Alteration of Benzodiazepine Binding to Platelets in Spontaneously Hypertensive Rats

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The brain benzodiazepine receptors have been investigated intensively since their discovery in 1977 (1–3). The pharmacologically distinguishable peripheral-type benzodiazepine binding sites, which initially were found in the kidney, liver and lung (4), have not been studied as thoroughly. We reported the detailed characterization of these sites in rat mast cells (5), rat blood platelets (6), mouse thymocytes (7) and rat heart and kidney (8). The peripheral-type benzodiazepine binding sites have no known physiological consequences. Recently, Wang et al. (9) suggested that these sites are involved in the regulation of thymoma cell proliferation.

Platelets from hypertensives have been reported to have multiple abnormalities: they exhibit increased adherence to glass beads (10), increased sensitivity to aggregation by ADP (11), decreased sensitivity to aggregation by in vitro or in vivo exposure to noradrenaline (12), and increased rate of noradrenaline efflux (13).

The spontaneously hypertensive rat (SHR) strain provides a useful model for studying hypertension. We have examined the peripheral-type benzodiazepine binding sites in platelets from SHR and found differences from the Wistar/Kyoto (WKY) normotensive control (14). This report compares the peripheral-type benzodiazepine binding sites in platelets from two types of hypertension, SHR and DOCA-salt uninephrectomized hypertensive rat, with their normotensive controls. Imipramine binding was also investigated in these animals to determine whether changes noted in the binding were limited to the benzodiazepine sites.

Age matched male SHR and WKY normotensive controls were supplied by Taconic Farms Inc. (Germantown, New York, U.S.A.). Left unilateral nephrectomy was performed on Sprague-Dawley (150 g) rats. Twenty of the rats received a single subcutaneous implantation of DOCA (25 mg tablet/rat). Ten of the DOCA treated rats also received drinking water containing 1.0% NaCl. Controls were sham-operated age-matched Sprague-Dawley rats. Treatment was maintained for three weeks. Systolic arterial blood pressure was recorded by tail plethysmography. Blood was withdrawn via the portal vein from each rat, and the platelets were isolated as described previously (6). [3H]-diazepam binding and [3H]imipramine binding were measured by a filtration assay as previously described (5).

The results in Table 1 indicate that at 4, 8, 12 and 20 weeks of age, the B$_{\text{max}}$ of [3H]diazepam binding to the platelets of SHR was significantly higher than that of WKY. The K$_{d}$, however, was not significantly different for any of these ages (Table 1). Using another ligand, [3H]imipramine, there were no significant differences in either B$_{\text{max}}$ or K$_{d}$ between SHR and WKY: 4–30 weeks old WKY, K$_{d}$=10.6±1.4 nM, B$_{\text{max}}$=328±22 fmoles/10$^8$ platelets (N=6); 4–30 weeks old SHR, K$_{d}$=9.6±1.0 nM, B$_{\text{max}}$=369±30 fmoles/10$^8$ platelets (N=5).

We found that in the experimentally induced hypertensive animal model, namely, DOCA-salt uninephrectomized rats, the B$_{\text{max}}$ and K$_{d}$ values in platelets were not significantly different from controls, while the
Table 1. [3H]diazepam binding to platelets from SHR and WKY rats of different ages

<table>
<thead>
<tr>
<th>Age</th>
<th>N</th>
<th>Blood pressure (mmHg)</th>
<th>Bmax (fmole/10⁶ platelets)</th>
<th>Kd (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 weeks</td>
<td>8</td>
<td>114±3</td>
<td>137±16</td>
<td>28.0±1.0</td>
</tr>
<tr>
<td>SHR</td>
<td>8</td>
<td>116±4</td>
<td>229±17*</td>
<td>29.1±1.0</td>
</tr>
<tr>
<td>8 weeks</td>
<td>6</td>
<td>99±6</td>
<td>147±8</td>
<td>29.7±2.7</td>
</tr>
<tr>
<td>SHR</td>
<td>8</td>
<td>133±5*</td>
<td>243±21*</td>
<td>37.5±2.7</td>
</tr>
<tr>
<td>12 weeks</td>
<td>8</td>
<td>121±5</td>
<td>283±28</td>
<td>34.4±4.4</td>
</tr>
<tr>
<td>SHR</td>
<td>8</td>
<td>181±7*</td>
<td>378±20*</td>
<td>34.7±4.1</td>
</tr>
<tr>
<td>20 weeks</td>
<td>10</td>
<td>113±4</td>
<td>183±16</td>
<td>28.8±2.0</td>
</tr>
<tr>
<td>SHR</td>
<td>10</td>
<td>191±6*</td>
<td>300±16*</td>
<td>29.5±1.7</td>
</tr>
</tbody>
</table>

Results are shown as the mean±S.E. Statistical analysis was done using Student’s t-test. Significantly different from WKY at *P<0.01.

Table 2. [3H]diazepam binding to platelets of DOCA-salt rats

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Blood pressure (mmHg)</th>
<th>Kd (nM)</th>
<th>Bmax (fmole/10⁶ platelets)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOCA-salt-uninephrectomized rat</td>
<td>10</td>
<td>188±10</td>
<td>29.6±1.4</td>
<td>700±38</td>
</tr>
<tr>
<td>DOCA-uninephrectomized rat</td>
<td>5</td>
<td>132±3</td>
<td>24.7±2.7</td>
<td>693±31</td>
</tr>
<tr>
<td>Control rat</td>
<td>10</td>
<td>124±2</td>
<td>25.5±2.3</td>
<td>730±43</td>
</tr>
</tbody>
</table>

Results are shown as the mean±S.E.

The blood pressure of DOCA-salt uninephrectomized rats was significantly higher than that of the controls (Table 2).

Changes in the peripheral-type benzodiazepine Bmax were noted in the platelets of the SHR prior to the onset of hypertension. The imipramine binding sites in the platelets remain unchanged, which might imply a certain degree of specificity for the increased Bmax of peripheral-type benzodiazepine.

The failure of the DOCA-salt animals to show similar changes would imply that blood pressure per se was not a causative factor for the changes noted. The genetic composition of the SHR and DOCA-salt rats differs and may be a factor responsible for the differences observed.

Abnormalities in net Na+ and K+ fluxes have been observed in erythrocytes of SHR (15). An abnormally low ratio of Na+/K+ net fluxes in Na+–loaded/K+-depleted erythrocytes of human essential hypertensives have been reported (16). However, studies of secondary hypertension have not shown a similar membrane abnormality (17).

The results in this study suggest that the alteration of platelet benzodiazepine binding may represent biochemical markers of primary hypertension. However, studies on the benzodiazepine binding to platelets of human essential hypertensives are required.

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References


