Central and Peripheral Autonomic Mechanisms Involved in the Circulatory Actions of Methyldopa

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SUMMARY Intracisternal (i.e.) and intravenous (i.v.) administration of methyldopa in conscious rabbits produced closely similar changes in hemodynamics, heart rate, and falls in plasma norepinephrine levels. Two weeks after giving i.c. 6-hydroxydopamine (6-OHDA), when there is widespread destruction of central noradrenergic neurons, the effects of i.c. methyldopa virtually were abolished. This suggests that noradrenergic neurons are the major central site of biotransformation into active metabolites. The circulatory and norepinephrine effects of i.v. methyldopa were attenuated but not completely abolished after giving i.c. 6-OHDA. Hence, in the rabbit about 70% of the action of methyldopa was central and about 30% was peripheral in the human therapeutic range of methyldopa concentrations. Preliminary lesion experiments suggest that the A5 nucleus plays an important role in the bradycardia. Two weeks after giving 5,6-dihydroxytryptamine (5,6-DHT) to destroy serotonergic (5HT) neurons the effects of i.c. methyldopa on mean arterial pressure (MAP) and heart rate were attenuated to approximately 50% of control effects. Therefore, some of the central effects of methyldopa apparently are mediated through 5HT pathways. We also compared the effects of i.c. methyldopa with those of i.c. clonidine (an α₂-adrenergic receptor agonist) and with the effects of transmitter release from the endings of noradrenergic and 5HT neurons during the first few hours after either 6-OHDA or 5,6-DHT administration. Our findings suggest that after biotransformation of methyldopa its active metabolites increase the activity of the bulbospinal noradrenergic neurons that control MAP and heart rate and reduce the activity of bulbospinal 5HT neurons.

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KEY WORDS • A5 nucleus • clonidine • methyldopa • noradrenergic neurons • serotonergic neurons • sites of biotransformation

MORE than 20 years after its discovery methyldopa remains one of the most important drugs in the treatment of hypertension. After a single dose there is a 1- to 2-hour latency before the blood pressure falls, which is due to its biotransformation into active metabolites. The long latency probably has been a factor in the drug's neglect in experimental studies, particularly those that involve its central administration. Earlier studies on the effects of methyldopa in the presence of centrally and peripherally acting dopa-decarboxylase inhibitors have suggested that the CNS is the major site of action of methyldopa.2-3 The noradrenergic neurons of the CNS are believed to contribute to the drug's central action, because in the rat the fall in blood pressure produced by intraperitoneal (i.p.) administration was prevented by central administration of the selective neurotoxic drug 6-hydroxydopamine (6-OHDA) a few days earlier.4 Biotransformation of methyldopa in peripheral sympathetic neurons, however, also is believed to make some contribution to the drug's antihypertensive action.5,6

This paper summarizes recent studies from our laboratory on the mechanisms of action of methyldopa. We first compared the effects of intracisternal (i.c.) and intravenous (i.v.) methyldopa on hemodynamics and baroreceptor-heart rate reflex properties in conscious rabbits. We next assessed the effects of destruction of central noradrenergic neurons on the circulatory and plasma catecholamine changes of i.c. and i.v. methyldopa. In addition, we studied the role of the central serotonergic (5HT) pathways on the effects of i.c. methyldopa. In the last part of the paper we discuss the hypothesis that centrally administered methyldopa increases the activity of bulbospinal noradrenergic neurons and decreases that of bulbospinal 5HT neurons.
Methods

The experiments were performed in male and female rabbits crossbred from a multicolored English strain, which weighed from 2.2 to 3.4 kg. Preliminary operations were performed under halothane anesthesia, after induction with propanidid (Epontol). At these operations one or more of the following was implanted in each rabbit: (1) a fine polyvinylchloride (PVC) catheter for intracisternal drug injections was inserted into the cisterna magna through the atlanto-occipital membrane; (2) a Doppler (6 mm i.d.) flow transducer was placed on the aortic root for measuring cardiac output by the continuous Doppler method; (3) perivascular balloons were placed around the thoracic descending aorta and inferior vena cava to transiently raise and lower intravascular pressures to characterize the baroreceptor-heart rate reflex.

Plasma norepinephrine and methyldopa were analyzed by high performance liquid chromatography with electrochemical detection (Jackman, Oddie, Skews, and Bobik, unpublished observations).

Results and Comment

Comparison of Intracisternal and Intravenous Effects of Methyldopa

We obtained methyldopa dose-mean arterial pressure (MAP)-response curves in conscious rabbits after intracisternal and intravenous administration of the drug (Figure 1). The fall in MAP followed a similar time course when the drug was administered by either route (Figure 1). After i.e. methyldopa a given fall in MAP was produced by 1/300 to 1/400 of the i.v. dose. With equipotent near-maximal intracisternal and intravenous doses the fall in MAP was approximately 50% due to reduction in cardiac output and approximately 50% due to reduction in total peripheral resistance (TPR).

The same intracisternal and intravenous doses produced similar reductions in resting heart rate and similar changes in the baroreceptor-heart rate reflex properties. The reflex was characterized by sigmoid function curves that related MAP to heart period (HP, pulse interval); to construct the curves MAP was altered from the resting state by transiently inflating the aortic or caval perivascular balloons for 30 seconds and observing the evoked changes in HP. In the presence of methyldopa there was an increase in HP range (between the upper and lower curve plateaus) and an increase in gain. These changes were due mainly to vagal efferent facilitation during transient rises in MAP.

Central Circulatory Effects of Methyldopa After Selective Destruction of Noradrenergic and 5HT Neurons

Three groups of rabbits were studied prior to and 14 days after one of the following treatments: (1) 6-OHDA (600 µg/kg i.e.), (2) 5,6-dihydroxytryptamine (5,6-DHT) (633 µg/kg i.e.), which is a selective neurotoxic drug for 5HT neurons, or (3) 0.9% NaCl vehicle. None of the treatments produced significant changes from the initial control values in resting MAP and heart rate. The falls in MAP and heart rate produced by methyldopa in the control study that preceded these treatments were similar in all three groups of rabbits. Similarly, there was good agreement in the vehicle-treated group of rabbits between the control responses and those obtained 14 days after treatment (Figure 2, left panel).

After 6-OHDA treatment, the normal falls in MAP and heart rate produced by methyldopa in the control study that preceded these treatments were similar in all three groups of rabbits. Similarly, there was good agreement in the vehicle-treated group of rabbits between the control responses and those obtained 14 days after treatment (Figure 2, left panel).

After 6-OHDA treatment, the normal falls in MAP and heart rate produced by methyldopa virtually were absent on day 14 (Figure 2, middle panel). The average changes from resting rates were close to 0 (i.e.,
A MAP from ANOVA; * indicates synthetic enzymes fall to a minimum after administration of the neurotoxic drugs in these experiments was relatively specific. For example, after 6-OHDA administration, spinal cord noradrenergic neurons and 3,4-dihydroxyphenylethylene glycol (DHPG) concentrations averaged 26% and 10% respectively of the values observed in the vehicle treatment: filled rectangles represent results obtained 14 days after i.c. administration of methyldopa (0.4 mg/kg i.e.). Open rectangles represent control responses before any treatment; filled rectangles represent results obtained 14 days after administration of 5,6-DHT, 633 ng/kg (left), 6-hydroxydopamine (6-OHDA), 600 μg/kg (middle), and 5,6-dihydroxytryptamine (5,6-DHT), 633 μg/kg (right). Bar in control rectangle is 1 SEM fromanova; * indicates p < 0.05 for significance of difference within animals. (Based on data of Head et al., 1983.)

MAP = -0.7±2.3 mm Hg, ns; heart rate = -4.8±2.6 beats/minute, 0.1 > p > 0.05).

After 5,6-DHT treatment, the hypotension and bradycardia were attenuated to approximately 50% of the initial control responses (Figure 2, right panel). The attenuation was thus less marked than after 6-OHDA.

The results of neurochemical analysis of different brain regions suggested that the action of each neurotoxic drug was relatively specific. For example, after 6-OHDA administration, spinal cord noradrenergic neurons and 3,4-dihydroxyphenylethylene glycol (DHPG) concentrations averaged 26% and 10% respectively of the corresponding values observed in vehicle-treated rabbits; there were small reductions in spinal cord dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations, but no significant changes were observed in the concentrations of 5HT or of its metabolite 5-hydroxyindoleacetic acid (5HIAA). After administration of 6-OHDA, 5,6-DHT, the spinal cord concentrations of 5HT and 5HIAA averaged 41% and 28% respectively of the values observed in the vehicle group; noradrenergic neurons, DHPG, dopamine, and DOPAC concentrations were not altered significantly.

The 14-day observation period after the administration of the neurotoxic drugs in these experiments was considerably longer than that observed in most previous studies. It corresponds approximately to the time at which concentrations of transmitter and biosynthetic enzymes fall to a minimum after administration of the selective neurotoxic drugs or after an electrolytic lesion of a particular pathway.

These studies indicate that noradrenergic neurons of the CNS are critically important for the hypotension and bradycardia that occur after centrally administered methyldopa. The most likely explanation is that the noradrenergic neurons are the major central site of biotransformation of methyldopa into active metabolites.

The attenuation of the circulatory effects of methyldopa after 5,6-DHT administration suggests that central 5HT pathways also mediate part of the central action of methyldopa. Because it is unlikely that 5HT neurons are involved in the biotransformation of methyldopa, these neurons must be in series with noradrenergic neurons where methyldopa is converted into active metabolites (see Figure 7). The 5HT neurons are pressor (i.e., excitatory to preganglionic sympathetic motoneurons, see below), so that the most likely effect of the active metabolite of methyldopa is inhibition of their activity.

We also compared in the same rabbits the MAP and heart rate responses to different intracisternal doses of the α₁-agonist clonidine before and 14 days after administration of 6-OHDA, 5,6-DHT, or vehicle (Figure 3). After 6-OHDA administration the fall in MAP at the highest dose of clonidine was attenuated by approximately 50% of the control response before administration of the neurotoxic drug. This result contrasts with the complete abolition after 6-OHDA administration of the fall in MAP evoked by methyldopa. After 5,6-DHT administration, however, the hypotensive responses to clonidine and to methyldopa were both reduced by approximately 50%.

In these experiments we used equi potent hypotensive doses of i.c. methyldopa and clonidine. Clonidine acts on bulbo spinal α₁-adrenergic receptors of the CNS. Hence, the significant residual response to this drug after 6-OHDA administration suggests that only some of the α₁-adrenergic receptors are located on noradrenergic neurons; others are located on other types of neurons (e.g., 5HT neurons). The active metabolite of methyldopa is considered to act on the same central α₁-adrenergic receptors as clonidine. Clonidine therefore can be considered a marker for the neurons on which the methyldopa metabolite acts distal to the site of central biotransformation. If this hypothesis is correct methyldopa and clonidine should alter autonomic function in a closely similar manner (i.e., on the assumption that they acted on similar pathways in the CNS). We have found that intracisternal administration of the two antihypertensive drugs produces similar changes in MAP, cardiac output, and TPR; both facilitate the vagal component of the baroreceptor–heart rate reflex and inhibit cardiac sympathetic motoneurons that do not receive projections from baroreceptor afferents. The only observed difference in their action to date relates to those cardiac sympathetic motoneurons that receive projections from the baroreceptors; these were inhibited by clonidine, but were virtually unaffected by methyldopa. Thus, apart
from this relatively minor difference the results are consistent with an action by both drugs on similar central autonomic pathways.

**A5 Lesions**

In preliminary experiments to pinpoint the cell groups involved in the biotransformation of methyl-
dopa we have made bilateral cathodal lesions of the A5 nucleus in the pons. These lesions destroyed an average 90% of noradrenergic cells. We compared the effects of i.v. methyldopa in these rabbits with the responses of sham-operated rabbits. The fall in heart rate after A5 lesions was only approximately 30% of the response observed in sham-operated animals (Figure 4). The reduction in MAP was slightly greater between 2 and 4 hours after methyldopa administration, but this did not account for the attenuation of the heart rate response, which was already evident during the first 2 hours after giving methyldopa when the falls in MAP were the same in the lesioned and sham-operated groups.

**Fraction of Intravenous Methyldopa that Undergoes Biotransformation in Peripheral Sympathetic Neurons**

On the basis of the experiment in Figure 2 we have assumed that all the biotransformation of methyldopa into active metabolites that takes place in the CNS occurs in neurons destroyed by 6-OHDA (i.e., mainly noradrenergic neurons). Therefore, if after administration of i.v. 6-OHDA adequate time is allowed for degeneration of the terminals, any residual response to intravenous administration of the drug can be considered to be caused by its biotransformation in peripheral sympathetic neurons.

We used another group of seven rabbits to study the falls in MAP and heart rate that occur after methyldopa (0.6 mg/kg i.v.), which is slightly larger than the dose used in the experiment in Figure 2; on another day we determined the effects of 50 mg/kg i.v. methyldopa.
Both responses and the changes in plasma norepinephrine levels were studied before and 2 to 3 weeks after administration of 6-OHDA. In four other rabbits we examined the effects of a smaller intravenous dose of methyldopa (25 mg/kg), and again those of 0.6 mg/kg i.e. With the larger intravenous dose the plasma concentrations of methyldopa were approximately double the upper limit of the human therapeutic range (Table 1), but with 25 mg/kg they were within this range (1.56 ± 0.15 μg/ml). Administration of i.e. 6-OHDA had no effect on the resting variables but abolished the fall in MAP and in plasma catecholamine concentration produced by i.e. methyldopa and greatly attenuated the heart rate changes (Figure 5, left panel; Table 1). By contrast, the changes produced by intravenous administration of methyldopa were only attenuated, not abolished. With 25 mg/kg. i.v. of methyldopa the falls in MAP and heart rate were about 30% and 45% respectively of the control responses (Figure 5, middle panel). With a dose of methyldopa of 50 mg/kg i.v. the falls in MAP and heart rate averaged 50% and 60% of the control responses and the fall in plasma norepinephrine levels averaged 30% of control (Figure 5, right panel; Table 1).

These results suggest that the effects produced by an intravenous dose of methyldopa that raises plasma levels to the upper level of the human therapeutic range are approximately 70% caused by its central action and approximately 30% by its biotransformation in peripheral sympathetic neurons. At higher intravenous doses a greater fraction of the drug’s action appears to be due to peripheral biotransformation. We do not at present know whether the same proportion of central to peripheral neuronal mechanisms accounts for the hypotensive action of methyldopa in other species.

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<th>Table 1. Results Obtained in 7 Rabbits Showing Resting Values and Circulatory and NE Responses to Methyldopa (0.6 mg/kg i.c.; 50 mg/kg i.v.) Before and 2-3 weeks After 6-OHDA (600 μg/kg i.c.)</th>
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MAP = mean arterial pressure; HR = heart rate; NE = norepinephrine; ∆ MAP, ∆ HR, ∆ NE = changes produced by methyldopa; SED = standard error of difference from control by analysis of variance.

For difference from control: * < 0.05; † < 0.001.
Central Action of Methyldopa in Relation to Changes in Activity of Noradrenergic and 5HT Pathways

There has been much controversy about the circulatory functions mediated through the noradrenergic and 5HT pathways of the CNS.22 We have found that the circulatory effects that occur during the first few hours after injecting 6-OHDA or 5,6-DHT are due to release of the transmitter from the nerve endings of noradrenergic and 5HT neurons respectively.2, 15, 16, 25-27

In rabbits with intact CNS i.e. 6-OHDA produces, after some latency, a rise in MAP and bradycardia, both of which reach their peak about 2 to 3 hours after injection (Figure 6, left panel). These effects apparently are mediated through CNS α-adrenergic receptors, as they are abolished or attenuated by i.c. phentolamine.25 In pontine (decerebrate) rabbits, where only bulbospinal pathways are intact, there are early falls in MAP and in heart rate, with the peak responses at 0.5 to 1.0 hour (Figure 6, middle panel).

We wondered why the short latency falls in MAP and heart rate, mediated through bulbospinal pathways, were not seen in the rabbit with intact CNS in which the long latency rise in MAP was mediated through a suprapontine pathway (Figure 7). From recent findings of the effects of 6-OHDA in sinoaortic denervated (SAD) rabbits it appears that in intact rabbits the early bulbospinal depressor response normally is buffered through the baroreceptor reflex. In SAD rabbits we regularly observed the characteristic short latency falls in MAP and heart rate, followed by the late pressor response (Figure 6, right panel). The early changes were thus similar to those found in pontine rabbits. But in SAD animals we found a late elevation in heart rate above resting rates in association with the pressor response (Figure 6, right panel). Comparison of the findings in intact and in SAD animals suggests that the late bradycardia in the former is reflexly produced in response to the rise in MAP. It apparently completely masks the centrally initiated rise in heart rate observed in SAD rabbits, which like the pressor response is mediated through a suprapontine pathway (Figure 7). We have not analyzed the role of the vagus and sympathetic nerves in the late tachycardia response of SAD rabbits. It is likely to be caused by increased sympathetic activity, as its timing coincides with the marked elevation of sympathetic constrictor activity that we have observed previously, which affects particularly the renal and mesenteric vascular beds.15

**Figure 6.** Time course of changes in mean arterial pressure (ΔMAP) and heart rate (ΔHR) after i.c. injection of 6-OHDA at arrow, in 9 rabbits with intact CNS (left), 5 pontine (decerebrate) rabbits (middle), and 5 sino-aortic denervated (SAD) rabbits (right). Bar is ± 1 SEM, from ANOVA.

**Figure 7.** Schematic representation of noradrenergic (NA) and serotonergic (5HT) pathways controlling blood pressure and heart rate. Note that in suprapontine pressor pathway, 5HT neuron is in series with NA neuron. Suprapontine NA heart rate pathway is based on data in sino-aortic denervated rabbits. Bulbar and spinal NA and 5HT pathways controlling blood pressure have a mixed parallel and series arrangement while those regulating cardiac vagal activity are arranged in parallel (For further description see text.)
We have also studied the effects of 5HT release in intact and pontine rabbits during the first few hours after i.c. 5,6-DHT administration. These experiments have shown that there are distinctive suprapontine and bulbospinal pressor pathways. We found that in the suprapontine pathway a 5HT neuron is in series with a noradrenergic neuron (Figure 7). This finding was deduced from experiments in which the acute effects of 6-OHDA (or 5,6-DHT) administration were studied after previous destruction of SHT (noradrenergic) neurons by intracisternal administration 1 week earlier of the other neurotoxic drug. The finding that the bulbospinal SHT pressor pathway and the noradrenergic depressor pathway have antagonistic effects on blood pressure (Figure 7) is in accord with results obtained by recordings from preganglionic sympathetic neurons after iontophoretic application of the appropriate transmitter. We have found that noradrenergic and 5HT neurons exert antagonistic effects on the vagal component of the baroreceptor–heart rate reflex, which are facilitated by noradrenergic release and inhibited by 5HT release (as shown schematically in Figure 7).

From this discussion it appears likely that central administration of methyldopa enhances the activity of bulbospinal noradrenergic neurons and reduces that of bulbospinal 5HT neurons. Our studies suggest that all the central biotransformation occurs in noradrenergic neurons and that subsequent changes result from stimulation by metabolites of a2-adrenergic receptors on bulbospinal noradrenergic and 5HT neurons, as is produced by clonidine. Hence, the arrangement of the bulbospinal pathways controlling blood pressure shown in Figure 7: one set of noradrenergic neurons exerts a direct inhibitory action on sympathetic preganglionic neurons; the other acts indirectly through a2-adrenergic receptors on cell bodies distal to the sites of uptake of the drug by central and peripheral noradrenergic neurons to injury. In: Fuxe K, Olsen L, Zotterman Y, eds. Dynamics of degeneration and growth in neurons. Oxford: Pergamon Press, 1974: 141-158.

We do not know at present why the action of methyldopa appears to be confined to bulbospinal noradrenergic and 5HT neurons and why the corresponding suprapontine pathways are not also affected. Possibly this is due to differences in uptake of the drug by different neurons or to differences in distribution of a2-adrenergic receptors on cell bodies distal to the sites of biotransformation of the drug. These remain challenging problems for the future.

Acknowledgments
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27. Korner PI, Head GA. Cardiovascular functions of central noradrenergic and serotonergic neurons in conscious rabbits; their contribution to the central action of clonidine. Chest 1983;83(suppl):3355–3385