Effects of Antihypertensive Drugs on Renal Function and Atrial Natriuretic Polypeptide in Spontaneously Hypertensive Rats with Renal Ablation

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YASUJIMA, M., ABE, K., KANAZAWA, M., YOSHIDA, K., KOHZUKI, M., SATO, M., TAKEUCHI, K., OMATA, K., TSUNODA, K., KUDO, K., OTA, K., KIMURA, T. and YOSHINAGA, K. Effects of Antihypertensive Drugs on Renal Function and Atrial Natriuretic Polypeptide in Spontaneously Hypertensive Rats with Renal Ablation. Tohoku J. Exp. Med., 1989, 158 (1), 85-94 — To determine whether pharmacological control of blood pressure could affect the renal function and levels of atrial natriuretic polypeptide (ANP) in spontaneously hypertensive rats (SHR) with renal ablation, and to ascertain the benefits of antihypertensive drugs, we studied effects of oral administration of captopril (50 mg/kg/day), an inhibitor of angiotensin converting enzyme, benidipine (3 mg/kg/day) and nilvadipine (10 mg/kg/day), newly developed blockers of calcium channel, and indapamide (10 mg/kg/day) for 14 days on systolic blood pressure, serum creatinine, blood urea nitrogen, and plasma ANP concentration in SHR subjected to surgical removal of the left kidney and infarction of two-thirds of the right kidney (5/6 nephrectomy) a week before. Three weeks after the surgery, systolic blood pressure (mmHg) in the untreated group was 253 ± 9 (n = 10), in the captopril group 156 ± 9 (n = 7, p < 0.05), in the benidipine group 197 ± 9 (n = 7, p < 0.05), in the nilvadipine group 146 ± 9 (n = 7, p < 0.05) and in the indapamide group 206 ± 5 (n = 7, p < 0.05). Serum creatinine (mg/100 ml) was lower in the captopril group (0.58 ± 0.02, n = 7, p < 0.05) and in the benidipine group (0.50 ± 0.03, n = 7, p < 0.05) but not in the nilvadipine group and in the indapamide group 3 weeks after 5/6 nephrectomy compared to the untreated group. Blood urea nitrogen was also lower in the captopril group and in the benidipine group but not in the nilvadipine group and in the indapamide group. Plasma ANP concentration was significantly reduced.

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by the treatment with captopril and benidipine but not with nilvadipine and indapamide. These results suggest that the reduction of blood pressure by the inhibition of angiotensin converting enzyme with captopril has the potential to ameliorate renal function of the SHR with remnant kidney, a model of chronic renal failure with hypertension, associated with the decreased concentration of plasma ANP. However, it remains to be determined whether the reduction of blood pressure by calcium channel blockers may be involved in the delayed progression of renal failure in this model since there were disparate effects on renal function and plasma ANP concentration with these two calcium channel blockers.

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Systemic hypertension is a well-known cause of progressive renal injury in both humans and experimental animals. In rats, a reduction of the functioning renal mass produces the elevation of systemic blood pressure and progressive renal failure (Chanutin and Ferris 1932; Loomis 1946). Studies of the renal function in rats after extensive ablation of renal mass suggest that an adaptive response is hyperfiltration by the remaining nephrons resulting gradually in the glomerulosclerosis (Hostetter et al. 1981; Brenner 1983). Strong evidence has been obtained of the potential participation of atrial natriuretic polypeptide (ANP) in modulating the adaptive renal function typically observed in the setting of reduced renal mass (Cole et al. 1985; Smith et al. 1986). In addition, elevated levels of circulating ANP have been reported in spontaneously hypertensive rats (SHR) (Gutkowska et al. 1986; Morii et al. 1986) which provides the best available animal model of essential hypertension in humans. However, it is unknown whether high blood pressure or deteriorated renal function per se is responsible for the elevated levels of circulating ANP.

More recently angiotensin converting enzyme inhibitors have been shown to be beneficial on hyperfiltration damage in remnant nephrons in chronic renal failure (Meyer et al. 1985; Jackson and Johnston 1988), whereas their major potential disadvantage is the deterioration of glomerular filtration rate in the stenotic kidney or kidneys when used in the treatment of renovascular hypertension (Farrow and Wilkinson 1979; Hricik 1985). Although calcium channel blockers are potent and well-tolerated antihypertensive agents, the potential role of calcium channel blockers to attenuate the progression of hypertensive renal disease or of chronic renal disease has not been well-documented.

The aim of the present study was to determine whether pharmacological control of blood pressure could affect the renal function and levels of ANP in SHR with renal ablation and to ascertain the benefits of captopril, an inhibitor of angiotensin converting enzyme, or benidipine (Kubo et al. 1985; Fujii et al. 1988) and nilvadipine (Ohtsuka et al. 1983), newly developed blockers of calcium channel, in this model of rats.
**MATERIALS AND METHODS**

Thirty eight male SHR, 6 week-old, were subjected to 5/6 nephrectomy by removal of the left kidney and infarction of two-thirds of the right kidney. This subtotal nephrectomy was performed under ether anesthesia. The right kidney was exposed via a midline abdominal incision and the two poles of the right kidney were excised by encircling them with loops of ligatures and then tightening the loops. Five days after, the left kidney was removed in total. Rats were housed in a metabolic cage designed to prevent feces-urine contact (model ST; Sugiyamagen, Tokyo). The rats were fed a regular diet (sodium 0.24%; potassium 0.69%; Oriental CMP, Oriental Yeast, Tokyo) and had free access to tap water. Seven days after the surgery, the rats were randomly allocated to receive captopril (Sankyo Co., Tokyo), benidipine (Kyowa Hakko Kogyo Co., Tokyo), nilvadipine (Fujisawa Pharmaceut., Co., Tokyo), indapamide (Sumitomo Pharmaceut., Co., Tokyo), or no treatment. The rats were studied up to 14 days after the surgery. Captopril (50 mg/kg/day) and indapamide (10 mg/kg/day) were dissolved in drinking water. Benidipine (3 mg/kg/day) and nilvadipine (10 mg/kg/day) were dissolved in 75% polyethylene glycol 300 and administered by daily gavage.

Systolic blood pressure was monitored daily by indirect tail cuff method (Pfeffer et al. 1971). Urine was collected every day for determination of volume, and sodium concentration using flame photometry. Fourteen days after the initiation of drugs administration, rats were sacrificed and trunk blood was obtained for determination of blood urea nitrogen (BUN), serum creatinine and ANP levels.

For determination of ANP concentration, rats were killed by rapid decapitation and trunk blood was collected into heparinized tubes and centrifuged at 4°C. Plasma samples were immediately mixed with 0.1 N HCl and kept at -20°C. The concentration of ANP was measured by the modified method reported previously (Kimura et al. 1986). Radioimmunoassay was performed after extraction of acidified plasma through C18 sep-pak cartridges with 80% acetone-HCl (pH 1.5). Rat ANP antiserum (Mitsubishi Petrochemical Co., Ltd, Tokyo) cross-reacts completely with α-humam ANP (100%), and atriopeptin III, but not significantly with arginine vasopressin, ACTH, angiotensin II, substance P, oxytocin and methionine-enkephalin. Fifty μl of synthetic α-rat ANP or extracted sample, the antiserum (final dilution, 1 : 90,000) and 125I-human ANP (specific activity, -2,000 μCi/μg, Amersham Japan Co., Tokyo), and 250 μl of the buffer were incubated in a non-equilibrium method. The lowest detectable level of ANP was 7 pg/tube and 50% inhibition of binding was 50 pg/tube. The recovery rate of added α-rat ANP (125 pg) was 74.2± 13.3 (n =15) and inter-and intra-assay coefficients were 17.9% and 13.9%, respectively. The serial dilutions of the extracted plasma sample paralleled the standard curve.

All results were expressed as means±s.E. The data were statistically analysed by using unpaired t-test. In the data on systolic blood pressure, urine volume and urinary sodium excretion among groups, the statistical analysis was performed by two-way analysis of variance for repeated measurements. Statistically significant differences on each day were isolated by the Newman-Keuls test for multiple comparisons of each value.

**RESULTS**

Subtotal nephrectomy was well tolerated in all rats. Body weight, systolic blood pressure, fluid intake, urine volume and urinary sodium excretion were not significantly different among the groups prior to commencing treatment (Table 1). As shown in Fig. 1, captopril, benidipine, nilvadipine and indapamide treatment resulted in significant falls in systolic blood pressure. After 7 days of antihypertensive therapy, systolic blood pressure was 221±5 mmHg in untreated rats (n =
TABLE 1. Initial parameters before treatments with captopril, benidipine, nilvadipine or indapamide in spontaneously hypertensive rats (SHR) with reduced renal mass

<table>
<thead>
<tr>
<th></th>
<th>Captopril group</th>
<th>Benidipine group</th>
<th>Nilvadipine group</th>
<th>Indapamide group</th>
<th>Untreated group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 7)</td>
<td>(n = 7)</td>
<td>(n = 7)</td>
<td>(n = 7)</td>
<td>(n = 10)</td>
</tr>
<tr>
<td>BW (g)</td>
<td>179 ± 5</td>
<td>192 ± 3</td>
<td>180 ± 8</td>
<td>179 ± 4</td>
<td>185 ± 8</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>176 ± 4</td>
<td>178 ± 3</td>
<td>193 ± 7</td>
<td>184 ± 5</td>
<td>177 ± 4</td>
</tr>
<tr>
<td>FI (ml/day)</td>
<td>27.0 ± 1.0</td>
<td>32.0 ± 1.2</td>
<td>28.0 ± 1.8</td>
<td>28.0 ± 0.8</td>
<td>22.3 ± 2.3</td>
</tr>
<tr>
<td>UV (ml/day)</td>
<td>13.2 ± 1.2</td>
<td>14.7 ± 1.1</td>
<td>18.5 ± 1.2</td>
<td>17.5 ± 0.3</td>
<td>13.6 ± 1.7</td>
</tr>
<tr>
<td>U_{Na}V (mEq/day)</td>
<td>0.78 ± 0.06</td>
<td>0.64 ± 0.05</td>
<td>0.53 ± 0.07</td>
<td>0.72 ± 0.08</td>
<td>0.81 ± 0.06</td>
</tr>
</tbody>
</table>

Results are expressed as means ± s.e. BW, body weight; SBP, systolic blood pressure; FI, fluid intake; UV, urine volume; U_{Na}V, urinary sodium excretion.

Fig. 1. Systolic blood pressure in spontaneously hypertensive rats with reduced renal mass. The SHR received captopril (50 mg/kg/day) (○), benidipine (3 mg/kg/day) (●), nilvadipine (10 mg/kg/day) (▲), indapamide (10 mg/kg/day) (●) or no treatment (■). Results are means ± s.e. Analysis of variance for repeated measurements revealed a significant change in systolic blood pressure in SHR with reduced renal mass received captopril (p < 0.01), benidipine (p < 0.01), nilvadipine (p < 0.01) or indapamide (p < 0.01) compared to that in the untreated group. It also revealed a significant change in systolic blood pressure in SHR with reduced renal mass received captopril (p < 0.01) compared to that in benidipine- or indapamide-treated rats, and received nilvadipine (p < 0.01) compared to that in benidipine- or indapamide-treated rats.
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10), 145±8 mmHg in captopril-treated rats (n = 7), 175±8 mmHg in benidipine-treated rats (n = 7), 150±9 mmHg in nilvadipine-treated rats (n = 7) and 193±13 mmHg in indapamide-treated rats (n = 7). Systolic blood pressure in the captopril-, benidipine-, nilvadipine- and indapamide groups of rats remained significantly lower than the untreated group throughout the 14 days treatment period (Fig. 1).

Captopril, benidipine, nilvadipine and indapamide treatment did not induce any significant changes in urine volume and urinary sodium excretion throughout the 14 days treatment period, compared to those in untreated rats. As shown in Table 2, serum creatinine levels were lower in the captopril- and benidipine-treated rats but not in the nilvadipine- and indapamide-treated rats compared to the untreated-rats. Similarly, BUN was also lower in the captopril- and benidipine-treated rats but not in the nilvadipine- and indapamide-treated rats. Plasma ANP concentration was significantly reduced by the treatment with captopril and benidipine but not with nilvadipine and indapamide 3 weeks after 5/6 nephrectomy, compared to the untreated-rats.

**DISCUSSION**

The SHR remnant kidney model combining genetic hypertension with subtotal nephrectomy appears to be a model of accelerated hypertension and nephropathy as determined by functional parameters. In the present study, the effect of angiotensin converting enzyme inhibition to preserve remnant kidney function in rats by blood pressure reduction was extended to the SHR remnant
kidney model. However, the beneficial effects of calcium channel blockers on remnant kidney in this model of rats were not confirmed. Additionally, the present experiments clearly demonstrated that the blood pressure reduction and amelioration of renal function was associated with the decreased levels of circulating ANP in the SHR remnant kidney model, suggesting that ANP might play some potential roles in the regulation of blood pressure and renal function in this model of rats.

Captopril and nilvadipine treatments were associated with comparable reductions of blood pressure during the 2 weeks of treatment. But, there were disparate effects on the preservation of remnant kidney function between these 2 drugs. Captopril treatment was associated with lower serum creatinine and BUN levels than those observed in untreated rats. In contrast, nilvadipine-treated rats had similar serum creatinine and BUN levels to untreated rats. Thus, pharmacological control of systemic hypertension did not uniformly improve the progressive course of declined function of remnant kidney that follows subtotal nephrectomy. It is interesting to note that benidipine, another blocker of calcium channel, reduced serum creatinine and BUN levels to the similar levels in captopril-treated rats whereas unexpectedly it failed to achieve a comparable reduction in blood pressure with captopril and nilvadipine. Therefore, the disparate effects on serum creatinine and BUN levels among the dihydropyridine calcium channel blockers nilvadipine and benidipine might be well-explained by the difference in blood pressure reduction, although it remains to be ruled out that there may be disparate effects on the preservation of remnant kidney function between benidipine and nilvadipine. These effects on the remnant kidney function in this model of rats following blood pressure reduction by calcium channel blockers are consistent with the previous reports (Jackson et al. 1986; Jackson and Johnston 1988). Treatment of hypertension with indapamide, possessing both natriuretic and direct vasodilatory activity (Van Zwieten 1984), or the calcium channel blocker benidipine, produced a milder and comparable decrease in blood pressure, but had dissimilar effects on renal function in SHR with reduced renal mass. The apparent differences in the results on renal function between these two drugs were not due to different degrees of systemic blood pressure. Taken together the study of benidipine with that of nilvadipine, treatment of hypertension with calcium channel blockers might produce a beneficial effect on renal function assessed by serum creatinine and BUN levels if the blood pressure reduction was not excessive although the critical levels of blood pressure during the treatment was not defined. However, the excessive reduction of blood pressure induced by calcium channel blockers in this model of rats would cancel the beneficial renal effects produced by a milder reduction of blood pressure following the treatment with this line of drugs. Further studies are required to determine if the milder reduction of blood pressure associated with the amelioration of renal function would be advantageous to prevent cardiovascular accidents.
An explanation for the specific beneficial effects of angiotensin converting enzyme inhibitors on renal function would be the inhibition of endogenous angiotensin II formation in the kidney. Angiotensin II mainly constricts the efferent arteriole, thus contributing to the maintenance of glomerular capillary pressure, and glomerular filtration (Zusman 1984; Raji and Keane 1985). Anderson et al. (1986) demonstrated that reduction of efferent arteriolar resistance and elevation of glomerular capillary ultrafiltration coefficient with angiotensin converting enzyme inhibitors are consistent with the inhibition of endogenous angiotensin II. These evidences suggest that antihypertensive drugs which lower efferent arteriolar resistance may be of special benefit to patient at risk for progressive renal disease. However, angiotensin II has many other actions within the kidney (Hall 1986; Heller and Horacek 1986), which may be also involved in the mechanism of deteriorated renal function in the rat remnant kidney model. Therefore, the exact actions of angiotensin converting enzyme inhibitors in this model of rats remain to be elucidated.

Since the filtration fraction was unchanged in the previous studies with calcium channel blockers (Sunderrajan et al. 1986; Reams et al. 1987), it has been assumed that the increase in glomerular filtration rate and effective renal plasma flow may be related predominantly to the decrease in afferent arteriolar resistance. These assumption would be responsible for the differences in the outcome of angiotensin converting enzyme inhibitors and calcium channel blockers treatments in their renal effects. In addition, several investigators demonstrated that calcium channel blockers attenuate the intrarenal effects of exogenously administered angiotensin II and norepinephrine. (Bauer and Reams 1987; Romero et al. 1987). It would also be assumed that calcium channel blockers induced a reversal of the direct effect on angiotensin II or norepinephrine of the mesangium or efferent arteriolar resistance. However, we could not determine the exact mechanisms of calcium channel blockers in renal effects in the rats with remnant kidney model in the present experiments.

Previous reports have shown that the ANP secretion may play an important role in promoting compensatory changes in renal function in chronic renal failure (Cole et al. 1985; Smith et al. 1986). In the present study, amelioration of renal function assessed by serum creatinine and BUN levels, produced by captopril and benidipine, was associated with the decreased plasma ANP levels. In contrast, nilvadipine did not induce any significant change in plasma ANP levels, which was associated with the reduction in blood pressure but not with the amelioration of renal function. Thus, decreased plasma ANP levels after the effective antihypertensive drugs may represent a reversed mechanism that occurs in response to ameliorated renal function. Whether the reduction in blood pressure after the effective antihypertensive drugs are associated with the decreased plasma ANP remain to be elucidated. It has been argued that the increased levels of circulating ANP may represent a compensatory mechanism that operates in
response to relative elevation of blood pressure in experimental models of hypertension (Gutkowska et al. 1986; Morii et al. 1986) and in patients with essential hypertension (Sugawara et al. 1985; Sagnella et al. 1986). The unchanged levels of plasma ANP in the treatment with nilvadipine associated with the significant reduction in blood pressure may indicate that elevation of plasma ANP levels in SHR with remnant kidney model is not related only to elevated blood pressure, but depends on the severity of impaired renal function.

In conclusion, the present study demonstrated that the reduction of blood pressure by the inhibition of angiotensin converting enzyme with captopril has the potential to ameliorate renal function in the SHR remnant kidney model of chronic renal failure associated with the decreased concentration of circulating ANP. However, it remains to be determined whether the reduction of blood pressure by calcium channel blockers may be beneficial in delaying the progression of renal failure in this model. Finally, it is also suggested that elevation of circulating levels of ANP in the SHR with remnant kidney model may depend on the severity of impaired renal function.

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References


