Some Histochemical Observations on Tissue of Renal Hypertensive Animals

TADAYOSHI FUJITA, SHIGERU AOKI, MICHIO NAGAHAMA, AND NOBUYOSHI ITO

The 1st Department of Surgery, Faculty of Medicine, Kobe University
(Director: Prof. Takao Mitsuno)

We have reported on experimental renal hypertension and its surgical procedure, especially in Reno-portal-shunt.

The present paper deals with renal hypertension and its surgical procedure with reference to the disturbance of ATP-ATPase system.

We found disturbance of ATP-ATPase in hypertensive dogs in contrast with normotensive and Reno (portal) anastomized dogs.

A number of papers have been so far published on experimental renal hypertension and its surgical therapy. However, there has been a few reports on the biochemical mechanism in the disturbance of ATP-ATPase system found in clinical cases.

The present paper deals with renal hypertension regarding it’s surgical treatment, with special reference to the disturbance of ATP-ATPase system.

Materials and Methods

Male mongrel dogs, weighing about 10kg were used, and have all been well trained. They were divided into three groups, the first group being normotensive dogs with blood pressure of 120 mmHg level. The second group, hypertensive dogs of 160 mmHg level and the third group, Reno-portal anastomized dogs with blood pressure of 120–130 mmHg. Hypertension of the dogs were caused by left nephrectomy and right renal artery constriction, according to the method by Goldblatt9. (Operation methods of three group animals are shown in Fig. 1)

The hypertensive animals were maintained in this condition for over three months after operation. Some of the hypertensive dogs were then operated to reduced the blood pressure to the normal level by20 Reno-portal shunt as described above.

Homogenate Studies

Immediately after the animals were killed by intravenous administration of pentobarbital sodium in the amount of 30–40mg per 1kg

(Received for Publication, June 21, 1965)

Japanese Circulation Journal Vol. 30, March 1966 199
body weight, (injection time being approximately 20") the liver, kidney, aorta and femoral artery were sliced and homogenized with appropriate amount of bidistilled water (0°C) in a Potter-Elevichem Homogenizer.

The homogenate was then freeze-dried and thawed with CO₂/aceton to complete the homogenation (Dounce 1951). For the ATP-ase activity assay, one ml of the homogenate was incubated with a mixture solution of 0.05 M Tris-buffer (pH 7.3), 1.3 × 10⁻³ M ATP (Boehringer), 0.18 M potassium sodium tartrate (Merck p. a.), 1 × 10⁻² M magnesium chloride, and 5 × 10⁻³ M lead nitrate (British Drug House, anal. quality). The mean final pH value was 6.8 ± 0.2, after 20 minute at room temperature (22–24°C), 0.2 ml of perchloric acid was added to the incubation mixture in 2N concentration, and subjected to the phosphorus analysis according to the Allen's method.

Detection of Histochemical ATP-ase

Tissue blocks (kidney, liver, arterial wall) were immediately frozen in a electric freezer, and sectioned with the aid of a microtome.

The sections were then placed in the following incubation mediums. (Table 1)

The constituents were mixed in the above order. During incubation, the pH was adjusted to 7.2 over 50–60min. The sections were removed from the medium and rinsed with distilled water. The rinsed sections were then dipped into a solution of 1% yellow ammonium sulfide for 1 min. and again rinsed with water. For microscopic observation the stained sections are mounted under glycerin jelly.

Results and Discussion

The ATP-ase activity of various tissue of the three group dogs was almost equal with their homogenates, also there was not found significant difference in the amounts of Pi in normotensive, hypertensive and Reno-Portal anastomized dogs, as given in Table (2).

Fig. 2, 3, however suggest an important histochemical facts, showing localization of Pi which was distinctly stained in arteriole's wall of hypertensive dogs.

This finding was extremely in contrast with the results found in tissue of the other animals.

There is a possibility that renal hypertension may be caused by disturbances of the ATP-ase system in the arteriole's wall. It has been assumed by many investigators that the increase in diastolic pressure is caused by a constant increase in the resistance of the arterioles, resulting in the muscular contraction of the arterioles. It is certain that ATP-ase is responsible for the breakdown

| Table (1) |
| Final concentration |
| 20 ml of 125 mg% ATP (disodium sulf) | 8.3 × 10⁻⁴ M |
| 20 ml of 0.2 M Tris buffer (pH 7.2) | 8.2 × 10⁻³ M |
| 3 ml of 2% Pb(NO₃)₂ | 3.6 × 10⁻³ M |
| 5 ml of 0.1 M MgSO₄ 7H₂O | 1 × 10⁻² M |
| 2 ml of distilled water |

<p>| Table (2) |</p>
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Normotensive dogs</th>
<th>Hypertensive dogs</th>
<th>Reno-Portal-anastomized dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (control)</td>
<td>0.059 mg</td>
<td>0.052 mg</td>
<td>0.057 mg</td>
</tr>
<tr>
<td>Liver</td>
<td>0.077 mg</td>
<td>0.045 mg</td>
<td>0.072 mg</td>
</tr>
<tr>
<td>Kidney (control)</td>
<td>0.047 mg</td>
<td>0.037 mg</td>
<td>0.044 mg</td>
</tr>
<tr>
<td>Kindney</td>
<td>0.067 mg</td>
<td>0.066 mg</td>
<td>0.063 mg</td>
</tr>
<tr>
<td>Aorta (control)</td>
<td>0.019 mg</td>
<td>0.013 mg</td>
<td>0.019 mg</td>
</tr>
<tr>
<td>Aorta</td>
<td>0.043 mg</td>
<td>0.038 mg</td>
<td>0.040 mg</td>
</tr>
<tr>
<td>Middle artery (control)</td>
<td>0.018 mg</td>
<td>0.017 mg</td>
<td>0.019 mg</td>
</tr>
<tr>
<td>Middle artery</td>
<td>0.032 mg</td>
<td>0.038 mg</td>
<td>0.030 mg</td>
</tr>
</tbody>
</table>

Japanese Circulation Journal Vol. 30, March 1966
of ATP into ADP, to supply the energy required for muscular contraction. Recently, it has been pointed out by Maekawa\textsuperscript{79} that a relatively high activity of ATP-ase is found in the blood serum of hypertensive patients and animals. Stein and Harris\textsuperscript{9} have also reported that the arteriosclerotic wall possesses a high activity of ATP-ase which is easily detected by the staining method.

Such results are not surprising, because the ATP-ase activity determined elsewhere are not solely dependent on arterioles wall. Since the ATP-ase activity were determined from the entire tissue homogenates, it is quite obvious that the results obtained from the arterial wall would be similar to that of other tissues. The ATP-ase of various tissue in hypertensive animals were distinctly stained when compared with those of other groups, as shown in Figs. 2–5. In experimental renal hypertension, as

---

*Fig. 2. ×400  Kidney (Hypertensive dogs)*

*Fig. 3. ×400  Kidney (Hypertensive dogs)*

\textit{Japanese Circulation Journal  Vol. 30, March 1966}
shown in Fig. 6; the disturbance is mainly seen in ADP + Pi → ATP formula, rather than in the ATP → ADP + Pi formula. It is assumed that experimental renal hypertension does not occur from a simple mechanism, such as high ATP-ase activity.

**SUMMARY**

1. The surgical procedure of renal portal anastomosis for experimental renal hyperten-

---

*Fig. 6.*

---

*Fig. 4.* ×400 Kidney (Normotensive dogs)

---

*Fig. 5.* ×400 Kidney (Reno-portal-anastomized dogs)

---

sion is effective.
2. The disturbance of ATP-ATPase system occurs in renal hypertensive animals.
3. The disturbance of ATP-ATPase system do not occur in reno-portal anastomized and normotensive animals.

REFERENCES