Effect of Central Serotonin Depletion on Blood Pressure and the Renin System in Rats

LIDIA E. MIKULIC, MARÍA L. KURNJEK, RITA RUSSO, MARÍA R. TROLLIET, and NIDIA BASSO

SUMMARY In the present study we examined the effect of depletion of central nervous system serotonin by 5,7-dihydroxytryptamine on blood pressure in male Wistar rats. We also analyzed the relationship between the serotonergic and renin-angiotensin systems. Blood pressure was determined before and after intracisternal administration of 5,7-dihydroxytryptamine, 200 μg in saline with 1 mg/ml ascorbic acid (n = 9). Control rats (n = 8) received intracisternal vehicle. Before sacrifice, blood and cerebrospinal fluid samples were obtained. The brain was dissected in several areas. Serotonin, norepinephrine, angiotensinogen, and reninlike concentrations were determined in the brain parenchyma; angiotensinogen concentration was evaluated in cerebrospinal fluid and plasma samples; plasma renin activity was also measured. Treatment produced a significant decrease in blood pressure (~10 mm Hg; p < 0.025) and, simultaneously, a high depletion of serotonin stores in the studied central areas (p < 0.001), except in the cerebral cortex. Reninlike concentration was increased in the medulla oblongata (p < 0.005) and the brainstem (p < 0.02) after 5,7-dihydroxytryptamine treatment. Angiotensinogen concentration was decreased in the hypothalamus and elevated in the spinal cord. Angiotensinogen concentration in cerebrospinal fluid, plasma angiotensinogen concentration, and plasma renin activity did not change with treatment. Serotonin concentration in the cerebrospinal fluid remained unchanged, while the 5-hydroxyindoleacetic acid level was diminished (~47%; p < 0.001). Intracisternal administration of 5,7-dihydroxytryptamine produced a hypotensive effect in normal rats and several modifications of the renin-angiotensin complex, suggesting a relationship between the monoaminergic and peptidergic systems.

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KEY WORDS • angiotensinogen • serotonin • blood pressure • cerebrospinal fluid

MANY studies have focused on the role of serotonin (5-hydroxytryptamine, 5-HT) in the central control of arterial blood pressure (BP). The results of such studies have been contradictory. In the early works, Bogdanski et al.1 observed that in dogs the administration of 5-hydroxytryptophan (5-HTP), a precursor of 5-HT, induced hypertension and tachycardia. On the other hand, some authors reported that 5-HTP produced a hypotensive response in conscious rats2 and anesthetized dogs and cats.3,4 When central 5-HT stores were pharmacologically reduced in both normotensive and hypertensive animals, however, different results were obtained.5–8 Possible explanations for this discrepancy are the use of drugs that affect serotonin stores differently, the relative lack of specificity of these drugs, and the different designs of the experiments. The central nervous system seems also to be involved in the regulation of renin secretion. Some experiments that analyzed the role of brain monoamines in the regulation of renin secretion indicated that intravenously administered 5-HTP increased plasma renin activity (PRA).9 Therefore, in the present study we further analyzed the effect of central 5-HT depletion by intracisternal administration of 5,7-dihydroxytryptamine (5,7-DHT) on the level of blood pressure and the relationship between the serotonergic and renin-angiotensin systems.

Materials and Methods

Male Wistar rats of the CbcbTHOM albino strain, weighing about 250 g, were used. The animals were fed a standard Purina rat chow diet and were offered tap water ad libitum to drink; they were kept in an automatically lighted room (from 0700 to 1900) at a constant temperature (22 ± 1°C). Systolic BP was measured by a plethysmographic tail-cuff method (Narco Bio-Systems, Houston, TX, USA) in the conscious rats before and 14 days after treatment (each determination represented the mean of three readings). Blood samples were obtained at the latter time by cut-
ting the tip of the tail in the conscious, unrestrained animals. Injection of the 5,7-DHT (200 μg) dissolved in 10 μl saline containing 1 mg/ml ascorbic acid into the cisterna magna was performed in nine animals under ether anesthesia; eight sham rats received 10 μl of the vehicle. Fifteen days later, the animals were anesthetized with sodium pentobarbital (40 mg/kg i.p.) and a cerebrospinal fluid sample was obtained from the cisterna magna. The central nervous system was removed and dissected over ice, and the following areas were separated: cerebral cortex, hypothalamus, brainstem, medulla oblongata, and spinal cord. The mesenteric artery, heart, and adrenal glands were also excised. Angiotensinogen concentration (AoC) and reninlike concentration in tissues, plasma renin activity (PRA), plasma AoC, and cerebrospinal fluid AoC were determined. Norepinephrine concentration was measured in the heart and mesenteric artery, and both norepinephrine and epinephrine were evaluated in the adrenal glands. Serotonin concentration was determined in the brain and cerebrospinal fluid, and 5-hydroxyindoleacetic acid (5-HIAA) level was measured in the latter samples.

**Determination of Angiotensinogen and Reninlike Concentrations**

The hypothalamus and the medulla oblongata were homogenized in 200 μl of 8 mM EDTA solution in saline; 25% homogenates were prepared with the other brain areas. All the homogenates were centrifuged at 1000 g for 30 minutes at 4°C. The supernatants were separated, and an aliquot was incubated in the presence of an optimal concentration of angiotensinase inhibitors with either an excess of semipurified angiotensinogen from plasma of nephrectomized rats for reninlike concentration determination or an excess of hog kidney renin for AoC evaluation. Plasma AoC and cerebrospinal fluid AoC were estimated by incubating conveniently diluted samples with an excess of hog kidney renin; PRA was determined by incubating the samples with adequate angiotensinase inhibitors. Incubations were conducted in the presence of 0.04 mM Tris chloride buffer (pH 7.2) for 60 minutes for brain AoC and for 3 hours for reninlike concentration and PRA at 37°C. The presence of angiotensin I in all samples was evaluated by radioimmunoassay (Phadebas, Pharmacia Diagnostics, Uppsala, Sweden).

**Determination of Catecholamines**

After dissection, all the samples were kept frozen in an acid solution (HClO4/EDTA/Na2SO3, 100:1:1) at -70°C. The tissues were homogenized in the same solution and centrifuged at 1000 g for 30 minutes at 4°C. The supernatants were used to separate the catecholamines using chromatographic alumina columns. The eluated amines were evaluated fluorometrically in the adrenal glands by Crout’s method, and norepinephrine concentration was determined in all the other tissues using the method described by Laverty and Taylor.

**Determination of Serotonin and 5-Hydroxyindoleacetic Acid**

Serotonin was evaluated in the cerebrospinal fluid samples and in an aliquot of the supernatants used for determination of norepinephrine in the central nervous system areas; 5-HT as well as 5-HIAA were measured by high performance liquid chromatography using micro-Bondapak C18 columns and electrochemical detection with an electrochemical transducer and amperimetric detector (Bioanalytical Systems, West Lafayette, IN, USA).

Data are expressed as means ± SEM. Differences between groups were established by the Student’s t test.

**Results**

A decrease in BP was observed in rats injected intracisternally with 5,7-DHT (before treatment, 106 ± 3 mm Hg; after 5,7-DHT, 96 ± 3 mm Hg; p<0.0025). The sham group showed no changes in BP (113 ± 2 mm Hg, before and after intracisternal vehicle injection). The drug produced a significant depletion of 5-HT concentration in all the brain areas except the cerebral cortex (Table 1). On the other hand, treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cerebral cortex</th>
<th>Medulla oblongata</th>
<th>Brainstem</th>
<th>Hypothalamus</th>
<th>Spinal cord</th>
<th>Cerebrospinal fluid</th>
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</thead>
<tbody>
<tr>
<td>5-HT (ng/g)</td>
<td></td>
<td></td>
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<tr>
<td>Saline (n = 8)</td>
<td>295.3 ± 12.3</td>
<td>329.0 ± 19.6</td>
<td>362.9 ± 9.3</td>
<td>338.3 ± 21.0</td>
<td>318.0 ± 21.0</td>
<td>0.77 ± 0.06 ng/ml</td>
</tr>
<tr>
<td>5,7-DHT (n = 9)</td>
<td>278.7 ± 25.8</td>
<td>97.0 ± 11.6*</td>
<td>67.1 ± 9.6*</td>
<td>202.7 ± 21.8*</td>
<td>145.8 ± 15.9*</td>
<td>0.79 ± 0.1 ng/ml</td>
</tr>
<tr>
<td>RC (ng Ang I/μl)</td>
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<tr>
<td>Saline (n = 8)</td>
<td>56.1 ± 3.2</td>
<td>45.5 ± 1.2</td>
<td>48.9 ± 3.1</td>
<td>91.5 ± 3.6</td>
<td>53.8 ± 4.0</td>
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<tr>
<td>5,7-DHT (n = 9)</td>
<td>51.3 ± 3.4</td>
<td>54.4 ± 2.7</td>
<td>60.9 ± 2.7</td>
<td>94.4 ± 8.4</td>
<td>58.8 ± 2.8</td>
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<tr>
<td>AoC (ng Ang I/μl)</td>
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<tr>
<td>Saline (n = 8)</td>
<td>34.2 ± 1.5</td>
<td>53.4 ± 2.2</td>
<td>83.9 ± 5.0</td>
<td>99.2 ± 9.2</td>
<td>26.2 ± 1.6</td>
<td>45.5 ± 2.4*</td>
</tr>
<tr>
<td>5,7-DHT (n = 9)</td>
<td>32.2 ± 1.4</td>
<td>60.3 ± 3.5</td>
<td>72.3 ± 6.0</td>
<td>71.2 ± 8.9</td>
<td>30.7 ± 2.1*</td>
<td>49.0 ± 2.7</td>
</tr>
</tbody>
</table>

Values are means ± SEM. 5-HT = serotonin; RC = reninlike concentration; Ang = angiotensin; AoC = angiotensinogen concentration. *p<0.001; †p<0.005; ‡p<0.02; §p<0.05. [Expressed as ng Ang I/μl].

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**TABLE 1. Concentration of Serotonin, Renin, and Angiotensinogen in the Central Nervous System and Cerebrospinal Fluid in 5,7-Dihydroxytryptamine-Treated and Control Rats**
produced a depletion of the norepinephrine stores only in the spinal cord (232.3 ± 21.7 vs 155.9 ± 26.7 ng/g; p<0.05). The norepinephrine concentration in the mesenteric artery was increased in 5,7-DHT-treated animals (3329.8 ± 117.2 vs 4013.3 ± 227.2 ng/g; p<0.025), while no changes were observed in the heart content. Both catecholamines (norepinephrine and epinephrine) in adrenal glands were unchanged by treatment. The drug did not induce any changes either in the PRA (control: 8.6 ± 1.2 vs treated: 7.1 ± 0.9 ng Ang I/ml/hr) or the AoC of plasma and cerebrospinal fluid. Reninlike concentration and AoC in the different areas of the brain are presented in Table 1. The 5-HT level in cerebrospinal fluid was similar in sham and treated rats, but the metabolite 5-HIAA was diminished after 5,7-DHT administration (~47%; p<0.001).

Discussion

The role of central serotonergic neurons in regulating BP is not clear. Reduction of central 5-HT stores induced by intraperitoneal and intracisternal administration of p-chlorophenylalanine has been reported to produce an increase in systolic BP in rats. On the contrary, a similar depletion of the amine due to intraperitoneal injection of p-chlorophenylalanine in rabbits and oral administration of p-chlorophenylalanine in rats has been shown to cause a decrease in mean arterial pressure. Moreover, Wing and Chalmers reported that intracisternal administration of 5,6-DHT caused depletion of 5-HT stores in the spinal cord and resulted in a lowering of mean arterial pressure in conscious normotensive rabbits and in rabbits with neurogenic hypertension. Otherwise, previous studies suggested that the hypotension observed after serotonergic stimulation is mediated by a bulbospinal serotonergic pathway whose origin is localized in the medulla oblongata. On the other hand, stimulation of the serotonergic mesencephalic nucleus provoked a hypertensive effect. These results seem to indicate that BP changes after serotonergic stimulation could depend on the central 5-HT areas or pathways involved.

Present knowledge suggests that central serotonergic projections induce opposite effects and have different roles in cardiovascular control. Intravenous 5-HTP and L-tryptophan produce an increase in PRA apparently due to the release of serotonin within the central nervous system.9 It has also been found that angiotensin induces the synthesis and release of 5-HT in hypothalamic tissue. Furthermore, the infusion of 5,7-DHT into both medial forebrain bundles that selectively depleted the forebrain 5-HT levels blunted the pressor effect of angiotensin II infused into the anterior hypothalamic preoptic region.

Present results show that depletion of 5-HT in hypothalamus, brainstem, medulla, and spinal cord reduces the BP level in normal rats. Simultaneously, reninlike concentration was increased in the medulla oblongata and in the brainstem, where the most important 5-HT depletion (~70.5% and ~81.5%, respectively) was found.

Our results show that 5-HT depletion induces a decrease of AoC in the hypothalamus and an increase in the spinal cord. Both effects could be due to modifications in the novosynthesis of the protein or to its release from potential precursors, since reninlike concentrations remained unchanged in those areas. On the other hand, reninlike concentration was significantly enhanced in the medulla oblongata and brainstem, while AoC did not change. These data suggest an increased activity of the peptidergic system in the main serotonergic nucleus of the raphe as well as in the spinal cord.

Present results seem to indicate that 5-HT depletion in the main serotonergic nuclei of the central nervous system induces a decrease in BP, suggesting a role for this amine in cardiovascular control. Furthermore, the data add information on the possible interrelationship between the serotonergic and the renin-angiotensin systems. The postulated increase in peptidergic activity could be triggered by the hypotensive effect of 5-HT depletion and could be acting through increased peripheral sympathetic activity.

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