Acute Changes in the Renin-Angiotensin System Modify Bradykinin and Angiotensin Reactivity and Metabolism in Conscious Rats

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SUMMARY We have shown that angiotensin I (AI) conversion as well as bradykinin (BK) inactivation and reactivity are altered in chronic renal hypertensive rats. In the present experiments we tested the possibility that acute renal hypertension or AI and All infusion cause alterations in both systems. Pulmonary inactivation of BK was estimated by comparing intravenous and intraaortic equipressor doses (20 mm Hg), and the extent of AI conversion was assessed by determining the equipressor doses of AI and All that produced a 20 mm Hg rise in mean arterial pressure (MAP). Acute renal hypertension was produced by unclamping the renal pedicle (URP) occluded for 5 hours in conscious rats. Before URP, the MAP was already increased (131 ± 2 mm Hg) and captopril (10 mg/kg, i.v.) produced a fall of 27 ± 8 mm Hg, suggesting that the renin-angiotensin system was overactive. After URP, MAP rose to 151 ± 3 mm Hg, and captopril completely abolished the hypertension. Before URP, reactivity to BK was increased [doses 6 times smaller than control (C), 34 ± 5 ng], and URP produced no further elevation. Pulmonary BK inactivation (97.5% ± 4%) was the same before and after URP. Before URP, doses of All 5 times greater than C (2 ± 4 pmol) were necessary, and hyporeactivity to AI was markedly increased after URP (doses 300 times larger than C). After URP, the conversion was maximal (104% ± 2% vs 49% ± 3% in C), and it was already elevated before URP (82% ± 10%) when six of the nine rats studied had maximal extent of conversion. During infusion of different doses of AI and All in C rats, the vascular reactivity and pulmonary inactivation of BK was increased when compared to control period. Decrease in reactivity to All was observed during infusion of AI or All, but the extent of AI to All conversion remained unchanged. Therefore, hyperreactivity to BK was produced during acute hyperreactivity of the renin-angiotensin system (occlusion of the renal pedicle) as well during infusion of AI or All. Hyperreactivity to AI was accompanied by a marked increase in AI to All conversion before and after URP. Infusion of AI or All caused hyporeactivity to All but did not affect the extent of conversion. (Hypertension 5 (supp V): V-172-V-176, 1983)

KEY WORDS • converting enzyme • bradykinin inactivation • angiotensin conversion • renal hypertension • kidney unclamping

CONVERTING enzyme (CE) is known to be a common link between the renin-angiotensin system (RAS) and the kallikrein-kinin system. Moreover, CE is present in many tissues and in plasma, and most of the CE activity has been found to be widespread in vascular endothelium. Plasma CE activity has been measured under different pathophysiological conditions over the last decade. Plasma CE is increased in sarcoidosis and decreased in lung disease of other etiology. During the development of one-kidney, one clip perinephritis hypertension, plasma CE activity decreases in rabbits. Plasma CE activity was unchanged in women receiving oral contraceptive and in normal pregnancy and increased in Gaucher's disease. The extent of angiotensin I (AI) conversion to angiotensin II (All) and bradykinin (BK) degradation was shown to be increased in one-kidney, one clip hypertensive rats (1K1C), and these hypertensive rats are hyperreactive to BK. The possibility that the alterations observed depended only on an increase in the CE activity was not supported by the observation that 20 hours after unclipping the alterations in BK degradation and vascular reactivity to BK returned to normal, whereas the increase in AI conversion persisted. Moreover the finding that two-kidney, one clip hyper-
tensive rats (2K1C) exhibit hyperreactivity to BK accompanied by elevated pulmonary inactivation of BK, but show normal or elevated AI conversion depending on the duration of hypertension. It is another indication that the mechanism underlying alterations in AI conversion and BK degradation can be partially dissociated.

Since the in vivo blood pressure response method permits the simultaneous evaluation of AI conversion and BK inactivation in conscious rats, we undertook to study the alterations produced in both systems by acute renal hypertension and by AI and All infusion. Although acute hypertension produced by unclamping the renal pedicle occurred for a few hours has been studied in anesthetized rats, it was necessary to standardize this model of hypertension in conscious rats.

**Methods**

Male and female Wistar rats weighing 180–280 g were used. One day before the experiment a plastic cannula (PE-10 connected to PE-50) was inserted into the abdominal aorta through the femoral artery under ether anesthesia for measuring direct pressure. For intravenous injections, a cannula was implanted into the femoral vein and intraarterial injections were made through a cannula inserted into the ascending aorta through the left carotid artery. The catheters emerged through the back of the rat for recording arterial pressure; the cannula from the femoral artery was attached to a Hewlett-Packard 1280 pressure transducer and Hewlett-Packard Model 7754A multichannel recorder. During measurements, the rats were placed on a small platform where they remained unrestrained and usually quiet. Pulmonary inactivation of BK was estimated by comparing intravenous and intraarterial doses that produced a 20 mm Hg fall in mean arterial pressure (MAP). AI to All conversion was assessed by determining the equipressor doses of AI and All that produced a 20 mm Hg rise in MAP. Similar results were obtained when the extent of BK inactivation and AI conversion were determined by single injection or by infusion methods. The hypotensive responses to BK were compared with those produced by sodium nitroprusside. AI, All, and BK were synthesized by Dr. A.C.M. Paiva (Escola Paulista de Medicina, São Paulo). Amino acid composition and concentration of each stock solution were determined by amino acid analysis after hydrolysis with 6 N constant boiling HCl for 22 hours at 110°C. The substances were injected as a single bolus in a volume of 0.1-0.2 ml.

Results obtained in normotensive control rats were compared to those obtained in rats with acute renal hypertension produced by releasing renal pedicle occluded for 5 hours. In another series of experiments, normotensive rats were studied before and during infusion of AI and All. The peptides were infused (0.2 ml/min) at two different doses: a low dose (0.2–2.0 ng/min) that produced an increase in MAP of 5 to 10 mm Hg and a high dose (20–100 ng/min) that produced an increase in MAP of 20 to 45 mm Hg.

Acute renal hypertension in anesthetized rats produced upon unclamping of the renal pedicle that had been occluded for a few hours has been described before. To apply this model of hypertension to conscious rats, the following procedures were carried out. Laparothomy was performed under ether anesthesia and a thick cotton thread was passed over the left renal pedicle. Using a straight suture needle both ends of the thread were exteriorized through the back of the rat. A piece of latex tubing was placed on the external side of the skin and the thread tied tightly above the latex tubing. The tubing prevented changes in the extent of occlusion caused by the movements of the unrestrained conscious rat. Right nephrectomy was performed simultaneously. During a preliminary series of experiments, the renal pedicle was released either 3 or 5 hours after occlusion. The renal pedicle was released by cutting the thread and pulling it out. This procedure did not produce responses common to painful stimulation: flight reaction, vocalization, or autonomic response. Usually only a startle reaction was observed. To assess RAS activity, the responses of MAP to intravenous injection of the CE inhibitors (CEI) BPP₆, (SQ20,475, 1 mg), BPP₅, (SQ20,881, 1 mg), and captopril (SQ14,225, 10 mg/kg) were studied, BPP₆, and BPP₅, were products of Schwarz/Mann, Orangeburg, New York, and captopril was provided by Squibb. Plasma renin activity (PRA) was determined using a commercial angiotensin radioimmunoassay kit in normotensive and hypertensive rats.

Results are reported as means ± standard error of the mean (SEM). The statistical significance of differences between groups or within a group at different times was analyzed by unpaired and paired t tests. Changes were considered to be significant at p < 0.05.
Effect of captopril injection on the pressure response produced by releasing the renal pedicle occluded for 5 hours in conscious rats. A. Evolution of mean arterial pressure after unclamping. B. Effect of captopril (10 mg/kg, i.v.) given 15 minutes after unclamping. C. Effect of captopril (10 mg/kg, i.v.) given 5–10 minutes before unclamping. Asterisk indicates statistically significant difference, p < 0.05.

Control rats). PRA levels of six rats 15 minutes after unclamping were significantly increased (32.3 ± 6 vs 3.0 ± 0.6 ng/ml/hr in 10 normotensive rats).

Bradykinin Inactivation and Reactivity During Acute Renal Hypertension

MAP after 5 hours of occlusion was already increased (131 ± 2 vs 115 ± 2 mm Hg in the control group) and rose to 151 ± 3 mm Hg after unclamping. Reactivity to BK was elevated before unclamping (fig. 2). Compared to control rats, the animals with 5 hours of pedicle occlusion needed approximately 6 times less BK injected intraaortically (5.5 ± 1 vs 33.6 ± 5 ng) or intravenously (0.27 ± 0.04 vs 1.4 ± 0.2 μg) to produce the standard −20 mm Hg response. No increase in hyperreactivity to BK was observed after unclamping. Since similar alterations were observed after BK injections intraaortically and intravenously, no significant change in the extent of pulmonary BK inactivation (97.5%–98.5%) was demonstrable. In another series of experiments, reactivity to intraaortically injected BK and nitroprusside was compared in six normotensive rats before occlusion and when they were hypertensive after unclamping. Before occlusion (109 ± 2 mm Hg), the responses to BK (10, 20, and 50 ng) were 12 ± 1, 17 ± 1, and 23 ± 2 mm Hg, respectively, and markedly increased after unclamping (165 ± 3 mm Hg) to 26 ± 1, 32 ± 2, and 40 ± 2 mm Hg respectively. To nitroprusside (0.1, 0.2, 0.5, and 1.0 μg), the responses observed were not statistically different from those observed after unclamping (9 ± 3, 12 ± 2, 18 ± 2, and 26 ± 3 mm Hg vs 6 ± 1, 9 ± 1, 13 ± 1, and 22 ± 2 mm Hg).

Angiotensin I Conversion and Reactivity to Angiotensin II During Acute Renal Hypertension

Before unclamping the renal pedicle (MAP = 129 ± 2 mm Hg), reactivity to AI was decreased. A dose 5 times larger (9.7 ± 2 pmol) than that used for the control normotensive rats was necessary to produce the standard pressure response. Hyporeactivity to AI was proportionally smaller (17.3 ± 3 vs 4.7 ± 0.7 pmol); thus the extent of AI to All conversion was markedly increased (82% ± 10% vs 49% ± 3%). Before unclamping, six of the nine rats studied already had the maximal extent of conversion (100%). After releasing the renal pedicle (MAP = 150 ± 4 mm Hg), reactivity to AI was drastically decreased, requiring doses 300 times larger (718 ± 10 vs 2.3 ± 0.4 pmol) to produce a standard 20 mm Hg drop in mean arterial pressure (MAP) when injected intraaortically (BK-v) or intraaortically (BK-a) in a control group of rats (C), and in rats with the renal pedicle occluded for 5 hours (PO) and after unclamping during the acute hypertension (AH). The extent (%) of pulmonary inactivation of BK in each circumstance is depicted at the top of the figure. The number of rats is given in parentheses. Statistically significant different (p < 0.05) from C (*) or from PO (†).
Figure 3. Comparison of the doses of angiotensin I (AI) and angiotensin II (AII) that produced a standard 20 mm Hg increase in mean arterial pressure (MAP) in the control group of rats (C) and in rats with the renal pedicle occluded for 5 hours (PO) and after unclamping during the acute hypertension (AH). The extent (%) of AI to AII conversion is depicted at the top of the figure. The number of rats is given in parentheses. Statistically significant different (p < 0.05) from C (*) or from PO (†).

The standard pressure response, and AI to AII conversion was maximal in all animals, i.e., the doses of AI and AII were similar (fig. 3).

Alterations Produced by Infusion of Angiotensin I and II

As shown in table 1, the reactivity to BK was enhanced during infusions of two different doses of AI and AII that produced MAP increases to approximately 10 and 30 mm Hg, respectively. The effects produced by AI tended to be larger and more uniform (smaller SEM) than those produced by AII, but the differences were not statistically significant. During the infusion of high doses of AI and AII, the intraarterial doses of BK required to produce the standard hypotensive response (∼20 mm Hg) were only 14% and 30%, respectively, of the dose required by the same rats during the control period. The BK potentiation was usually greater when BK was administered intraarterially than intravenously, which indicates an elevation of the extent of the pulmonary inactivation of BK. For example, during infusion of high doses of AI the extent of pulmonary degradation of BK increased from 98.2% ± 0.5% to 99.4% ± 0.1%.

<table>
<thead>
<tr>
<th>Infusion</th>
<th>ΔMAP (mm Hg)</th>
<th>% of control dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low AI (7)</td>
<td>6 ± 1</td>
<td>79 ± 24</td>
</tr>
<tr>
<td>Low AII (8)</td>
<td>9 ± 1</td>
<td>59 ± 13</td>
</tr>
<tr>
<td>High AI (8)</td>
<td>34 ± 2</td>
<td>35 ± 11</td>
</tr>
<tr>
<td>High AII (7)</td>
<td>34 ± 2</td>
<td>45 ± 11</td>
</tr>
</tbody>
</table>

The number of rats is given in parentheses.

Changes in reactivity to AI and AII and the extent of AI conversion were also studied during the infusion of low and high doses of AI and AII. A parallel decrease was observed in the reactivity of injected AI and AII, which was proportional to the dose of AI and AII infused, with no alteration in the extent of AI conversion. During infusion of the high dose of AI, MAP rose from 118 ± 1 to 150 ± 3 mm Hg and the doses that produced the standard pressure effect increased from 5.8 ± 2 to 164 ± 36 pmol of AI and from 2.8 ± 0.8 to 97 ± 12 pmol of AII.

Discussion

The data of the present study show for the first time that reactivity to BK injected intraaortically can be markedly potentiated in conscious rats by infusion of AI and AII and during increase in RAS activity as observed before and after unclamping the renal pedicle. The maximum potentiation observed was obtained when the dose required to produce the standard response was approximately 10% of the dose used before infusion of AI. The same maximum extent of potentiation was observed before and after unclamping the renal pedicle, despite the fact that before unclamping the MAP was only slightly elevated and the responses to CEI were smaller. Therefore, it was observed that acute changes in RAS activity produced a potentiation of intraaortically injected BK smaller than that previously observed in rats with chronic hypertension. Doses approximately 30 times smaller than that required for control normotensive rats were used in chronic 1K1C and 2K1C rats, whereas in the acute hypertension of the present study the doses were only 10 times smaller. Potentiation of BK injected intravenously was also observed, which confirms observations in conscious rats during infusion of AI to AII. Hyperreactivity was of the same magnitude to BK injected intraaortically or intravenously, thus not changing the calculated pulmonary inactivation of BK in the rats with a clamped kidney. Pulmonary degradation of BK was unexpectedly increased during infusion of AI and AII because of the potentiation of intraaortic BK was larger than the potentiation of intravenous BK. Thus, the potentiation of BK injected intravenously demonstrated in the present experiments as well as that observed in chronic renal hypertension occurs in the peripheral vessels and is not due to a decrease in pul-

<table>
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<th>ΔMAP (mm Hg)</th>
<th>BK-v</th>
<th>BK-a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low AI (7)</td>
<td>6 ± 1</td>
<td>79 ± 24</td>
<td>32 ± 7</td>
</tr>
<tr>
<td>Low AII (8)</td>
<td>9 ± 1</td>
<td>59 ± 13</td>
<td>43 ± 13</td>
</tr>
<tr>
<td>High AI (8)</td>
<td>34 ± 2</td>
<td>35 ± 11</td>
<td>14 ± 5</td>
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<td>High AII (7)</td>
<td>34 ± 2</td>
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</table>

TABLE 1. Hyperreactivity to Bradykinin (BK) Produced by Infusion of Angiotensin I (AI) or II (AII). Expressed as Percentage of the Dose Injected Intravenously (v) or Intraaortically (a) before Infusion to Produce the Standard Hypotensive Response (20 mm Hg)
Pulmonary inactivation that would allow more BK to reach the peripheral circulation. Hemodynamic studies in conscious I.KIC rats indicated that hyperreactivity was specific for BK since the response to nitroprusside was not modified. Moreover, the augmented hypotensive response to BK were due mainly to decreases in peripheral resistance.\(^\text{16}\)

One explanation for vascular hyperreactivity to BK when RAS activity is increased is in terms of the occupancy of CE (kininase II)\(^2\) by AI or All. Indeed, competition between AI, All, and BK has been demonstrated in vitro with purified CE.\(^1\) The existence of other bradykininases in the lung besides CE may account for the fact that no decrease in the extent of pulmonary inactivation of BK was observed when BK hyperreactivity was demonstrated. An alternative hypothesis is that CE activity, which is widespread throughout vascular endothelium,\(^2\)\(^-\)\(^3\) may be qualitatively different from pulmonary CE, since it has been shown that extrapulmonary CE is more resistant to long-lasting blockade by SQ 20,881.\(^19\)

As expected from previous studies,\(^28\) the infusion of AI and All greatly decreased the pressure reactivity to All in conscious rats. Doses 50–100 times higher than during the control period were required to produce the same standard response. Hyporeactivity to AI was of the same magnitude as hyperreactivity to All, thus resulting in no change in extent of AI to All conversion. However, conversion was greatly increased during acute renal hypertension. After unclamping the renal pedicle, when larger doses of AI and All had to be injected because reactivity was markedly decreased, the rate of conversion was 100%. However, 5 hours after occlusion and immediately before unclamping, when reactivity to AI was only moderately decreased, six of nine rats already showed maximal rates of conversion (100%). It should be stressed that at this time RAS activity was already elevated (although not as much as after unclamping), as indicated by the moderate increase in MAP and the responses to CEI.

The increase in the circulating levels of AI alone may not account for the increase in the conversion rate of AI observed in the present experiments, before and after unclamping, since no change was detected upon infusion of AI. Furthermore, in perfused rat lung,\(^11\) increasing AI from 10\(^{-6}\) to 10\(^{-4}\) M in the perfusion medium produced no changes in the percentage of AI removed (and therefore presumably the percentage of conversion). As pointed out by Ryan,\(^22\) factors other than the kinetics of CE are necessary to explain the increase in the rate of AI to All conversion in the in vivo preparation. Besides physical factors (dilation of the vessel, increase in perfusing pressure, shunting of blood, etc.) or changes in the shape and surface of endothelial cells that can increase the efficiency of AI hydrolysis, endogenous inhibitors\(^6\) could play an important role. The effect of the renal pedicle occlusion and of its release upon AI to All conversion described here and the absence of an effect produced by infusion of AI and All, suggest that the kidney may modulate the CE activity.
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