Development of Renovascular Hypertension After Central Serotonin Depletion

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The participation of the central serotonergic system in the development of two-kidney, two clip (2K2C) Goldblatt renovascular hypertension in the rat has been examined. Half of the rats were treated with desmethylimipramine intraperitoneally and 5,7-dihydroxytryptamine intracisternally; the other half received only desmethylimipramine and the 5,7-dihydroxytryptamine vehicle. Two days later, a silver clip was placed in both renal arteries in half of the rats of each group. A sham operation was performed in the remaining rats. Blood pressure was recorded during the 5 weeks after treatment. At the end of the experiment, blood and cerebrospinal fluid samples were obtained. The brain was dissected into several areas and kept frozen. Norepinephrine, serotonin, angiotensinogen, and renin-like concentration were evaluated in the brain areas. Plasma renin activity and angiotensinogen concentration in the plasma and cerebrospinal fluid were estimated. In the sham-operated groups, blood pressure was lower in the treated than in the control rats. The curve of blood pressure increase, as well as the final blood pressure, was similar in the treated and control 2K2C rats. Serotonin was significantly depleted by the 5,7-dihydroxytryptamine treatment in all brain areas. Treatment did not induce any changes in central norepinephrine concentration. Plasma renin activity was diminished in the treated sham-operated rats. These data indicate that the central serotonin depletion does not prevent the development of hypertension and confirm the role of the amine in normal blood pressure regulation. On the other hand, the peripheral renin-angiotensin system might participate in the development of high blood pressure in serotonin-depleted animals. (Hypertension 1990;15(suppl I):I-166–I-169)

The role of serotonin (5-hydroxytryptamine, [5-HT]) in the central control of blood pressure (BP) has been analyzed in numerous studies. Alterations in brain serotonin concentration can produce significant changes in cardiovascular function. The activation of cerebral serotonin receptors induces a pressor effect in normotensive rats. Moreover, depletion of central serotonin levels can retard the development of some experimental hypertension models and also diminish the BP of hypertensive animals. In this sense, a blocking agent of S2 receptors has shown a significant hypotensive effect in two-kidney, two clip (2K2C) renal hypertensive rats. Previous studies have shown that depletion of central nervous system (CNS) serotonin stores reduces the BP levels in normal rats. At the same time, changes in the central renin-angiotensin system were detected. Central serotonin mechanisms seem to be also involved in the control of peripheral renin secretion and in the regulation of plasma renin activity (PRA).

The purpose of the present experimental design was to investigate the effect of central serotonin depletion on the onset and development of 2K2C experimental renal hypertension in the rat and, furthermore, to analyze the effect of both conditions on the central and peripheral renin-angiotensin system.

Methods

Male Wistar rats of the albino strain, weighing about 250 g, were used. The rats were fed a standard Purina rat chow diet (Cabeza, Buenos Aires, Argentina) and were offered tap water ad libitum to drink; they were kept in an automatically lighted room (from 7:00 AM to 7:00 PM) at a constant temperature (22±1°C).

Treated Rats

5,7-Dihydroxytryptamine (5,7-DHT) (Sigma Chemical Co., St Louis, Missouri) (200 µg) was dissolved in 10 µl of a 0.1% ascorbic acid solution in
saline and injected into the cisterna magna of 16 ether-anesthetized rats that were pretreated with desmethylimipramine (DMI) (25 mg/kg i.p.), 30 minutes before the 5,7-DHT injection, to protect brain norepinephrine neurons. Control rats (n=18) received 10 μl of 0.1% ascorbic acid in saline into the cisterna magna under the same conditions. A silver clip (0.25-mm width) was placed in both renal arteries in half of the rats of the two groups. A sham operation was performed in the remaining rats.

Systolic blood pressure was measured by a plethysmographic tail-cuff method (Narco Biosystems, Houston, Texas) in the conscious rats before treatment and once a week for 5 weeks after treatment. Each determination represented the mean of three readings.

At the end of the experimental period, the rats were anesthetized with sodium pentobarbital (40 mg/kg i.p.), and a cerebrospinal fluid sample was obtained from the cisterna magna. The brain was removed and dissected over ice, and the hypothalamus, brainstem, medulla oblongata, and spinal cord were separated. Angiotensinogen concentration (AoC) and renin-like concentration in tissues, plasma renin activity (PRA), plasma, and cerebrospinal fluid (CSF) angiotensinogen concentration were determined. Serotonin and norepinephrine concentrations were determined in the brain areas. 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) levels were measured in the CSF.

**Determination of Angiotensinogen and Renin-like Concentrations**

The hypothalamus and 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 1,000g 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 renin-like concentration or an excess of hog kidney renin for AoC evaluation. Plasma and CSF angiotensinogen concentration were estimated by incubating 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 M Tris chloride buffer (pH, 7.2) for 60 minutes for AoC and during 3 hours for renin-like concentration and PRA at 37° C. The presence of angiotensin I (Ang I) in all the samples was evaluated by radioimmunoassay (Phadebas, Pharmacia Diagnostics, Uppsala, Sweden).

**Determination of Norepinephrine**

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 1,000g for 30 minutes at 4° C. The supernatants were used to separate the catecholamine with chromatographic alumina columns. The eluted amine was evaluated fluorometrically in all the brain areas with the method described by Laverty and Taylor.

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

Serotonin was evaluated in the CSF samples and in an aliquot of the supernatants used for norepinephrine determination in the brain areas; 5-HT as well as 5-HIAA were measured by high-performance liquid chromatography (Waters-Millipore, Milford,
Results

Blood pressure in sham-treated rats was significantly lower than in the control rats at the end of the experiment ($p<0.05$) (Figure 1). BP remained lower in treated sham-operated rats than in the control rats during the whole experimental period. Hypertension developed in a similar manner in 2K2C treated and control rats. No significant difference between both groups was observed in the curves of BP increase, and it climbed to the same final value at the end of the fifth week.

Norepinephrine concentration in all the brain areas was not affected by the pharmacologic treatment with DMI and 5,7-DHT. On the other hand, 5-HT concentration was significantly depleted in all the analyzed brain areas in the rats that received the pharmacologic treatment (Table 1). The 5-HT level in CSF was similar in control and treated normotensive and hypertensive rats, however, the metabolite 5-HIAA was diminished in the treated groups (-45% in control and -50% in 2K2C rats; $p<0.001$).

In both untreated groups (vehicle injected), no significant changes in PRA were observed (sham-operated, 8.6±2.0 vs. 2K2C, 11.5±2.0 ng Ang I/ml/hr). The same level of PRA was present in untreated and treated sham-operated rats. These results confirm a previous report done in normal rats. On the other hand, failure to induce hypertension after pharmacological depletion of brain serotonin has been described in the spontaneously hypertensive rat (SHR). Angiotensinogen concentration in the hypothalamus was also higher in hypertensive rats (sham, 79.3±5.1 vs. 2K2C, 112.1±6.6; sham–5,7-DHT, 75.5±3.2 vs. 2K2C–5,7-DHT, 123.3±10.0 ng Ang I/g). Differences were highly significant between hypertensive and normotensive rats ($p<0.01$). No significant differences in angiotensinogen concentration were observed among the different experimental groups in all other brain areas.

Table 1. Concentration of Serotonin in the Central Nervous System and Cerebrospinal Fluid in 5,7-Dihydroxytryptamine–Treated and Control Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Medulla oblongata (ng 5-HT/g)</th>
<th>Brainstem (ng 5-HT/g)</th>
<th>Hypothalamus (ng 5-HT/g)</th>
<th>Spinal cord (ng 5-HT/g)</th>
<th>CSF (ng 5-HT/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated control</td>
<td>451.3±52.5</td>
<td>454.8±32.6</td>
<td>421.8±66.0</td>
<td>548.6±23.1</td>
<td>2.9±1.0</td>
</tr>
<tr>
<td>Sham-operated treated</td>
<td>54.1±7.0*</td>
<td>91.6±11.5*</td>
<td>175.7±21.3*</td>
<td>232±5.6*</td>
<td>2.8±0.6</td>
</tr>
<tr>
<td>2K2C control</td>
<td>467.9±23.8</td>
<td>452.7±34.2</td>
<td>662.4±77.8</td>
<td>532.8±20.6</td>
<td>2.0±0.4</td>
</tr>
<tr>
<td>2K2C treated</td>
<td>95.8±15.2*</td>
<td>135.3±24.3*</td>
<td>146.8±35.6*</td>
<td>35±5.9*</td>
<td>2.1±0.6</td>
</tr>
</tbody>
</table>

Values are mean±SEM. CSF, cerebrospinal fluid; 2K2C, two-kidney, two clip Goldblatt hypertension.

* $p<0.01$, with respect to control groups.

Discussion

This experiment has shown that depletion of CNS serotonin by intracisternal administration of 5,7-DHT produced a significant decrease in the level of BP of control rats, whereas it failed to prevent the development of experimental 2K2C Goldblatt renovascular hypertension in the rat. Effectiveness of 5,7-DHT treatment was confirmed by determination of serotonin levels in all of the analyzed brain areas that were depleted 60–90% of normal levels, 5 weeks after intracisternal injection of the neurotoxin. Administration of 5,7-DHT after pretreatment with DMI, to protect brain norepinephrine neurons, did not modify the catecholamine concentration in the brain; thus, central sympathetic mechanisms do not seem to be involved in BP reduction in untreated, sham-operated rats. These results confirm a previous report done in normal rats. On the other hand, failure to induce hypertension after pharmacological depletion of brain serotonin has been described in the spontaneously hypertensive rat (SHR). Present findings are at variance with other reports that show no influence of serotonin depletion on BP of normal rats or demonstrate a hypotensive effect of brain serotonin decrease in hypertensive rats. In this sense, some authors using the same pharmacological combination used by us reported a decrease in systolic BP in the SHR.
that lasted up to 10 days after injection. The reasons for this discrepancy, as well as the disparity in response between our normotensive and hypertensive animals, are not readily apparent. In general, CNS 5-HT seems to be a pressor agent in rats and rabbits because of its ability to increase peripheral sympathetic outflow. Increased sympathetic outflow could be involved in the maintenance of spontaneous hypertension but does not seem to be necessary for the onset and development of high BP in 2K2C Goldblatt renovascular hypertension in the rat. In contrast, peripheral sympathectomy lowers BP in control rats. Present results have shown some alternatives; they have confirmed that the long-term stage of hypertension is not dependent on an increased amount of circulating renin activity because untreated hypertensive rats had the same PRA levels as sham-operated rats. In 5,7-DHT–treated rats, however, renin appeared to participate in the maintenance of high BP because the level of PRA was significantly lower in treated sham-operated rats than in serotonin-depleted hypertensive rats. The role of brain serotonin in the maintenance of PRA in the conscious rat has been described by other authors. It seems likely that lower PRA in 5,7-DHT control rats is the result of a diminished rate of renin secretion because the level of plasma angiotensinogen was not affected by the pharmacologic treatment. As previously mentioned, central serotonin pressor effect could be mediated through increased peripheral sympathetic outflow, which would modulate renal renin secretion. Present results closely resemble those obtained with peripheral sympathectomy, supporting the participation of peripheral sympathetic effects as mediators of central serotoninergic activation.

Although changes in central angiotensinogen concentration were independent of serotonin depletion, increased levels of renin substrate suggest the participation of the brain renin-angiotensin system in the long-term stage of hypertension. In this sense, increased angiotensinogen could mean enhanced release of Ang I and, if the converting enzyme was necessary for the onset and development of high BP. Present data have shown that significant central serotonin depletion does not prevent the development of 2K2C Goldblatt renovascular hypertension although this pharmacologic treatment induced a significant reduction in BP in normal rats. Changes in PRA suggest that the peripheral enzymatic complex might participate in the maintenance of hypertension in central serotonin-depleted animals. On the other hand, increased prohormone concentrations in the hypothalamus seems to play a role in the maintenance of hypertension in treated and untreated animals.

References

KEY WORDS • norepinephrine • renin • angiotensinogen • central nervous system • cerebrospinal fluid
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