ agonist in brain might well be expected to down regulate the number of $\alpha_2$-adrenergic receptors. This phenomenon has been observed by us and others. If the mechanism for blood pressure reduction is related to $\alpha_1$ agonist formation, then dose-related reductions in blood pressure are likely to be associated with dose-related changes in $\alpha_1$ adrenergic receptor number. Both $\alpha_1$ and $\alpha_2$-adrenergic receptors have been described pre- and postsynaptically. A simple model based on studies of peripheral tissues such as the isolated cat spleen suggests that stimulation of presynaptic $\alpha_2$-adrenergic receptors inhibits the release of transmitter. The reduction in transmitter release leads to reduced stimulation of the postsynaptic $\alpha_1$-adrenergic receptor. On a long-term basis, this lowered stimulation leads to increases in $\alpha_1$-adrenergic receptor number. Similar $\alpha_2$-adrenergic receptor supersensitivity has been produced with central sympathectomy using 6-hydroxydopamine or median forebrain bundle lesions.
Biochemical differences between spontaneously hypertensive rats (SHR) and normotensive Wistar Kyoto rats (WKY) have been shown for catecholamine synthetic enzymes, catecholamine concentrations, and α-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 α-methyldopa. We have compared the concentrations of dopamine and norepinephrine and their α-methylated analogues in multiple brain regions of the animals and also measured changes in α, and α2-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 3H-prazosin for α1-adrenergic receptors. α2 agonist (high-affinity state) and α2-antagonist (low-affinity state) binding were determined with the 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

![Graph showing blood pressure reduction](image)

**Figure 1.** Reduction in mean blood pressure in spontaneously hypertensive rats (closed circles) and normotensive Wistar-Kyoto rats (open circles) after 72-hour intravenous infusion of methyldopa. Each point is the mean and SEM of at least five animals.

<table>
<thead>
<tr>
<th>Table 1. Adrenergic Receptor Assay Conditions</th>
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<tr>
<td><strong>Adrenergic Receptor</strong></td>
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</tr>
<tr>
<td>α-1</td>
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<tr>
<td>α-2(L)</td>
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<td>α-2(H)</td>
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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 methylnorepinephrine. Furthermore, the increase in methylnorepinephrine 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 methylnorepinephrine were statistically significant (p < 0.05).
ylnorepinephrine 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 $\alpha$- 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. $\alpha_2$-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 $^{3}H$-$p$-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-
ing, not antagonist binding, which suggests that there was only a reduction in high-affinity states with no change in the total number of \( \alpha \)-adrenergic receptors. It is unlikely that differences in residual agonist concentrations led to observed changes as membranes were washed and preincubated before \( \alpha \)-adrenergic agonist receptor assays were performed. Furthermore, experiments using cerebral cortical membranes preincubated with \( \alpha \)-methylnorepinephrine showed an increase rather than a decrease in \( \alpha \)-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.\(^{25,26}\)

The down regulation of \( \alpha \)-receptors (high-affinity state) was likely due to the presence of the \( \alpha \)-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.\(^{27}\)

Cantor et al. have reported that \( \alpha \)-adrenergic receptor binding measured by WB-4101 is greater in hypothalamus of 16-20-week-old SHR compared with control animals and that there is no change in adrenergic receptor binding after treatment with clonidine.\(^{18}\)

Although we did not see a difference in baseline hypothalamic \( \alpha \)-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 \( \alpha \)-agonist and decreased \( \alpha \)-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 hypotensive changes. We cannot say whether receptor changes modulate the hypotensive action of methyldopa. Because there is down regulation of \( \alpha \)-receptor high-affinity states and up regulation of \( \alpha \)-adrenergic receptors, these changes would tend to reduce the hypotensive efficacy of an \( \alpha \)-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 methyldopamine, 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 methyldopamine production but only a constant concentration and turnover rate of methylnorepinephrine at all hypotensive doses of methyldopa.\(^{3}\) In the present experiments we have seen the same plateau in methylnorepinephrine concentra-

Acknowledgments

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