Angiotensin II Generation in Mesenteric Arteries in Rats: Effects of Nephrectomy, Deoxycorticosterone and Dexamethasone

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Abstract

Angiotensin II (ANG II) generation in the mesenteric arteries was studied in four groups of rats: deoxycorticosterone (DOCA)/salt treated, glucocorticoid treated, nephrectomized and control rats. Basal plasma renin activity (PRA) was undetectable in the nephrectomized group and suppressed in the DOCA/salt treated rats, but was increased in the rats treated with glucocorticoid. The Basal plasma ANG II concentration changed comparably with PRA in all four groups of rats. In the control rats, ANG II was released from the mesenteric arteries at a rate of 43.0±12.0 pg/h, and it was not decreased by nephrectomy. In DOCA/salt rats and glucocorticoid rats, ANG II release significantly decreased to 12.8±7.1 and 6.9±1.5 pg/h, respectively. Captopril treatment significantly reduced ANG II release from the mesenteric arteries in both controls and nephrectomized rats, but did not influence ANG II output in DOCA/salt rats or in glucocorticoid treated rats. In nephrectomized rats, captopril lowered blood pressure in association with a significant reduction in the mesenteric ANG II formation. These results indicate that the renal and vascular renin-angiotensin system (RAS) may be independently regulated, and in nephrectomized animals the vascular RAS contributes in part to the maintenance of blood pressure. The present results also suggest that volume expansion per se and/or pharmacological intervention by DOCA and glucocorticoid could modulate vascular ANG II generation.

All components of the RAS have now been shown to exist in the blood vessels of various animal species. Neither the precise mechanism of its synthesis in the blood vessels nor the physiological significance of locally generated ANG II for cardiovascular homeostasis is clear. Previously, ANG II release from isolated rat mesenteric artery preparation has been reported to be enhanced by sympathetic nerve stimulation and a sympathomimetic drug (Nakamaru et al., 1986), and ANG I generation is increased by renin substrate added to the hindquarter perfusion (Oliver and Sciaccia, 1984). More directly, angiotensins have

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been shown to be synthesized and secreted in cultured bovine aortic endothelial cells (Kifor and Dzau, 1987). These findings and the recently recognized tissue-specific angiotensinogen mRNA (Ohkubo et al., 1986; Kalinyak and Perlman, 1987) suggest the possibility that renal and local RAS may be independently regulated.

The present study was undertaken to elucidate the relationship between renal and vascular RAS, using ex vivo perfusion of the mesenteric arteries in rats with various renin levels in the circulation. Furthermore, in order to assess whether vascular RAS play a role in the hypotensive effect of angiotensin converting enzyme (ACE) inhibitors, we attempted to study the effects of captopril on vascular ANG II generation in rats.

Materials and Methods

Male Wistar rats each weighing 180–200 g were used. All rats were placed in individual cages and maintained in an environmentally controlled room having a humidity level of 55 ±5%, a temperature of 23°C and light from 9:00 to 20:30. All animals were fed normal rat chow (CRF-1, Charles River, U.S.A.) which contained 0.012 m mol/g of sodium, 0.224 m mol/g of potassium and 0.3 m mol/g of calcium for one week prior to the experiment. All rats were allowed to drink water ad libitum and were randomly allocated into four groups. Ten animals constituted each group. Silastic rubber strips impregnated with DOCA (Sigma Chemicals Co. St. Louis, U.S.A.) or dexamethasone (Wako Chemicals Co. Osaka, Japan) were prepared according to the method of Berecek and Bohr (1977) as reported previously (Miyamori et al., 1985). Silastic rubber strips containing no drugs were given to the two groups of rats which were allowed free access to tap water: one group served as the control, and the other group received total nephrectomy 72 hours prior to the perfusion study. DOCA was given at a dose of 50 mg/kg and these animals were given a 1% NaCl solution to drink for 28 days. For the treatment with glucocorticoid, the animals were given tap water containing 5 mg/l of dexamethasone, the daily dose being calculated by the amount of water consumed. Thus it was estimated that each animal ingested 50–100 μg of dexamethasone per day. Half of the animals in each group were given 10 mg/Kg captopril (Squibb, Princeton, NJ, U.S.A.), orally for 2 days. Blood pressure (BP) and heart rate were recorded by the tail-cuff method previously reported (Miyazaki et al., 1980).

PRA was measured in the tail arterial blood samples before the animals were anesthetized (Miyamori et al., 1980). Then all the animals received an intraperitoneally 0.5 mg/g of Inactin-Bky anesthesia (Byk Gulden, Konstanz, Federal Republic of Germany), and the mesenteric artery was immediately excised and prepared for perfusion following the method originally described by McGregor (1965) with the minor modifications previously reported from our laboratory (Miyamori et al., 1983). Briefly, the arteries were perfused with Krebs-Ringer solution, pH 7.4, prewarmed and oxygenated with a 95% O2±5% CO2 gas mixture at a constant rate of 4 ml/min. The perfusion pressure was constantly monitored by means of a pressure transducer connected to a polygraph (RM 600, Nihon-koden, Tokyo, Japan) and recorded. After 30 min of equilibration, 480 ml of the perfusate was collected over 120 min and half of the samples were applied to a Sep-Pak C18 cartridge column (Waters Associates, Milford MA, U.S.A.) prewashed with 3 ml of methanol and 10 ml of Krebs-Ringer solution. The recovery of authentic ANG II dissolved in 100 ml of Krebs-Ringer solution at a concentration of 1 pg/ml during the effluent collection, extraction and condensation process is approximately 92±3%. The recovery was not corrected in subsequent experiments. The time dependency of the ANG II release was tested in four fractions of perfusates in independent experiments. The latency and viability of the tissue preparations were tested by pressure responsiveness to noradrenalin in the perfusion line.

The ANG II absorbed by the Sep-Pak C18 column was extracted with methanol/water/trifluoroacetic acid 80/19.9/0.1 (v/v) and measured by RIA. The tyrosyl-125-I ANG II was obtained from New England Nuclear (MA, U. S.A.) and reconstituted in distilled water at a concentration of approximately 1850 kBq/ml (50 μCi/ml). The solution was aliquoted and
dissolved once before use. The antiserum for ANG II was purchased from 1 gG Corporation (Nashville, TN, U.S.A.). The antibody is directed toward the carboxyl terminus of ANG II which reacted fully with ANG II amide and the hepta-, hexa- and penta-derivatives. Less than 0.1% of both angiotensinogen and angiotensin I crossreacted with the ANG II antibody. ANG II standard, purchased from the Peptide Institute (Osaka, Japan), was dissolved in water and prepared for RIA at a concentration ranging between 1.56 and 200 pg/tube. The sensitivity of the assay was 1.0 pg/tube. The intra- and inter-assay variances were 8.5% and 12% respectively.

High performance liquid chromatography (HPLC) was carried out in selected samples from the nephrectomized rats for ANG II identification. The HPLC system (LC 5A, Shimadzu Scientific Instrument Inc., Kyoto, Japan) with a Hiber RP-18 column (4×215 mm, E. Merk Dermstadt F.R.G.) was used, combined with methanol gradient system; methanol was linearly increased from 20–80%, at a flow rate of 0.5 ml/min, fractions were collected for 30–60 seconds and dried in a vacuum centrifuge. Each sample was resolved in 0.2 M Tris acetate buffer, 0.02% sodium azide, pH 7.4, and subjected to radio-immunoassay of ANG II.

The data were expressed as the mean±SEM in the text. Statistical analysis of the results was done by two way ANOVA analysis followed by Seffe’s multiple comparison, and p<0.05 taken as a significant level.

Table 1. Body weight (Bwt), systolic blood pressure (sBP), heart rate (HR), plasma renin activity (PRA) and plasma angiotensin II concentration (p-ANG II) in control, nephrectomized (Nex), deoxycorticosterone acetate-treated (DOCA) and dexamethasone-treated (Dex) rats.

<table>
<thead>
<tr>
<th></th>
<th>Bwt (g)</th>
<th>sBP (mmHg)</th>
<th>HR (bpm)</th>
<th>PRA (ng/ml/h)</th>
<th>p-ANG II (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>193±4</td>
<td>119±1</td>
<td>412±10</td>
<td>9.4±0.9</td>
<td>395.5±20.3</td>
</tr>
<tr>
<td>Nex</td>
<td>203±5</td>
<td>142±12**</td>
<td>388±11</td>
<td>U.D.**</td>
<td>683.2±65.1**</td>
</tr>
<tr>
<td>DOCA</td>
<td>323±5**</td>
<td>141±2**</td>
<td>402±8</td>
<td>1.6±0.5**</td>
<td>110.4±14.6**</td>
</tr>
<tr>
<td>Dex</td>
<td>165±3**</td>
<td>166±2**</td>
<td>402±9</td>
<td>17.1±1.9**</td>
<td>1107.4±298.5**</td>
</tr>
</tbody>
</table>

** Significantly different from the control values (p<0.01).
All values represent the mean±S.E.M. Ten animals constituted each group.

Results

Table 1 shows the body weight, blood pressure (BP), heart rate, PRA and plasma ANG II concentrations in the four groups of rats. Approximately half of the rats in each group were given captopril. In nephrectomized rats, the basal plasma ANG II and PRA decreased, the latter to an undetectable level. In the DOCA/salt treated rats, PRA and ANG II were markedly suppressed but were still measurable, and BP and body weight were higher than in the control rats. In dexamethasone treated rats also, the BP rose but the PRA and plasma ANG II levels were markedly higher than in the three other groups of rats. There was no significant difference in heart rate among the four groups of rats.

Captopril administration induced a slight but significant decrease in BP in the control, nephrectomized and dexamethasone treated rats. In the DOCA/salt treated rats, BP was not significantly influenced by oral captopril administration. The heart rate decreased significantly in the nephrectomized rats in response to captopril (Figure 1). In the nephrectomized rats and in DOCA/salt rats, PRA did not change with captopril administration.
The HPLC profile of authentic ANG II, analysis of the effluent from nephrectomized rat samples, and their correspondence to radioimmunoassay results are shown in Figure 2. The peak of immunoreactive ANG II coincided with authentic ANG II. There were no obvious time dependent changes in the ANG II concentrations in the four fractions of perfusate. The amount of ANG II released from the mesenteric arteries in the perfusion circuit in the four groups of rats is shown in Figure 3. These animals received either vehicle alone or captopril for 2 days. The ANG II output in the effluent of the isolated mesenteric arteries was 43.0±12.0 pg/h in the control rats and was decreased to 20.6±6.5 pg/h by pretreatment with captopril (P<0.05). In nephrectomized rats, 55.6±9.3 pg/h of ANG II was released in the effluent. This value was not significantly different from that in control rats. Captopril treatment decreased vascular ANG II release in the nephrectomized rats to 19.0±5.5 pg/h (P<

Fig. 1. Effect of angiotension converting enzyme inhibition by captopril on systolic blood pressure (sBP) and heart rate (HR) in control, nephrectomized (Nex), deoxycorticos- terone acetate (DOCA)-treated and dexamethasone (Dex) treated rats. The open columns indicate sBP before captopril, and the shaded columns indicate sBP changes after captopril administration. Each column and point represents mean±S.E.M. * p<0.05; ** p<0.01
Each column represents mean ± S.E.M. * p < 0.05; ** p < 0.01. Decreased ANG II in control and in nephrectomized rats. ANG II generation was significantly different in the nephrectomized rats. Captopril significantly decreased ANG II (DOC) rats. ANG II generation was significantly decreased in the nephrectomized rats (DOC) and dexamethasone (DEX) rats. ANG II generation from the isolated mesenteric arteries in control, nephrectomized (Nex), and dexamethasone (DOMA) rats. ANG II generation from the isolated mesenteric arteries (Nex, DOMA, Control).
Basal ANG II output in the DOCA/salt rats and dexamethasone treated rats was 12.8 ± 7.1 pg/h and 6.9 pg/h, respectively which was significantly lower than control and nephrectomized rats (P < 0.01). Captopril administration changed ANG II output to 11.7 ± 0.7 pg/h and 4.7 ± 1.3 pg/h in DOCA/salt and dexamethasone rats, respectively.

Discussion

It has previously been shown that ANG II is continuously produced in the mesenteric and hindlimb preparations, and it has been suggested that it plays a role in the local regulation of vascular tone (Dzau, 1986). The present study confirmed arterial release of ANG II in the rat mesenteric artery, and further demonstrated that this release is augmented by nephrectomy, suggesting that the renal and vascular RAS may be under independent regulation.

The mechanism of increased vascular ANG II generation produced by nephrectomy is not evident from the present study. It may be postulated first that the circulating ANG II is negatively related to the RAS in the vascular tissue. Thus, a release from the negative feedback by the RAS in the circulation after nephrectomy could explain the increase in vascular ANG II production. However, the decreased vascular ANG II production in DOCA salt rats, in which the plasma renin level is suppressed by expanded plasma volume, makes the above possibility less likely. Secondly, the potassium ions accumulated following nephrectomy may stimulate vascular renin production as demonstrated in adrenal renin which is increased after potassium loading in the presence of a suppressed plasma renin level (Nakamura et al., 1985). Thirdly, a role of the sympathetic nerve activation in the stimulation of vascular ANG II generation (Nakamura et al., 1986) following nephrectomy has yet to be proven.

It may be argued that ANG II released in the perfusate would originate from the circulation which was bound to the endothelium. In the present study the ANG II concentration in the fractionated perfusion samples showed no time-dependent decline up to 120 min, supporting the view that it reflects in part newly synthesized ANG II locally. More directly, the cellular synthesis and secretion of ANG II and ANG III has been reported in cultured bovine aortic endothelial cells (Kifor et al., 1987).

The physiological significance of the RAS locally generated in the vascular wall is not well understood. It is hypothesized that the system may play a role in the maintenance of vascular tone (Oliver and Siacca, 1984; Zau 1986) and blood pressure regulation (Thurston et al., 1979; Loundon et al., 1983; Okamura et al., 1986) in hypertensive animal models, either directly or indirectly by facilitating sympathetic nerve transmission (Ziogas et al., 1985; Malik et al., 1976).

Although it is now evident that peripheral tissues produce angiotensins and only a portion of the plasma concentration is produced by the renal RAS (Dzau, 1986; Campbell, 1985), it is not clear from this study to what extent vascular ANG II contributes to the plasma concentration. Thus, vascular RAS activity appears unrelated to the plasma level. In fact, an increased aortic renin-like activity in the presence of a normal or decreased circulating renin level is reported in spontaneously hypertensive rats (Assad and Antonaccio, 1982) and the chronic phase of Goldblatt twokidney hypertension rats (Garst et al., 1979). This dissociation of the local and circulating RAS suggests that the local RAS may contribute little to the circulating RAS level. However, few studies have been reported which directly tested the vascular ANG II generation in various experimental
hypertensive models.

In DOCA salt rats, the sympathetic tone is activated (Reid et al., 1975) and this may facilitate ANG II formation (Nakamura et al., 1986). ANG II generated in the arterial wall is therefore felt to be increased in this model. In the present study, however, ANG II release from the mesenteric artery are suppressed in DOCA/salt rats. Therefore, it is unlikely that vascular RAS activation may participate in the pathogenesis of hypertension in this model. In keeping with this finding, there is also evidence that aortic renin is decreased in DOCA salt rats (Swales et al., 1983). It is speculated that a common mechanism of RAS regulation such as expanded plasma volume, in kidneys and vessels may be operative in DOCA salt rats.

In glucocorticoid hypertensive rats, the RAS is increased in the circulation and suppressed in the vascular wall. Tissue-specific angiotensinogen mRNA has recently been demonstrated to be expressed in various tissues, e.g., liver, mesentery, aorta, atria, kidney, brain, spinal cord, large intestine, adrenal, and lung (Ohkubo et al., 1986). Glucocorticoid administration has been reported to increase the accumulation of angiotensinogen mRNA in the liver and the kidney (Kalinyak et al., 1987).

In another study, coadministration of dexamethasone, estradiol and triiodothyronine accumulated mRNA in several tissues including the mesentery (Campbell and Habener 1986). On the basis of these findings, it may be anticipated that mesenteric ANG II generation will be increased in rats given dexamethasone. The present results, however, do not suggest the existence of local RAS stimulation in this animal. Although we have no evidence for the quantitative changes in the local angiotensinogen levels, it may be that increased mRNA does not necessarily induce angiotensinogen accumulation. In this context the quantitative analysis of renin mRNA expression revealed that mRNA does not correlate with renin activity (Suzuki et al., 1988). These authors explained the dissociation of mRNA and its related product by differences in translational efficiency among various organs, or differences in post-translational processing. It is not known whether the differences in processing could result in the altered renal and vascular angiotensinogen level in response to dexamethasone administration in rats.

Captopril administration decreased BP which was not associated with changes in mesenteric ANG II production in glucocorticoid treated rats. The hypotensive effect of captopril is most likely achieved by reducing the circulating ANG II in this model of hypertension. Although the ex vivo perfusion pressure observed in the above experiments may not be synonymous with the vascular tone in vivo, vasoconstrictors added to the perfusion circuit respond similarly to changes observed in vivo. Lastly, the possibility of ANG III formation as a consequence of the elimination of the aminoterminal aspertyl residue of ANG II, intracellularly or after secretion, might account for the apparently small amount of ANG II in the effluent. In the present assay system, however, ANG II and ANG III were not distinguishable. Taken together the results support a minor contribution of the locally released angiotensins in the circulation. We also speculate that in certain pathophysiological conditions, the local RAS may be important as an autocrine or paracrine for the control of vascular tone as suggested previously (Dzau 1984).

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References


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