Contrastive Profiles in Two Types of Renovascular Hypertension in Rabbits

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SUMMARY

Plasma renin activity, serum electrolytes and hematocrit were serially measured during the period from the 2nd to 70th day after renal artery clipping in rabbits with intact contralateral kidney (18 animals) and those with contralateral nephrectomy (16 animals). Hypertension developed in both groups. Plasma renin activity was increased in about half of animals with intact contralateral kidney during the first 14 days and in 4 of them on the 70th day, but it remained unchanged or rather reduced in animals with contralateral nephrectomy throughout the observation period. Serum sodium showed an initial increase only in animals with contralateral nephrectomy. Serum potassium was gradually decreased during the first 14 days in animals with intact contralateral kidney but remained unchanged in those with contralateral nephrectomy. Hematocrit was progressively increased from 40.6±0.7 (SE) % to 48.3±1.4% in animals with intact contralateral kidney, whereas it remained unchanged in those with contralateral nephrectomy. These results revealed striking contrasts in plasma renin activity, serum electrolytes and hematocrit between 2 types of renovascular hypertension.

Additional Indexing Words:
Hematocrit Hypokalemia Renin

There are 2 types of experimental models for renovascular hypertension. One is a type produced by constriction of one renal artery with an intact contralateral kidney and the other is a type produced by constriction of one renal artery with contralateral nephrectomy. Persistent hypertension can develop in both models in the rat.1),2) It has generally been believed that the constriction of one renal artery alone cannot produce persistent hypertension either in the dog or in the rabbit unless the opposite kidney is removed.3),4) However, recent studies5),6) have demonstrated that the constriction of one renal artery alone can produce persistent hypertension in these animals when the constriction is adequate in degree.

Increasing attention has been paid to a comparison between the 2 types

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of renovascular hypertension, because different mechanisms may be involved in the development of hypertension. There are differences in plasma renin activity (PRA), renin content of the clipped kidney, aldosterone secretion, effect of sodium restriction on blood pressure, and sodium balance between the 2 types of renovascular hypertension in rats. A recent study from our laboratory showed that renal artery clipping led to remarkable contrasts in PRA, serum potassium and water intake between rabbits with and without an intact contralateral kidney. However, the time course of these parameters has not been completely revealed and the problems are still in controversy. Serial measurement of parameters may involve technical difficulties, especially in rats. The present study was attempted to obtain further information on the differences between the 2 types of renovascular hypertension. A comparison was made between rabbits with and without an intact contralateral kidney during the course of 70 days after renal artery clipping. The results revealed remarkable differences not only in PRA and serum electrolytes but also in hematocrit between the 2 types of hypertension.

Methods

Male rabbits weighing 2.5 to 3.0 Kg were fed standard pellets for the rabbit (CR 1, Japan Clea Ltd) which contained 14 mEq of sodium and 26 mEq of potassium per 100 Gm. Each animal was given 100 Gm of the pellets per day and water ad libitum. Animals were divided into 2 groups. Group A consisted of 18 animals in which the left renal artery was clipped by a silver circular clip of 0.9 mm id and the right kidney was left intact. All animals of group A survived over 70 days. Group B consisted of 16 out of 21 animals in which the left renal artery was clipped by a silver circular clip of 1.2 mm id about 4 weeks after removal of the right kidney. Five of the 21 animals died of vascular lesions including cerebral hemorrhage during the course of 70 days. These 5 animals were discarded. All animals of both groups except for one took the total amount of the pellets offered to them throughout almost the whole course. They did not always take the total amount of the pellets during the postoperative 1 or 2 days. One animal of group B did not take the total amount of the pellets during the second week. The animal had a transient azotemia. Serum urea nitrogen was increased to 156 mg/100 ml at that time.

The operative procedures were performed through a flank incision under sodium pentobarbital anesthesia (30 mg/Kg) after a 2-week control period. Blood pressure was measured on the central ear artery by an indirect method without anesthesia, every day during the first 6 days and once a week subsequently. PRA and serum sodium and potassium were measured before, and 2, 4, 6, 14, and 70 days after renal artery clipping. Serum urea nitrogen and hematocrit were measured before, and 6, 14, and 70 days after the clipping. About 6 ml of blood were taken from the marginal ear vein into a dry syringe in resting animals without anesthesia. Three ml of blood were collected into a chilled heparinized plastic tube and immediately
The plasma was frozen until measurement of renin activity. The remaining blood was used for measurement of serum sodium, potassium and urea nitrogen, and hematocrit. Blood count was made only once on the 70th day.

PRA was determined by angiotensin I radioimmunoassay with a commercial kit (CEA-CEN-SORIN). The procedure was based on the method of Haber et al. with a minor modification which was indicated by Kurihara (personal communication). 0.5 ml of plasma was incubated for 3 hours at 37°C with added 0.01 ml of a solution of disodium ethylenediamineacetate (5 Gm per 100 ml), 0.01 ml of an 8-hydroxyquinolin solution (0.33 Gm per 10 ml), 0.001 ml of a dimercaprol solution (10% in benzylbenzoate (W/V)), and 0.05 ml of phosphate buffer (0.5 M, pH 6.0). Other steps followed the instructions. Values for renin activity were expressed as nanogram angiotensin I equivalents generated per 1 ml of plasma per hour. A coefficient of variation was 22.1% for a pooled plasma. A single sample was determined by both radioimmunoassay and bioassay. The latter was performed by a method of Pickens et al with a minor modification. A highly significant correlation was present between the 2 assays (r = +0.916 N = 23).

**RESULTS**

Changes after renal artery clipping in body weight, blood pressure and serum urea nitrogen are illustrated in Fig. 1. The average of initial body weight was slightly greater in group B than in group A, but the difference was not significant. Subsequent changes in body weight showed a similar tendency in the 2 groups. The average of blood pressure showed a rapid rise during the first 6 days and a slow rise subsequently in both groups. Persistent hypertension developed in animals with an intact contralateral kidney (group A) as well as in those with contralateral nephrectomy (group B). There was a striking similarity in the course of blood pressure between the 2 groups. Changes in serum urea nitrogen showed a close parallelism between the 2 groups, although the average was slightly greater in group B than in group A at any time of the course. The difference in serum urea nitrogen might depend upon the presence or absence of an intact contralateral kidney.

The results on PRA are illustrated in Fig. 2. Before renal artery clipping PRA ranged from 0.1 to 4.7 ng in all 18 animals of group A and from 0.1 to 4.1 ng in 15 out of 16 animals of group B. After renal artery clipping PRA was increased above 5 ng in about half of the animals with an intact contralateral kidney (group A) during the first 14 days and in 4 of those still on the 70th day. On the contrary PRA remained below 5 ng in most of animals with contralateral nephrectomy (group B) throughout the observation period. An increase above 5 ng in PRA was found only in 1 or 2 animals at any time of the course. PRA was under 2 ng in 12, 10, 11, 10, and 14 out of 16 animals of group B at the 2nd, 4th, 6th, 14th, and 70th day respectively, whereas in 5, 4, 3, 4, and 7 out of 18 animals of group A at the corresponding times.
respectively. There were significant differences between the 2 groups at any point of the observation period (p<0.05, respectively). An exceptionally high value of 35.8 ng was observed on the 14th day in one of animals of group B. It might be due to transient renal insufficiency. As previously described, serum urea nitrogen was increased to 156 mg/100 ml on the 14th day in this animal.

Changes after renal artery clipping in serum sodium and potassium, and hematocrit are illustrated in Fig. 3. Serum sodium was slightly increased during the first 6 days in animals with contralateral nephrectomy (group B). The difference between group A and group B was significant on the 6th day (p<0.05). The difference tended to disappear in the subsequent course. Changes in serum potassium showed a pronounced contrast between animals with and without an intact contralateral kidney. During the first 14 days the average of serum potassium was gradually decreased from 4.06±0.04 (SE) mEq/L to 3.50±0.09 mEq/L in animals with an intact contralateral
Fig. 2. Plasma renin activity after renal artery clipping. Open circles (on left side) represent animals with intact contralateral kidney (group A, N=18), and solid circles (on right side) represent animals with contralateral nephrectomy (group B, N=16).

Fig. 3. Serum sodium and potassium, and hematocrit after renal artery clipping. Open circles represent animals with intact contralateral kidney (group A, N=18), and solid circles represent animals with contralateral nephrectomy (group B, N=16).
Table I. Results of Blood Counts on the 70th Day after Renal Artery Clipping

<table>
<thead>
<tr>
<th></th>
<th>Normal Animals (N=30)</th>
<th>Animals with Intact Contralateral Kidney (Group A, N=18)</th>
<th>Animals with Contralateral Nephrectomy (Group B, N=16)</th>
<th>p-Value between Group A and Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>39.0±0.4</td>
<td>48.3±1.4</td>
<td>40.3±0.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hemoglobin (Gm/100ml)</td>
<td>12.5±0.1</td>
<td>15.9±0.4</td>
<td>13.2±0.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Red Blood Cell (10⁶/mm³)</td>
<td>5.82±0.10</td>
<td>7.25±0.20</td>
<td>5.92±0.14</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Color Index</td>
<td>0.67±0.00</td>
<td>0.69±0.01</td>
<td>0.70±0.01</td>
<td>n.s.</td>
</tr>
<tr>
<td>Reticulocyte (%)</td>
<td>21.9±1.9</td>
<td>23.1±1.9</td>
<td>30.1±4.2</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

kidney (group A) and it was insignificantly increased 4.08±0.03 mEq/L to 4.14±0.05 mEq/L in animals with contralateral nephrectomy (group B). The difference between the 2 groups was highly significant on the 14th day (p<0.01). The difference became less remarkable in the subsequent course, but a significant difference was still present on the 70th day (p<0.05).

An impressive contrast was observed in changes in hematocrit. The average of hematocrit was about 40% in both groups before the surgery. After renal artery clipping it was progressively increased from 40.6±0.7% to 48.3±1.4% during the period of 70 days in animals with an intact contralateral kidney (group A), but it was transiently decreased on the 6th day and subsequently returned to the control level in animals with contralateral nephrectomy (group B). The difference between the 2 groups was highly significant both on the 14th and on the 70th day (p<0.01, respectively). The transient decrease in hematocrit which was observed in group B might be due to repeated withdrawal of blood during the first 6 days. The results of blood counts on the 70th day were presented in Table I. Normal values which were obtained in 30 preoperative animals were also indicated. Hematocrit, hemoglobin content and red cell count were significantly greater in group A than in group B. However, there was no significant difference in reticulocyte count between the 2 groups. Results on blood counts in animals of group B were almost the same as those in the normal animals. Correlation coefficients were calculated on group A between hematocrit and other parameters that were measured on the 70th day. There were no significant correlations between hematocrit and blood pressure (r=+0.41), between hematocrit and PRA (r=+0.06) and between hematocrit and serum potassium (r=−0.28).

DISCUSSION

Regoli et al[1] first noticed an important difference between the 2 types
of renovascular hypertension. They found renin content of the clipped kidney to be increased in hypertensive rats with an intact contralateral kidney but to remain unchanged in those with contralateral nephrectomy. Subsequent studies have shown that a similar difference is found in PRA between the 2 types of renovascular hypertension.\(^9\),\(^10\),\(^15\) However, there is no detailed information on the time course of PRA. In the present study we produced the 2 types of renovascular hypertension in rabbits, in which serial measurement of PRA was carried out by the use of radioimmunoassay. Increase in PRA was observed during the first 14 days in about half of animals with an intact contralateral kidney. The increase was still found on the 70th day in some of the animals. On the other hand PRA was unchanged or rather decreased throughout the course of 70 days in animals with contralateral nephrectomy. However, it cannot exclude the possibility that PRA is transiently increased before the 2nd day in animals with contralateral nephrectomy. A transient increase in PRA is observed during the first few days after renal artery clipping in dogs and sheep with contralateral nephrectomy.\(^18\)\)\(^20\) The present study revealed that PRA was sometimes increased in the chronic stage of renovascular hypertension. This condition was limited to animals with an intact contralateral kidney. These results agree with the observation by Miksche et al\(^10\) and correspond with the clinical observations that PRA is increased in some patients with renovascular hypertension.

A slight increase in serum sodium was observed during the first 6 days in animals with contralateral nephrectomy. The results might reflect a positive sodium balance. Swales et al\(^14\) found that sodium balance was progressively positive in rats with contralateral nephrectomy and progressively negative in those with an intact contralateral kidney during the development of hypertension. Hypokalemia is sometimes observed in both patients and animals with renovascular hypertension.\(^10\),\(^21\)\)\(^24\) However, no particular attention has been paid to the presence or absence of an intact contralateral kidney. A recent study from our laboratory\(^15\) showed that changes after renal artery clipping in serum potassium exhibited a remarkable contrast between animals with and without an intact contralateral kidney. Hypokalemia was observed only in animals with an intact contralateral kidney. However, the observation was limited to the acute stage of hypertension. The present study confirmed the previous ones and provided more detailed information on the period from the 2nd to the 70th day after renal artery clipping. The hypokalemia observed in animals with an intact contralateral kidney might be induced by aldosterone secretion which results from the increased release of renin. Singer et al\(^13\) demonstrated a significant increase in aldosterone secretion only in hypertensive rats in which a clip had been
applied on one renal artery and the other kidney had been left intact, and no significant changes in aldosterone secretion in hypertensive rats in which one kidney had been removed and a clip had been applied on the artery of the remaining kidney. However, they did not measure either serum potassium or PRA.

Changes after renal artery clipping in hematocrit were also contrastive between the 2 groups. Hematocrit was progressively increased in animals with an intact contralateral kidney, whereas it was unchanged in animals with contralateral nephrectomy. Ledingham et al observed that hematocrit was rather decreased during the first 3 weeks after renal artery clipping in rats with contralateral nephrectomy, but they did not study animals with an intact contralateral kidney. Gross et al recently reported fluid turnover and the activity of the renin-angiotensin system in renal hypertensive rats with an intact contralateral kidney. At the end of the 5th week after renal artery clipping they found slightly increased hematocrit, decreased serum sodium and elevated PRA. A remarkable increase in both hematocrit and PRA associated with negative sodium balance was observed in rats with a blood pressure higher than approximately 180 mmHg. Gross suggests that both the increased hematocrit and the elevated PRA may result from a decrease in plasma volume which occurs as a consequence of pressure-induced sodium loss in the animals with severe hypertension. The present study did not necessarily support the possibility, because no significant correlation was found either between hematocrit and blood pressure or between hematocrit and PRA. Tarazi et al noticed that the incidence of high hematocrit was significantly greater in patients with renal artery stenosis than in those without renal artery stenosis. Subsequently they demonstrated that plasma volume was significantly decreased in patients with renovascular hypertension. Dissmann et al showed that increase in red cell volume did not appear to play an essential role in the increase in hematocrit. In the present study the increase in hematocrit was not always associated with an increase in reticulocytes.

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