Bulbospinal Serotonin Pressor Pathways and Hypotensive Action of Methyldopa in the Rat

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SUMMARY The administration of methyldopa (200 mg/kg i.p.) induced a green fluorescence typical of catecholamine fluorescence, in regions of the brain stem which coincided with all the major serotonin cell groups, including the B1, B2, and B3 cell groups in the medulla. Prior administration of 5,7-dihydroxytryptamine (5,7-DHT), a neurotoxin relatively specific for serotonin neurons, prevented the appearance of this methyldopa-induced fluorescence. Electrical stimulation of the ventrolateral medulla in areas that coincided with the lateral elements of the B1 and B3 serotonin cell groups evoked pressor responses recorded via cannulae in the abdominal aorta. The pressor responses were frequency-dependent and could be markedly attenuated by prior administration of 5,7-DHT either intracerebroventricularly (i.c.v.) or directly into the cervical cord to ablate descending serotonin nerve fibers. Microinjection of methyldopa (4–16 µg) directly into the region of the B1 and B3 cells in the ventrolateral medulla evoked a dose-dependent fall in arterial pressure observed for 4 hours. Here too, prior administration of 5,7-DHT either intracerebroventricularly or directly into the cervical cord largely prevented the hypotensive action of the microinjections of methyldopa. The administration of 5,7-DHT produced a highly selective depletion of serotonin stores without reducing the concentrations of norepinephrine. These experiments suggest that the activity of serotonin nerves descending into the spinal cord from the B1 and B3 cells in the ventrolateral medulla serves to elevate or maintain arterial pressure. They also suggest that these descending serotonin neurons may contribute to the hypotensive action of methyldopa. (Hypertension 6 (Suppl. II) II-16-II-21, 1984)

Key Words • ventrolateral medulla • arterial blood pressure • hypertension • antihypertensive drugs • 5,7-dihydroxytryptamine • 6-hydroxydopamine

THERE has been much debate about the role of central serotonin nerves in the control of blood pressure. Although many studies have suggested that activity of central serotonin nerves elevates arterial pressure, others appeared to indicate a depressor effect.1, 2 This controversy has been aggravated by the tendency to treat the serotonin neurons as a homogeneous network subserving a single function and by a failure to recognize that there are numerous distinct serotonergic projections with diverse roles in cardiovascular control.

Recent studies from our laboratory have focused on the contribution of a single projection of serotonin neurons descending from the B1 and B3 groups in the caudal medulla and terminating in the intermediolateral cell column of the spinal cord.3 Although most groups of central serotonin cells are confined to the midline, the B1 and B3 groups have both a midline component and more lateral elements adjacent to the ventrolateral surface of the medulla oblongata as shown in Figure 1.4 In the experiments presented here we have studied the effects of electrical stimulation of that area of the ventrolateral medulla that contains the B1 and B3 serotonin neurons as recently reported elsewhere.5 In addition, we have examined and compared the effects of microinjections of the hypotensive agent methyldopa into these ventrolateral areas of the medulla oblongata, as earlier studies have demonstrated that methyldopa is taken up extensively by serotonin cells in the brain stem.6

Methods

Adult male rats were used in these experiments. Rats used for histological studies were injected intraperitoneally (i.p.) with methyldopa as a suspension in 0.9% NaCl at a daily dose of 200 mg/kg for 5 days and then killed 3 hours after the fifth injection. Monoaminergic neurons in transverse sections of the brain were localized with the Faglu method for catecholamines7, 8 and with a modified Faglu method for serotonin.9

Arterial blood pressure was measured with a fine catheter inserted into the abdominal aorta without occluding blood flow. Electrical stimulation of the ventrolateral medulla was performed with a fine monopolar electrode lowered stereotaxically through a
craniotomy hole in anesthetized rats, as described previously. Microinjections of methyldopa into this same area were carried out using a 30-gauge needle inserted stereotaxically with the same coordinates; the drug was administered in 1 μl of 0.9% NaCl over a 30-second period. Histological verification of lesion and injection sites was carried out after each experiment on serial 50-μm sections of the medulla prepared with a Vibrotome (Oxford Laboratories, U.S.A.), after the tissues had been soaked in a mixture of 4% formaldehyde and 0.5% gluteraldehyde (Faglu).

The selective neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) was given intracerebroventricularly (i.c.v.) to produce a generalized destruction of central serotonin nerves. In other experiments it was given by direct intraspinal injection at the level of C2-C3 to produce a more restricted destruction of serotonin nerves descending to the spinal cord as described previously and illustrated in Figure 2. Control animals received an equivalent volume of vehicle.

The specificity of 5,7-DHT ablation of serotonin neurons was checked by measurements of both serotonin and norepinephrine in the brain and spinal cord at the end of each experiment, as described previously. Statistical analysis was performed using analysis of variance or the unpaired Student’s t-test.

Results

Fluorescence Histochemistry of Ventrolateral Medulla After Administration of Methyldopa

Administration of methyldopa (200 mg/kg i.p. for 5 days) resulted in the appearance of green fluorescence with the standard Faglu technique (Figure 3A) in regions that do not normally exhibit such fluorescence (Figure 3B). Using a modification of the Faglu tech-

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**Figure 1.** Schematic diagram showing B1 to B9 serotonin cell groups in sagittal section of rat brain (top) and B1, B2, and B3 cell groups in transverse section of rostral medulla (bottom left) and caudal medulla (bottom right).

**Figure 2.** Schematic diagram showing location of lesions produced with injections of 5,7-dihydroxytryptamine in the cervical spinal cord. C2 = second cervical vertebra; C3 = third cervical vertebra.

**Figure 3.** Photomicrographs of transverse sections of the caudal medulla oblongata, showing the midline area of the ventral medulla. A. Faglu fluorescence for catecholamines in animals treated with methyldopa (200 mg/kg i.p. daily for 5 days). B. Faglu fluorescence for catecholamines in control animals (not treated with methyldopa). C. Modified Faglu fluorescence for serotonin in control animals (not treated with methyldopa). D. Faglu fluorescence for catecholamines in animals given methyldopa (200 mg/kg i.p. for 5 days) after pretreatment with 5,7-dihydroxytryptamine i.c.v. Sections were fixed and stained with the Faglu method for catecholamine fluorescence (A, B, and D) or the modified Faglu method for serotonin fluorescence (C).
 technique designed to reveal serotonin fluorescence (Figure 3C) it became apparent that the methyldopa-induced green fluorescence coincided with the normal location of serotonin neurons, as first described by Dahlstrom and Fuxe in 1964. Prior administration of 5,7-DHT (200 μg i.c.v.) to destroy serotonin neurons markedly reduced the green fluorescence induced by methyldopa in these regions (Figure 3D).

Changes in Blood Pressure Produced by Electrical Stimulation or Microinjections of Methyldopa

Electrical stimulation of the ventrolateral medulla, which coincided with the B1 and B3 groups of serotonin cell bodies, was performed in control rats with 5-second trains of stimuli at frequencies of 10, 30, and 50 Hz with a current of 200 μAmp. A typical response to stimulation at 50 Hz is shown in Figure 4. Stimulation produced a frequency-dependent increase in both systolic and diastolic pressures (Figures 5 and 6 (left panels)), with the greatest pressor effect always obtained at 50 Hz. The pressure returned rapidly to baseline after cessation of stimulation (Figure 4).

Microinjections of 4, 8, and 16 μg of methyldopa into this same area of the ventrolateral medulla produced gradual and dose-dependent reductions in blood pressure that were observed for a period of 4 hours and ranged from approximately 10 mm Hg up to 50 mm Hg. The time course of the fall in blood pressure evoked by 8 μg of methyldopa is shown in Figures 5 and 6 (right panels). Injection of saline alone did not lower the pressure.

Blood Pressure Responses to Electrical Stimulation or to Microinjections of Methyldopa After 5,7-DHT (i.c.v.)

Two weeks after pretreatment with 5,7-DHT (200 μg i.c.v.) the pressor responses produced by electrical stimulation were markedly decreased as compared with controls. An example of the effect of methyldopa microinjections is shown in Figure 6. A larger pressor response was evoked by methyldopa in the vehicle-injected control group than in the 5,7-DHT group, thus reducing the magnitude of the difference in pressor responses to electrical stimulation. The effect of methyldopa microinjections was most evident at frequencies of 20 and 30 Hz.

![Figure 4. Typical record showing change in intraaortic pressure evoked by electrical stimulation of the ventrolateral medulla in a region that coincides with the B1 and B3 serotonin cell groups.](image)

![Figure 5. Effects of electrical stimulation (left) or microinjection of methyldopa (right) of the ventrolateral medulla in regions coinciding with the B1 and B3 serotonin cell groups in animals given 5,7-DHT (i.c.v.) or vehicle injections (i.c.v.). Values are means ± sem. Δ MAP = change in mean arterial pressure; * = significantly different from vehicle injected control, p < 0.05.](image)
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FIGURE 6. Same effects as for Figure 5, except that 5,7-DHT was administered by direct injection into the cervical spinal cord and not by i.c.v. injection.

Stimulation of the ventrolateral medulla were markedly attenuated at all three frequencies of stimulation (Figure 5, left panel).

In the same way, microinjections of 8 µg of methyl-dopa into the region of the B1 and B3 cells of rats pretreated with 5,7-DHT (200 µg i.c.v.), no longer produced the hypotensive responses seen in control animals (Figure 5, right panel). On the other hand, pretreatment with 6-hydroxydopamine (200 µg i.c.v.) did not modify the hypotensive responses to microinjections of methyl-dopa in the same region of the ventrolateral medulla.

Blood Pressure Responses to Electrical Stimulation or to Microinjections of Methyldopa After Intraspinal 5,7-DHT

Two weeks after intraspinal injection of 4 µg of 5,7-DHT to destroy the descending projections of the B1 and B3 serotonin neurons, the pressor responses to electrical stimulation of the ventrolateral medulla were again markedly reduced at all frequencies of stimulation (Figure 6, left panel). Furthermore, stimulation at 10 Hz actually resulted in a depressor response.

Intraspinal injection of 4 µg of 5,7-DHT also attenuated the effects of microinjections of methyl-dopa; the hypotensive response was reduced and the pressure returned to control levels within 4 hours (Figure 6, right panel).

Changes in Regional Serotonin and Norepinephrine Concentrations After Administration of 5,7-DHT

Two weeks after 200 µg of 5,7-DHT (i.c.v.) the concentration of serotonin was significantly reduced in the forebrain, midbrain, hypothalamus, and spinal cord, with the greatest reduction in the spinal cord where serotonin fell to less than 5% of the corresponding values in vehicle-injected controls (Figure 7). On the other hand, norepinephrine concentrations in these four regions were not significantly altered (Figure 7).

Intraspinal injection of 4 µg of 5,7-DHT produced a highly specific depletion of serotonin content in the spinal cord below the injection, with no significant change in the serotonin content of the three brain regions examined (Figure 7). Once again regional norepinephrine values were not reduced (Figure 7).

Discussion

These experiments provide strong evidence that the activity of serotonin neurons projecting from the ventrolateral elements of the B1 and B3 groups in the caudal medulla to the intermediolateral cell column in the spinal cord serves to elevate arterial pressure. The experiments also suggest that the hypotensive action of methyl-dopa is in part mediated through inhibition of the pressor activity of these descending serotonin neurons.
Ross et al.\textsuperscript{10} were the first to suggest that the cells projecting from this ventrolateral region of the caudal medulla into the spinal cord might be serotoninergic, and positive confirmation was then obtained by Loewy and McKellar.\textsuperscript{3} There is therefore a good anatomical basis for our attribution of the pressor responses to serotonin cells from B1 and B3 groups, which is further reinforced by the attenuation of these pressor effects by intracerebroventricular and intraspinal administration of 5,7-DHT (Figures 5 and 6) to produce selective depletion of serotonin stores (Figure 7).

The pressor nature of this descending serotoninergic projection from the ventrolateral medulla is consistent with our finding that microinjection of methyldopa into the region containing the B1 and B3 serotonin cell bodies lowers arterial pressure, and that this hypotensive action of methyldopa is also abolished or attenuated by i.c.v. and intraspinal 5,7-DHT (Figures 5 and 6). Furthermore, these results are consistent with our recent demonstration that intraperitoneal administration of methyldopa can induce a green fluorescence typical of catecholamine fluorescence in serotonin cell bodies in the brain stem (Figure 3A–C).\textsuperscript{6} In these experiments too, the methyldopa-induced green fluorescence manifested by the Faglu method was abolished by pretreatment with 5,7-DHT (Figure 3D).\textsuperscript{6} While the mechanism by which methyldopa acts on central serotonin neurons to lower blood pressure is not yet defined, it is well established that methyldopa does deplete serotonin stores by inhibiting the enzyme L-aromatic amino acid decarboxylase, or "dopa-decarboxylase."\textsuperscript{11} It therefore seems possible that methyldopa might be taken up by serotonin neurons in the brain stem and is then decarboxylated to form methylamphetamine, which in turn could be responsible for inducing the green fluorescence\textsuperscript{6} and for lowering the arterial blood pressure.

It should be stressed that the demonstration that one particular projection of central serotonin neurons exerts a pressor action, does not mean that all central serotonin nerves do likewise. Indeed, it is highly probable, given the controversy regarding the role of central serotonin nerves in the control of blood pressure, that there are other groups of central serotonin nerves that act in the opposite manner. It is even possible, for example, that the medial elements of these B1 and B3 serotonin cell groups contained within the medullary raphe nuclei have a depressor action, as previously suggested by Neumayr et al.\textsuperscript{12} We are currently pursuing this possibility using the experimental approach adopted in these experiments.
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