Effects of Methyldopa Metabolites on Amine Transmitters and Adrenergic Receptors in Rat Brain

WILLIAM J. LOUIS, ELIZABETH CONWAY, ROGER SUMMERS, PHILIP BEART, AND BEVYN JARROTT

SUMMARY Studies of catecholamine concentrations in defined nuclei from the anterior hypothalamic-preoptic regions and the medulla oblongata, known to contribute to cardiovascular control, were measured following acute or chronic methyldopa administration. These studies indicated that methyldopa was enzymatically converted to methyldopamine and methylnorepinephrine, and in some areas to methylepinephrine which replaced endogenous epinephrine. The predominant metabolite was methylnorepinephrine, which accumulated in concentrations higher than endogenous norepinephrine levels. (—)Methylnorepinephrine was found to be 6 times more potent and 75 times more selective for $\alpha_2$-adrenergic receptors than (—)norepinephrine, and it is suggested that this $\alpha_2$-adrenergic receptor action, particularly in the nucleus tractus solitarius, contributes to a major part of the antihypertensive effect of methyldopa. (Hypertension 6 [Suppl II]: 11-40-11-44, 1984)

KEY WORDS • methyldopa • $\alpha$-methyldopamine • $\alpha$-methylnorepinephrine • $\alpha_2$-adrenergic receptors • radioligand receptor assays • rat brain

THE central nervous system contains a variety of amine, amino acid, and peptide transmitters including dopamine, norepinephrine, epinephrine, and 5-hydroxytryptamine, acetylcholine, glycine, gamma-aminobutyric and glutamic acids. Other possible neurotransmitters include substance P, enkephalin, adenosine, histamine, and aspartic acid as well as various peptides. It appears that a balance between these transmitter systems in the central nervous system is necessary for proper regulation of brain function, and there is increasing evidence that in pathological disease states such as hypertension imbalances may occur and contribute to or be responsible for the elevated blood pressure.

The most readily identified and perhaps the most important of these transmitters in blood pressure regulation is norepinephrine, which mediates its action through both types of $\alpha$- as well as $\beta$-adrenergic receptors. Methyldopa, which interferes with central nervous system norepinephrine levels, has been an extremely effective antihypertensive agent since its introduction in 1960. Studies of its mode of action have contributed to the understanding of central noradrenergic control of blood pressure, although its mode of action has been the subject of considerable dispute. Methyldopa initially was introduced as a decarboxylase inhibitor and a derivative carbidopa has been used widely in the treatment of Parkinson's disease to prevent the peripheral decarboxylation of Levodopa. Because this mechanism did not satisfactorily explain the antihypertensive effect of methyldopa or its action on central nervous system transmitters, the false transmitter hypothesis was developed. Current knowledge indicates that methyldopa must be enzymatically converted to methylnorepinephrine, which has a highly selective action that is mediated through $\alpha_2$-adrenergic receptors.

Catecholamine Levels After Chronic Methyldopa Administration

Although clinical usage of the hypotensive agent methyldopa involves chronic administration, most studies on its mechanism of action have involved acute systemic or intracerebral injection of either the parent compound or its metabolites. Thus the evidence that the hypertensive response that follows methyldopa administration depends on the formation of methylnorepinephrine centrally and the activation of $\alpha$-adrenergic receptors by this metabolite is derived largely from acute studies. In addition, the possible central site of action has been suggested by reports that local injections of methylnorepinephrine into the anterior hypothalamic-preoptic region and the nucleus tractus solitarius (NTS) produce a fall in blood pressure in anesthetized rats.
In our studies (Figure 1) chronic administration of methyldopa to rats (40 mg/kg subcutaneously b.i.d. for 5 days) produced a profound depletion of norepinephrine and dopamine levels in nuclei in the anterior hypothalamic-preoptic region and in the medulla oblongata such that these amines were virtually undetectable 4 hours after cessation of drug treatment. At this time methylnorepinephrine was the predominant amine present in all of the nuclei and the levels were always higher than control norepinephrine levels in untreated rats. Much lower levels of methyldopamine also were present. In some nuclei the levels of methylnorepinephrine were as much as 6 times greater than the endogenous norepinephrine levels in untreated animals. Twenty-four hours after the last injection of methyldopa, methylnorepinephrine levels were still between 50% and 80% of four-hour values and norepinephrine levels were still less than 45% of control values.

These data support the contention that methylnorepinephrine is involved in mediating the hypotensive effect of methyldopa through actions in either the anterior hypothalamic-preoptic nuclei or the NTS in the medulla oblongata or through a combination of actions. The slow disappearance of methylnorepinephrine after cessation of the methyldopa treatment in part reflects the fact that methylnorepinephrine is not a substrate for monoamine oxidase and may explain the persistence of the hypotensive effect of this drug reported in both rats and humans.

**Major Site of Action of Methyldopa**

In acute studies (Figure 1) methyldopa administration to rats (200 mg/kg subcutaneously) produced a marked reduction in levels of norepinephrine and dopamine in anterior hypothalamic and preoptic nuclei implicated in catecholamine-mediated cardiovascular inhibitory functions. Similar effects were observed in...
mediated by guanyl nucleotides. This was also reflected in high levels of methylnorepinephrine in these nuclei. In most regions, however, methylnorepinephrine was highest 12 hours after cessation of methyldopa administration; thus the levels of methylnorepinephrine in medullary nuclei bore little relationship to the known time course of the antihypertensive response in these animals. By contrast the time course of accumulation of methylnorepinephrine in the NTS (Figure 1) closely correlated with the reported time course of the hypertensive effect of methyldopa, which suggests that this site is the major mediator of at least the acute antihypertensive effect of this drug. It is still possible, however, that the action of this metabolite in other areas may contribute to the fall in blood pressure during chronic administration.

Methylnorepinephrine

In recent years the understanding of α-adrenergic receptors has been facilitated both by in vitro and in vivo pharmacological studies and by the development of appropriate ligands. Adrenergic receptors exist as two pharmacologically distinct types designated α1 and α2, and it has been suggested that these receptors mediate selectivity by different mechanisms: α1 effects are due to elevation of intracellular calcium ions and α2 effects are caused by inhibition of adenylyl cyclase. The development in recent years of selective radioligands for α1- and α2-adrenergic receptors has provided tools that have facilitated the examination of the properties of adrenergic receptors at the molecular level. α1 Adrenergic receptors can exist in two affinity states designated α1H (high affinity) and α1L (low affinity). In contrast to antagonist ligands, agonist ligands labeled predominantly the fraction of α1-adrenergic receptors in the high-affinity state. The size of that fraction is governed by the ionic environment and by the presence of guanylnucleotides.

We have examined the relative potency for α1-adrenergic receptors of a series of clonidine-like compounds in membranes from rat cerebral cortex (Table 1). This and subsequent studies have demonstrated that drugs like prazosin and labetalol are highly α1-selective whereas drugs like clonidine have a high selectivity for α2-adrenergic receptors. An important observation was that guanfacine and CP14304-18 were even more selective for α2-adrenergic receptors than clonidine. We have also used [3H]-guanfacine to identify α2-binding sites in rat brain areas. The highest levels of binding, in descending order, were obtained in membranes prepared from cerebral cortex, hippocampus, hypothalamus, medulla oblongata, spinal cord, striatum, and cerebellum.

Table 2 compares inhibition constant (Ki) values for noradrenaline and methylnorepinephrine with a number of clinically important compounds that act on α-adrenergic receptors. (−)Methylnorepinephrine had a Ki value for [3H]-guanfacine similar to that of clonidine and was 7 times more potent than (−)norepinephrine. In addition, (−)methylnorepinephrine had much less effect on [3H]-prazosin binding than did (−)noradrenaline. When selectivity for α1-adrenergic receptors was expressed as the ratio of [3H]-prazosin to [3H]-guanfacine binding, methylnorepinephrine had a selectivity of more than 4000 and was the most selective compound tested. This selectivity contrasted with (−)norepinephrine's selectivity of 65. Thus methylnorepinephrine was 7 times more potent and 70 times more selective for brain α2-adrenergic receptors. Given the extremely high levels of methylnorepinephrine formed in cardiovascular inhibitory regions such as the anterior hypothalamus and NTS during chronic methyldopa administration, it seems likely that the major mechanism of its antihypertensive effect is mediated through its selective α2-adrenergic receptor actions in these regions.

Methylepinephrine

We previously have reported the presence of epinephrine and phenylethanolamine N-methyltransferase (PNMT) in various medullary and hypothalamic nuclei in rats. More recently, in vitro studies in homogenates of hypothalamus and medulla indicated that

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**Table 1. Displacement of [3H]-Prazosin and [3H]-Clonidine Binding from Membranes Prepared from Rat Cerebral Cortex by Clonidine-like Drugs**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Kᵢ [3H]-Prazosin</th>
<th>Kᵢ [3H]-Clonidine*</th>
<th>Kᵢ [3H]-Prazosin</th>
<th>Kᵢ [3H]-Clonidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guanfacine</td>
<td>6177 ± 310</td>
<td>1.9 ± 0.3</td>
<td>3220</td>
<td></td>
</tr>
<tr>
<td>CP14,304-18</td>
<td>2102 ± 173</td>
<td>0.8 ± 0.01</td>
<td>2627</td>
<td></td>
</tr>
<tr>
<td>Clonidine</td>
<td>593 ± 102</td>
<td>2.2 ± 0.2</td>
<td>269</td>
<td></td>
</tr>
<tr>
<td>Lofexidine</td>
<td>181 ± 49</td>
<td>2.3 ± 0.2</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>44-549</td>
<td>40 ± 2</td>
<td>1.8 ± 0.3</td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>

Inhibition constant (Kᵢ) values have been calculated from the (IC₅₀) for inhibition of binding with Cheng and Prusoff's equation IC₅₀ = Kᵢ (1 + D/K D), where IC₅₀ = concentration inhibiting specific binding by 50%, values given are means ± SEM for four to seven experiments conducted in duplicate.

* Modified from Summers, Jarrott, and Louis.
methylnorepinephrine was transformed to methylepinephrine by enzyme preparations from both regions but that methylnorepinephrine was a less effective substrate than norepinephrine. 21

In further experiments Sprague-Dawley rats were treated subcutaneously with methyldopa and the formation of methylepinephrine was studied in vivo. 21 Freshly dissected hypothalami and medullae oblongatae were analyzed for the presence of methylepinephrine with a high-performance liquid chromatography electrochemical detection system. These experiments indicate that methylepinephrine can be formed from methyldopa within the central nervous system. Moreover, formation was not significantly altered by the administration of the PNMT inhibitor SKF 64139, which suggests that the in vivo synthesis of methylepinephrine after the administration of methyldopa probably occurs by the action of nonspecific methyltransferases. 2 2 2 3

At this stage the functional significance of methylepinephrine is uncertain, but this amine may be involved in the depletion of epinephrine from medullary and anterior hypothalamic nuclei seen after chronic administration of methyldopa. 24 Moreover, the work of Dampney, 2 5 Blessing et al., 2 6 and Reis et al. 2 7 suggests that the Cl region, which is important in cardiovascular control, contains high concentrations of epinephrine. More recently Robertson et al. 2 8 have demonstrated that administration of methylepinephrine into either the lateral ventricle or the NTS produces a prolonged antihypertensive effect.

**Conclusion**

It is apparent that methyldopa administration produces marked alterations in the levels of catecholamines in all regions of the central nervous system. In the corpus striatum methyldopamine has been documented to replace the endogenous neurotransmitter dopamine in dopaminergic nerve terminals and is released in a manner similar to dopamine. 2 9 Similarly methylnorepinephrine replaces norepinephrine as a neurotransmitter in noradrenergic nerve terminals in other areas of the brain. Because it is not a substrate for monoamine oxidase, methylnorepinephrine accumulates both in the synaptic vesicles and in the soluble fraction of the cell, which explains in part the very high levels of methylnorepinephrine relative to control values of norepinephrine. In addition, binding studies indicate that methylnorepinephrine is considerably more potent and much more selective than norepinephrine for α 2 -adrenergic receptors in the central nervous system. Other reports indicate that methylnorepinephrine is more potent than norepinephrine in producing a fall in blood pressure after direct injection into the anterior hypothalamic-preoptic area and the NTS.

The mechanism by which the α 2 -agonists mediate their effect in the central nervous system is uncertain, however. The concept that presynaptic α 2 -adrenergic receptors modulate the amount of neurotransmitter released per pulse (see reviews by Langer 3 0 and Starke 3 1 ) is largely derived from studies in peripheral tissues but Rand et al. 3 2 have demonstrated such an action for clonidine in high concentrations in guinea pig hypothalamic slices, and similar results have been reported in rat cerebral cortex. 3 3, 3 4 It should also be noted, however, that clonidine still reduces sympathetic outflow and enhances the vaguely mediated cardiodepressor reflex after catecholamine depletion with reserpine and α-methyl-p-tyrosine. 3 5, 3 6 These latter results suggest that presynaptic effects in noradrenergic neurons cannot be the only mechanisms involved in the hypertensive response to clonidine and perhaps to other central α 2 -agonists.

More recently interest has focused on the presence of adrenergic neurons in the Cl region of the medulla oblongata. 2 6, 2 7 It is now apparent that methyldopa can be converted to methylepinephrine in both medullary and hypothalamic regions. 21 The significance of this observation is uncertain, in part because there are no clear data on the relative amounts of methylnorepinephrine and methylepinephrine formed and on whether methylnorepinephrine and methylepinephrine have different actions in these regions. Methyldopa administration also may affect other neurotransmitter pathways in the brain; for example, there is evidence that it can deplete serotonin pathways by replacing serotonin with methyldopamine. 3 7

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**Table 2. Relative Potencies of α-Adrenergic Receptor Drugs at α 2 -Adrenergic Receptors Labeled by [ 3 H]Prazosin and α 2 -Adrenergic Receptors Labeled by [ 3 H]Guanfacine in Membranes from Rat Cerebral Cortex**

<table>
<thead>
<tr>
<th>Drug</th>
<th>K i [ 3 H]-Guanfacine (nM)</th>
<th>K i [ 3 H]-Prazosin (nM)</th>
<th>K i [ 3 H]-Prazosin (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guanfacine</td>
<td>1.8</td>
<td>6210</td>
<td>3400</td>
</tr>
<tr>
<td>Clonidine</td>
<td>2.1</td>
<td>593</td>
<td>280</td>
</tr>
<tr>
<td>(-) α-Methylnorepinephrine</td>
<td>2.6</td>
<td>11,450</td>
<td>4400</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>6.9</td>
<td>7 2</td>
<td>1.04</td>
</tr>
<tr>
<td>(+) Norepinephrine</td>
<td>8.6</td>
<td>15,280</td>
<td>1777</td>
</tr>
<tr>
<td>(-) Norepinephrine</td>
<td>14.7</td>
<td>960</td>
<td>65</td>
</tr>
<tr>
<td>(+) Norepinephrine</td>
<td>400</td>
<td>39,580</td>
<td>99</td>
</tr>
<tr>
<td>Prazosin</td>
<td>12,000</td>
<td>0.11</td>
<td>0.000009</td>
</tr>
</tbody>
</table>

Source: Louis et al. 1 8
Acknowledgments

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