Initial Mechanisms in Hypertension After Bilateral Renal Ischemia in the Rat

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SUMMARY Cumulative water- and electrolyte balance, plasma creatinine (PC), plasma renin activity (PRA), urinary prostaglandins (PGs) $E_2$ and $F_2\alpha$, and kallikrein (K) were studied in 40 male Wistar CHbb THOM rats ($250 ± 4$ g SE). A solid silver clip (0.25 mm lumen) was applied to both renal arteries in 18 animals; 13 rats were sham-operated and nine remained intact. The analyses were performed during a control period and up to 10 days after surgery. Blood pressure (BP) recorded on the 10th and 12th day of the study increased significantly in clipped rats with respect to sham rats ($p < 0.001$); PC and PRA measured on the 11th day were not significantly different. A positive cumulative water "balance'' ($p < 0.01$) and sodium balance ($p < 0.02$) was found in clipped rats when compared with sham rats in the first 5 days of the experimental period. Significantly higher values of PGE$_2$ urinary excretion were observed in sham rats vs clipped rats on the 2nd and 5th day after surgery ($p < 0.02$). On the 2nd day after surgery, K urinary excretion was significantly lower in clipped rats than in sham rats ($p < 0.02$). No significant changes were observed in PGF$_2\alpha$ excretion. The absence of significant difference in PRA 10 days after bilateral renal artery stenosis points to a lack of any fundamental role of circulating angiotensin II at this stage of the development of hypertension. The significant water- and salt retention in the first 5 days after clipping suggests that it might be involved in the pathogenesis of this model. Early changes in PGs $E_2$ and $F_2\alpha$, and K appear to be related more to intrarenal adjustments soon after surgery than to the increase in BP. (Hypertension 3 (suppl II): II-205-II-210, 1981)

KEY WORDS • water-electrolyte balance • renin-angiotensin system • prostaglandin $E_2$ • prostaglandin $F_2\alpha$ • kallikrein • renovascular hypertension

THE pathogenesis of renovascular hypertension (RH) has been repeatedly analyzed but its primary mechanism has not yet been defined. Results in the literature studying different experimental models and stages of RH are conflicting.¹,² In this paper, the cumulative water- and salt balance, plasma renin activity (PRA), and urinary excretion of prostaglandins (PGs) $E_2$ and $F_2\alpha$, and kallikrein (K) were evaluated up to 10 days after clipping both renal arteries in the rat. The goal of this study was to examine the pathogenesis of this type of RH; two-kidney, two clip Goldblatt rats provide a suitable model to analyze the mechanisms involved after ischemia of the renal tissue.

Methods

Forty male Wistar CHbb THOM rats were studied. When the animals reached $250 ± 4$ g (SE), a solid silver clip (0.25 mm lumen) was applied on both renal arteries in 18 animals; 13 rats were sham-operated and nine remained intact. This last group was included so that the growing animals might be studied without interference from experimental maneuvers. Surgery was done under ether anesthesia. The animals were kept in individual metabolic cages with free access to food and demineralized water. Room temperature was maintained constant ($22° ± 1°$ C), and the air was adequately recycled; light was on from 7 am to 7 pm. After 7 days to adapt the rats to the experimental procedure, we measured sodium, potassium, and water balances on 3 consecutive days (control period) before the clips were applied, and during 10 days after surgery.

Blood pressure (BP) was indirectly recorded by a tail pulse pneumatic transducer connected to an oscilloscope, three times in each animal during the week of adaptation (before the balance control period) and twice at the end of the whole experimental period (on
the 10th and 12th day after clipping) and averaged. Blood samples were obtained between 2 and 4 pm by cutting the tip of the rat’s tail, 48 hours before the balance control period and on the 11th day after surgery; PRA was determined by radioimmunoassay, and plasma creatinine (PC) by the cinetic-colorimetric method.

**Balance Procedures**

The diet was specially prepared in our laboratory to assure a constant composition and adequate consistency. It contained equal amounts of sodium and potassium (100 μEq/g of the final semisolids) food and 40% w/w of water. During balance analysis, the animals were weighed daily and transferred to a new cage. Water and food intakes and urinary volumes were measured by weight differences; funnels of the old cages were rinsed with demineralized water, and washing water was separately collected. Aliquots of urine were separated for electrolyte determination, and the rest of the samples were stored at −70°C until PGs and K analyses were performed. Daily feces were dried during the night at 100°C and then weighed; afterward they were powdered and treated with HNO₃ in for 24 hours and diluted. Sodium and potassium in the samples were measured by a flame photometer with internal standard (Instrumental Laboratory 143). Sodium and potassium balances were calculated as the difference between intake and excretion.

Cumulative balances were calculated in the following periods: 1) control period, during 3 consecutive days just before surgery; 2) Days 1 to 5 after surgery; 3) Days 6 to 10 after surgery; and 4) total Days 1 to 10 after surgery. Cumulative balances were expressed per gram of body weight increase during each period; since it has been described that "sodium and potassium retention per gram of body weight increase was roughly the same at all levels of body weight gain," this expression was used to avoid the interference of growing in rats.

Urinary prostaglandins E₁ and F₂₅ were analyzed by the radioimmunoassay method developed by Dray et al. after the extraction, and chromatography through silicic acid columns (on the 2nd day of the control period and on the 2nd, 5th, and 10th day after the clips were applied). It has recently been reported that there is about 50% decrease in PG content in human urine samples when they are stored at 4°C for 24 hours; preliminary tests in our laboratory performed with PG standards and rat urine samples stored for 24 hours at constant room temperature (22±1°C), showed as much as a 12% decrease in PG content. Thus, under our experimental conditions, our samples allow us to compare accurately results between hypertensive and sham rats.

Urinary kallikrein excretion was analyzed using Roberts’ esterase assay activity method as modified by Nustad and Kirsten Vaaje, using α-N-benzoyl-L-arginine ethyl ester (BAEE) as substrate. Control K determinations were performed on 3 consecutive days before surgery and averaged; after surgery, the results were obtained on the 2nd, 5th, and 10th day.

Statistical significance of the data expressed as mean ± SE was determined by the Student’s t test.

**Results**

**Blood Pressure, Plasma Creatinine, and Plasma Renin Activity**

The absolute values for each group are given in table 1. The BP was significantly higher in clipped rats compared to sham rats (p < 0.001) at the end of the experimental period; PC and PRA were not significantly different. Since each rat was used as its own control, the results were also expressed as differences from control values in figure 1 (Δ mean ± SE). The increase in BP was significant in clipped rats compared to sham rats (p < 0.001). PC and PRA mean

*In = intact, Sh = sham; Cl = clipped. Means ± SE; ( ) = number of rats.*

*See Methods for timing of measurements. Student’s t test was used to determine statistical significance.*

**Table 1. Blood Pressure, Plasma Creatinine, and Plasma Renin Activity in Absolute Values**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control</th>
<th>After surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In</td>
<td>Sh</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>105</td>
<td>107</td>
</tr>
<tr>
<td>(mm Hg)</td>
<td>±2</td>
<td>±3</td>
</tr>
<tr>
<td>Plasma creatinine</td>
<td>0.71</td>
<td>0.71</td>
</tr>
<tr>
<td>(ml/100 ml)</td>
<td>±0.06</td>
<td>±0.04</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(11)</td>
</tr>
<tr>
<td>Plasma rennin activity</td>
<td>12.2</td>
<td>10.3</td>
</tr>
<tr>
<td>(ng Al/ml/hr)</td>
<td>±1.2</td>
<td>±1.9</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(9)</td>
</tr>
</tbody>
</table>
values were not statistically different. However, Δ mean value for PRA was positive in clipped rats while negative in sham and intact rats.

**Cumulative Water and Electrolyte Balance**

Significant positive cumulative balances, expressed as differences from control values, were found in the 1-5 day period for water \((p < 0.01)\) and sodium \((p < 0.02)\) in the hypertensive rats with respect to shams (figs. 2 and 3). The Δ mean higher values of water and sodium cumulative balances in hypertensive animals, when considering the whole 1-10 day period, were not statistically different. Cumulative potassium balance in hypertensive rats did not show any significant difference from that of the shams throughout this study.

**Urinary Excretion of Prostaglandins E\textsubscript{2} and F\textsubscript{2\alpha} and Kallikrein**

Absolute values in the control period and on Days 2, 5, and 10 after surgery are shown in table 2. Significant higher excretion of PGE\textsubscript{2} was observed in sham rats vs clipped rats on Days 2 and 5 after surgery \((p < 0.01)\); PGF\textsubscript{2\alpha} excretion did not show any statistical difference in this study. On the other hand, K excretion was statistically lower in clipped animals than in sham rats only on Day 2 after surgery. Nevertheless, since each rat was used as its own control, the experimental results were also expressed as a percentage of the control values (figs. 4 and 5). In the case of PGE\textsubscript{2}, significant higher values were obtained in sham vs clipped rats \((p < 0.02)\) on Days 2 and 5; no statistical changes were found for PGF\textsubscript{2\alpha}. Lower ex-
FIGURE 3. Changes in cumulative sodium balance observed during the 1st to 5th day, 6th to 10th day, and 1st to 10th day periods after surgery with respect to the control period (Δ mean ± se). Results were calculated as μEq retained per gram of body weight increase.

FIGURE 4. Changes in urinary excretion on prostaglandin E₂ (upper panel) and F₂α (lower panel) on the second, fifth, and tenth day after surgery as a percent of the control values (obtained on the second day prior to surgery) (Δ % means ± se).

TABLE 2. Urinary Prostaglandins and Kallikrein in Absolute Values

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>2nd day</th>
<th>5th day</th>
<th>10th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostaglandin E₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ng/24 hr)</td>
<td>32.2±6.0</td>
<td>26.6±4.1</td>
<td>30.4±4.4</td>
<td>26.6±4.4</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(11)</td>
<td>(11)</td>
<td>(7)</td>
</tr>
<tr>
<td>Prostaglandin F₂α</td>
<td>60.7±7.6</td>
<td>69.4±8.1</td>
<td>58.9±13.8</td>
<td>37.1±5.9</td>
</tr>
<tr>
<td>(ng/24 hr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(11)</td>
<td>(11)</td>
<td>(7)</td>
</tr>
<tr>
<td>Kallikrein</td>
<td>23.6±4.2</td>
<td>26.7±4.11</td>
<td>29.2±2.3</td>
<td>28.0±3.1</td>
</tr>
<tr>
<td>(EU/24 hr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(11)</td>
<td>(11)</td>
<td>(7)</td>
</tr>
</tbody>
</table>

In = intact; Sh = sham; Cl = clipped; EU = esterase units. Mean ± se; ( ) = number of rats.

Statistical significance determined by Student's t test. * = p < 0.01 (Sh vs Cl); † = p < 0.05 (Cl vs Sh).

In case of control, the values of PGs refer to urinary excretion on the second day prior to surgery, and the values of K represent the average of controls taken on the 3 consecutive days prior to surgery.
creatinine of K was observed on Day 2 after surgery in clipped rats vs sham rats (p < 0.02).

No correlation was found in the hypertensive animals, on Day 2, between \( \Delta \% \) of PGE\(_2\) excretion and \( \Delta \% \) of K excretion (r = 0.01), water excretion on Day 2 (r = 0.11), sodium excretion on Day 2 (r = 0.09), positive cumulative balance of water (r = 0.07) or sodium (r = 0.36) in the first 5 days after surgery. Urinary K on the second day after surgery did not correlate with any of the above-mentioned factors.

**Discussion**

This study of the early stages of two-kidney, two clip hypertension provides information on initial mechanisms disregarding compensatory responses that accompany prolonged hypertension. Bilateral artery clipping allows the analysis of the effect of pure renal ischemia excluding the function of an untouched kidney and avoiding unilateral nephrectomy.

**Plasma Renin Activity (PRA)**

The absence of significant differences in PRA 10 days after bilateral renal ischemia points to the absence of any fundamental role of circulating angiotensin II (AII) at this stage of the development of hypertension. Nevertheless, it has to be noted that PRA showed a tendency (NS) to be higher in hypertensive animals than in intact and sham rats (table I and fig. 1). Moreover, since the positive water- and salt balance observed in the first 1-5 day period does not seem to be totally reversed in the 6-10 day period, the renin/body fluid relationship could be inappropriately high and might suggest that circulating AII could still be participating in the maintenance of BP 10 days after bilateral clipping.

**Water- and Salt Cumulative Balance**

The significant water- and salt retention that appears in the initial phase of the development of hypertension might indicate that this factor contributes to the increase of BP in this model. As far as we know, there are no other available data on cumulative water- and salt balance in two-kidney, two clip hypertension. Either positive or negative water- and salt balance has been described in other Goldblatt models.\(^1\)-\(^4\) If fluid loading were a primary mechanism in RH, it should be present regardless of the Goldblatt model under consideration. Thus, water- and salt retention would rather constitute a contributory mechanism in hypertension derived from renal ischemia. This suggestion is also supported by recent papers in the literature.\(^15\)-\(^18\)

**Urinary Prostaglandins and Kallikrein**

There is general agreement that urinary excretion of PGs\(^{14}\) and K\(_1\) is a good index of the renal content of these substances. Moreover, although there is no unanimous agreement,\(^17\) PGE\(_2\) has been reported to be a vasoconstrictor in the rat.\(^18\) In the dog, it has been reported that PG synthesis is increased\(^20\) and K decreased\(^21\) after acute renal ischemia. On the other hand, the literature on rats with RH describes as much of a decrease in PGE-like material in the kidney perfusate (one-kidney, one clip)\(^22\) or PGE\(_2\) in slices of renal papilla (two-kidney, one clip)\(^23\) as in urinary K.\(^24\)-\(^26\) Nevertheless, results refer to hypertension in longer periods and in other experimental models than that studied here.

Sham rats showed the highest values of PGE\(_2\) and F\(_{\text{ins}}\) excretion on the second and fifth day; these results would agree with the literature in which the increase of PG synthesis after surgery and anesthesia has been described.\(^27\) On the other hand, if PGE\(_2\) and F\(_{\text{ins}}\) are
vasoconstrictors in the rat, the lower values in hypertensive rats in respect to shams (significant for PGE<sub>2</sub>) (fig. 4), could be related with early postclip vasodilation to improve renal blood flow. Later, on the 10th day, the absence of significant differences in Goldblatt rats would rule out any fundamental role of those PGs that at that time.

Urinary kallikrein excretion was significantly decreased in hypertensive rats on the second day (p < 0.02); nevertheless, this does not necessarily mean that there was a reduced ability for renal vasodilation after clipping. Actually, kinins are the end active products, and they were not evaluated in the present study; in this regard, it has been reported that changes in urinary excretion of kallikrein and kinins are not necessarily parallel.28

The significant lower excretion of PGE<sub>2</sub> and K in clipped rats on the second day could be the result of an impaired renal function after clipping the renal arteries. As an alternative, the early changes observed would support an as yet undefined contribution of these substances to local adjustments soon after surgical procedures.

In conclusion, PRA does not seem to play a fundamental role 10 days after bilateral renal artery stenosis. Water- and salt retention present in the initial phase of this model might participate in the genesis of hypertension although its precise role remains unclear. A definite relationship of PGE<sub>2</sub>, PGF<sub>ss</sub>, and K with the increase in BP has not been established.

Acknowledgments

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