Hyperreactivity to Bradykinin and Alterations in Angiotensin I Conversion and Bradykinin Inactivation in Renal Hypertensive Rats

MARIA CRISTINA DE OLIVEIRA SALGADO, M.S., AND EDUARDO MOACYR KRIEGER, M.D.

SUMMARY Low doses (1-10 ng) of angiotensin I (AI) injected intravenously into conscious rats produced greater pressure changes than when injected intraaortically. This finding again indicates the importance of the pulmonary circulation in the conversion of AI to angiotensin II (AII). At high doses of hormone (30 ng, which produces pressure rises of 40-50 mm Hg) the intravenous and intraarterial responses tended to be the same. However, the role of the lung for conversion is still indicated by comparing the time for onset of the pressure rise and the time to reach maximum pressure rise for the intravenous and intraaortic equipressor doses, was higher in conscious rats with chronic one-kidney renal hypertension (RHR; 217 ± 3 mm Hg) than in the normotensive control rats (NCR; 126 ± 3 mm Hg), 99.6% and 97.5%, respectively. Vascular hyperreactivity to bradykinin was seen in RHR in response to both intraaortic bolus injection and intraaortic infusion. The intraaortic equipressor dose was 15-30 times smaller in RHR than in NCR, while the differences with isoproteonel and nitroprusside were less pronounced. Indomethacin infusion (5 mg/kg/min) for 10 minutes had no effect on intraarterial hyperreactivity to bradykinin. The marked increase in vascular reactivity to bradykinin more than compensated for the larger pulmonary inactivation. The equipressor doses of bradykinin injected intravenously were 200 ± 24 ng in RHR and 1060 ± 143 ng in NCR. Conversion AI to AII, assessed by the equipressor doses of the hormones which produce 20 mm Hg rise in pressure, was also higher in the RHR than in the NCR, 55% vs 25%, with no change in vascular reactivity to AII. Hyperreactivity to bradykinin reverted entirely to normal 20 hours after unclipping the RHR, when blood pressure had also normalized. The responsiveness to nitroprusside tended to increase rather than decrease after unclipping. While the vascular hyperreactivity to bradykinin and the increased pulmonary degradation of bradykinin seen in the RHR were markedly affected by unclipping, the increased conversion of AI to AII and reactivity to AII remained unchanged after unclipping. These results suggest that the mechanism underlying the alterations in AI conversion and bradykinin degradation in one-kidney renal hypertensive rats can be partially dissociated. (Hypertension 4: 77-83, 1982)

KEY WORDS • hyperreactivity to bradykinin • angiotensin conversion • bradykinin inactivation • converting enzyme • pulmonary circulation

THE participation of the kallikrein-kinin system has been considered in addition to that of the renin-angiotensin system in recent studies dealing with the pathogenic mechanisms responsible for renal hypertension. The wide use in recent years of converting enzyme (CE) inhibitors to identify factors responsible for hypertension has focused interest on angiotensin conversion, but CE also affects bradykinin degradation. CE is thus a catalytic step common to the metabolism of peptide hormones of both kallikrein-kinin and renin-angiotensin systems. Few studies have been devoted to analyzing whether converting enzyme activity for AI and AII is modulated in vivo and in none of these was bradykinin degradation measured simultaneously. As estimated by bioassay, changes in total body sodium altered systemic conversion of AI in dogs and chronic oral contraceptive treatment in rats increased AI activation as measured in perfused lung preparation. Plasma CE activity remains unchanged in women receiving oral contraceptives and in normal pregnancy, although other components of the renin-angiotensin system are enhanced. Plasma CE activity is increased in sarcoidosis and decreased in a variety of pulmonary parenchymal diseases. Plasma CE ac-
tivity also decreases during the development of one-kidney perinephritis hypertension in rabbits.\textsuperscript{11}

The development of a method that permits the simultaneous evaluation of AI conversion and bradykinin inactivation in conscious rats described in the first part of this study prompted us to analyze these two parameters in conscious rats with one-kidney hypertension and during the phase after removal of the clip when arterial pressure returns to near normal levels.

Methods

Male Wistar rats weighing 200–250 g were used. Direct pressure was measured in unanesthetized rats by means of a plastic cannula (PE-10 connected to PE-50) inserted into the abdominal aorta through the femoral artery under ether anesthesia at least 24 hours before the acute experiment. For intravenous injection, a plastic 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 cannulas emerged through the back of the rat and, for recording arterial pressure, the cannula from the femoral artery was attached to a Statham P23-D pressure transducer and Hewlett-Packard, Model 7858A multichannel recorder. During injections and pressure measurements, the rats were placed on a small platform (10 × 20 cm and 30 cm high), where they remained unrestrained and usually quiet after an initial period of exploratory movements. Angiotensin I, All, and bradykinin were synthesized by Dr. A.C.M. Paiva (Escola Paulista de Medicina, São Paulo). Stock solutions of the peptides (1 mg/ml) were stored at -20°C. 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 volumes of 0.1–0.2 ml or infused at a rate of 0.1–0.2 ml/minute.

Intact animals were compared to rats with one-kidney renal hypertension. In another group of hypertensive rats, when control analysis was completed, the clip was removed from the renal artery under ether anesthesia and the tests were repeated 20 hours later on the unanesthetized animals. One-kidney renal hypertension was produced by applying a silver clip to the main left renal artery under ether anesthesia according to the technique described by Schaffenburg\textsuperscript{12} with simultaneous right nephrectomy. Periodic blood pressure measurements were made afterward by the tail plethysmographic method, and the rats were used for the acute experiment 2–3 months after the operation when the pressure had been stable for 2–3 weeks.

Bradykinin Inactivation

Bradykinin injected intravenously or intraarterially into conscious rats produced drops in mean arterial pressure which were proportional to the doses injected, provided that the dose was not large. Intravenous injection of 1 µg of bradykinin generally produced a pressure fall of 15–25 mm Hg lasting 8–15 seconds. Progressively increasing doses up to 5 µg produced proportionally larger falls in pressure (40–50 mm Hg), with only a small increase in the total duration of the responses. When this dose was exceeded, the duration of the response became progressively longer but without affecting the absolute magnitude of the fall in pressure. Therefore, to evaluate the extent of bradykinin inactivation in the pulmonary circulation of unrestrained conscious rats, we determined the equipressor doses that produced a standard pressure drop of 20 mm Hg when injected either into the vein or into the ascending aorta. In a series of experiments, bradykinin inactivation measured by bolus injection and infusion of bradykinin was compared in both normotensive and one-kidney renal hypertensive rats (fig. 1). Vascular reactivity to the hormone was analyzed by intraaortic administration of increasing doses given as a single bolus or as infusions to produce sustained hypotensive responses. The responses to bradykinin were compared with those produced by sodium nitroprusside and isoproterenol in conscious normotensive and renal hypertensive rats.

Extent of Angiotensin I Conversion

The extent of AI conversion was calculated from the equipressor doses of AI and All injected intravenously to produce the standard 20 mm Hg increase in mean arterial pressure. In a group of rats, the conversion obtained by bolus injection was compared to that calculated from the equipressor doses of the substances infused to produce a 20 mm Hg increase in mean arterial pressure for 2–3 minutes.

Results are reported as means ± standard error of the means (SE). The statistical significance of differences between groups or within one group at different times was analyzed by unpaired and paired t tests of the mean arterial pressure values. Changes were considered to be significant at $p < 0.05$.

Results

Importance of the Lung for Angiotensin I Conversion

The importance of the pulmonary circulation in the conversion of AI in unanesthetized normotensive rats was demonstrable when low doses of AI were injected. As reported for anesthetized rats,\textsuperscript{13}–\textsuperscript{16} we observed no significant difference in the pressor response of conscious rats when AI was given intravenously or intraarterially, provided the dose was high (30–50 ng) and produced a rise in pressure of 40–50 mm Hg. However, when small doses (1–10 ng) of AI were injected, the pressor responses obtained by intravenous injection were greater than the intraaortic ones (table 1). The pressor response to large doses of AI and All was investigated in a separate group of seven normotensive rats. With doses of 30–50 ng that produced pressor increases of between 40 and 50 mm Hg, there was no difference between the pressor responses to AI and All. However, the time-to-maximum-pressure response to AI was 6.1 ± 0.5 seconds for intravenous
Figure 1. Comparison of infusion and bolus injection methods. Left Panel: The extent (%) of bradykinin inactivation was measured from the doses infused or injected intravenously and intraarterially that produced a 20 mm Hg fall in mean arterial pressure. Normotensive control rats (NCR, 115 ± 3 mm Hg) and renal hypertensive rats (RHR, 210 ± 13 mm Hg). Right Panel: The extent (%) of conversion of angiotensin I (AI) to angiotensin II (AII) was calculated from the equipressor doses of AI and AII injected or infused intravenously that produced a standard 20 mm Hg rise in mean arterial pressure (NCR, 122 ± 3 mm Hg, and RHR, 181 ± 9 mm Hg).

Bradykinin Inactivation and Vascular Reactivity

The extent of bradykinin inactivation by the pulmonary circulation of the one-kidney renal hypertensive rat was much higher than that observed in normotensive animals (table 3). The difference was similar when bradykinin was infused intravenously rather than given by bolus injection (fig. 1). While 2.5% of the bradykinin injected intravenously into control rats reached the systemic circulation, only 0.4% of the injected hormone had a hypotensive effect on the hypertensive rats. Despite the fact that bradykinin degradation by the lung was higher in hypertensive rats, only one-fifth the dose of bradykinin injected intravenously was needed to produce the same hypotensive response in these animals as in normotensive rats. Thus, because they exhibited a marked vascular hyperreactivity to bradykinin, 34 times less hormone than needed in the normotensive rats was injected intraaortically to produce the same 20 mm Hg drop in pressure (table 3). The hyperreactivity to intraarterial injection of bradykinin was further analyzed in another series of experiments in which reactivity to other hypotensive agents was compared. Although the equidepressor dose of bradykinin was approximately 20 times lower in one-kidney hypertensive rats, for isoproterenol and sodium nitroprusside the doses were only half that used in normotensive rats (fig. 2). Hyperreactivity to bradykinin can also be observed when increasing doses of the hormone are infused intraaortically rather than injected.

<table>
<thead>
<tr>
<th>Dose (ng)</th>
<th>Responses (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>i.v.</td>
</tr>
<tr>
<td>1</td>
<td>12 ±0.9</td>
</tr>
<tr>
<td>5</td>
<td>20 ±0.9</td>
</tr>
<tr>
<td>10</td>
<td>32 ± 4.0</td>
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</tbody>
</table>

*Statistically significant difference, p < 0.05.

Table 2. Differences in the Time of Onset of Pressure Rise after Intravenous (i.v.) and Intraarterial (i.a.) Injection of Large Doses of Norepinephrine, Angiotensin II, and Angiotensin I (mean ± SE of Seven Rats)

<table>
<thead>
<tr>
<th>Dose</th>
<th>Onset of pressure rise (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>i.v.</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>2.9 ± 0.3</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>Angiotensin I</td>
<td>2.9 ± 0.3</td>
</tr>
</tbody>
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*Statistically significant difference, p < 0.05.

Table 3. Extent of Pulmonary Inactivation of Bradykinin Calculated from the Doses that Produce a Standard 20 mm Hg Drop in Arterial Pressure when Injected Intravenously (i.v.) or Intraarterially (i.a.) into Conscious Normotensive Control (NCR) and Conscious Renal Hypertensive (RHR) Rats

<table>
<thead>
<tr>
<th>Rats</th>
<th>Arterial pressure (mm Hg)</th>
<th>Bradykinin (ng)</th>
<th>Inactivation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCR (21)</td>
<td>126 ± 3</td>
<td>1096 ± 143</td>
<td>27 ± 5</td>
</tr>
<tr>
<td>RHR (21)</td>
<td>217 ± 3*</td>
<td>200 ± 24*</td>
<td>0.78 ± 0.3*</td>
</tr>
</tbody>
</table>

*Statistically significant difference, p < 0.05.
as a single bolus. The average dose of infused bradykinin per minute needed to produce a sustained 20 mm Hg drop in pressure was 18 times lower (83 ± 14 vs 1495 ± 272 ng/min) in the 10 hypertensive rats (198 ± 17 mm Hg) than in the eight controls (121 ± 3 mm Hg), while for sodium nitroprusside the dose was almost the same (3705 ± 589 vs 3600 ± 525 ng/min). In four renal hypertensive rats, indomethacin (5 mg/kg/min for 10 minutes) produced no changes in the enhanced responsiveness to intraaortic infusion of bradykinin.

Angiotensin I Conversion and Sensitivity to Angiotensin II

The extent of in vivo conversion of AI to All was estimated in the rat by comparing the hormonal doses that produce the standard 20 mm Hg rise in mean arterial pressure. As shown in table 4, conversion was significantly higher in conscious hypertensive rats than in normotensive controls (55% vs 25%). The extent of AI conversion calculated from infusion and bolus injection was similar (fig. 1). While vascular reactivity to All was similar in both groups (table 4), the hypertensive rats needed almost twice the dose of normotensive controls to produce the same pressor response as the normotensive ones, 56 ± 11 ng and 30 ± 5 ng, respectively.

Effect of Reversal of One-Kidney Hypertension

Seven rats with one-kidney renal hypertension (2–3 months) were studied during the control period (208 ± 5 mm Hg of mean arterial pressure) and 20 hours after removal of the clip from the left renal artery when blood pressure had returned to normal levels (126 ± 2 mm Hg). The equipressor dose of bradykinin injected intraaortically to produce the standard 20 mm Hg drop in pressure increased from 4 ± 2 to 23 ± 9 ng after unclipping, while the intravenous dose increased from 254 ± 72 to 617 ± 65 ng, at the same time. Consequently, there was not only a drastic reduction in hyperreactivity to bradykinin but also a significant decrease in pulmonary degradation of the hormone, from 98.7 ± 0.3% to 96.8 ± 0.9%.

Figure 3 shows the marked alteration seen 20 hours after clip removal in vascular reactivity to increasing doses of bradykinin infused intraarterially. In the control period, approximately 200 ng/minute of bradykinin produced the maximum depressor response that could be tolerated by the conscious one-kidney hypertensive rat. However, after pressure normalization doses as high as 800 ng/minute produced hypotensive responses that were very similar to those seen in the normotensive control rats (20 ± 1 mm Hg and 19 ± 1 mm Hg, respectively). In contrast with the marked reduction in sensitivity to bradykinin, the hypotensive responses to sodium nitroprusside after unclipping tended to be higher. However, differences were not statistically significant.

The influence of reversal of one-kidney hypertension on AI conversion was analyzed in another series of experiments (table 5). Unclipping produced...
no changes in the rate of AI activation, which was evaluated during the control period, nor in the vascular reactivity to All (the equipressor doses were not significantly different).

Discussion

Intravenous injection of 1-10 ng of AI into conscious rats (200-250 g) produced pressor responses almost twice as large as that obtained with the same dose injected intraaortically. This finding reinforces the concept that the lung is the major site of physiological activation of the hormone. As already observed in anesthetized rats, the same pressure responses were produced in conscious rats by intravenous or intraarterial injection of AI provided the doses were high (> 30 ng), producing a pressor rise of 40-50 mm Hg lasting several minutes. Before ruling out the possibility of the lung being the predominant site of conversion for large doses of AI in the unanesthetized rat, several physiological aspects of the circulation must be considered, especially the velocity of the circulation in this species. The onset of pressure rise following intravenous injection of norepinephrine and All occurred after 3 seconds, while only 1 second elapsed when the substances were injected intraaortically. Therefore, the circulation time between sites of i.v. and i.a. injection is 2 seconds. Since total circulation time in a conscious rat (250 g) should take no longer than 6-7 seconds, as calculated from previous studies where we found cardiac output to be more than 100 ml/min and blood volume to be 10-12 ml, the estimated transit time from the i.a. to the i.v. site is 4-5 seconds. These considerations are relevant to understand why intravenous and intraaortic injection of high doses of AI produce a pressor response which is independent of the site of injection. The only difference is that i.a. injection takes 3.7 seconds more than i.v. injection to reach maximum pressor effect, as seen in the present study.

A recent study has emphasized the importance of the extrapulmonary conversion of AI to All and suggested that the extrapulmonary CE could be more resistant to the inhibitory effect produced by a long-lasting CE inhibitor. Since the current study involved only small doses of AI, it is likely that the effects observed were due mostly to pulmonary CE. But, the observation that the delay for the onset of pressure rise was not significantly increased in conscious rats when angiotensin I was injected intraaortically rather than intravenously supports the idea that part of the AI is converted to All at or near the arteriole receptor sites, as indicated by other studies.

Our results illustrate the advantage of using conscious rats having a high sensitivity to the pressor effect of All; low doses of AI can be used in studies comparing pulmonary conversion of AI and inactivation of bradykinin. Since bradykinin becomes almost 97% biologically inactive in a single passage through the lungs, a very small dose of CE inhibitor will allow an additional 3% of bradykinin to reach the systemic circulation, but this will double the level of arterial bradykinin. This effect on bradykinin inactivation can be compared to changes in AI conversion only if the blood pressure response can detect small but significant changes in the extent of AI conversion.

The data reported here show for the first time a marked enhancement of the hypotensive response to bradykinin and pulmonary inactivation of bradykinin in hypertensive rats. The 15- to 30-fold increase in response to intraarterially injected bradykinin and 5-fold increase in response to intravenously injected
bradykinin in the one-kidney hypertensive rat, which was independent of indomethacin. It appears to be specific and related to the hypertensive state. The return of the sensitivity to bradykinin to the normal range after unclipping is significant and dramatic. In contrast, reactivity to isoproterenol and nitroprusside increased only twofold in the hypertensive state but then remained unaltered after unclipping. The increase in pulmonary inactivation of bradykinin demonstrable in hypertensive animals also seems to be specific and related to the hypertensive state for it too returned to near normal levels after removal of the clip. It should be noted that the increase in vascular reactivity to bradykinin more than compensated for the increased pulmonary inactivation, i.e., smaller doses of intravenous bradykinin are needed to produce a standard hypotensive response in hypertensive than in normotensive rats. The behavior of AI and All in this model differed significantly from that of bradykinin. Vascular reactivity to All (injected intravenously) was similar in both groups (but hypertensive rats needed almost twice the dose of norepinephrine). Conversion of AI was significantly higher (55%) in hypertensive rats than in normotensive controls (25%), but unclipping produced no change in AI activation nor in the vascular reactivity to All.

One-kidney hypertensive rats seem to be renin-dependent only for approximately one week and, contrary to the two-kidney rats, do not respond to converting enzyme blockade after hypertension is established. Therefore, there is not an obvious connection between alterations in the activities of the renin-angiotensin system and the increased AI conversion seen in the chronically hypertensive rats of the present study. Moreover, in view of our findings, it is a little surprising that the one-kidney hypertensive rats that exhibit such a marked hyperreactivity to bradykinin have not been reported to respond to CE inhibitor administration with a pressure decrease, since significant increases in plasma bradykinin have been observed after acute administration of CE inhibitor to hypertensive patients. It must be emphasized that the vascular hyperreactivity to bradykinin more than compensated for the higher degradation of the hormone in the lung, explaining why the hypertensive rats had enhanced hypotensive responses even when bradykinin was given intravenously. Comparison between the one-kidney rats of the present study, which showed hyperreactivity to bradykinin, and those of the previous study, which showed no response to CE inhibitor, might not be entirely valid since the level of hypertension was more severe in the rats used here.

It is interesting to note that a previous hemodynamic study using unanesthetized one-kidney hypertensive rats suggested that during the first hour after removal of renal artery constriction a vasodilator mechanism probably occurred which contributed to the process of pressure normalization. Since the kidney is an important source of the kallikrein-kinin system and since the one-kidney hypertensive rats of the present study showed such a remarkable vascular reactivity to bradykinin, this hormone might be considered a likely candidate for the humorally-mediated vasodilation occurring after unclipping. Other anti-hypertensive factors from the kidney, such as the neutral renomedullary lipid, could also mediate the effects. However, prostaglandins do not seem to be a major participant in the reversal of one-kidney hypertension in the rabbit.

The results show that both the sensitivity to and metabolism of bradykinin as well as the conversion of AI are modified in this model of renal hypertension. Although the angiotensin CE represents a crossover point in the metabolic pathways for both the renin-angiotensin and kinin-kallikrein systems, we cannot provide an explanation for these observations which is based simply on the occupancy of CE by circulating AI. Although this would account for the increased sensitivity to bradykinin by decreasing its inactivation it is not possible to explain the increased pulmonary inactivation of bradykinin and higher AI conversion by occupancy of CE. Other bradykininases in addition to CE have been demonstrated in the perfused lung and they might be involved in the observed change in bradykinin degradation. Moreover, unclipping of the renal artery produced no change in the extent of AI conversion which was elevated in the renal hypertensive rats, while bradykinin degradation decreased. This finding reinforces the idea that more than one mechanism is responsible for the alterations seen in these two systems.

Several crucial questions are left unanswered. We do not know if increased vascular sensitivity to bradykinin is due to a reduction in the enzymatic hydrolytic inactivation of the hormone, changes in sensitivity or number of bradykinin receptors, and/or other mechanisms related to indirect actions of bradykinin, such as catecholamine release. Furthermore, although the lung appears to be the main site of bradykinin inactivation, the quantitative measures of the pulmonary and extrapulmonary conversion of AI to All in normal and hypertensive models are not available. Finally, although it is shown that both bradykinin inactivation and AI conversion can be mediated by CE other enzymes in the pulmonary vascular bed can inactivate bradykinin but do not convert AI to All. Quantitative differences remain unknown and there is the possibility that these alternative kininases may also exist in extrapulmonary vascular beds.

The experimental approach used in this paper demonstrated that significant changes can occur in renin-angiotensin and kallikrein-kinin dynamics in intact, conscious animals; artifacts introduced by anesthesia and surgical intervention were minimized. However, further studies are needed to identify the individual components or systems responsible for these observations.

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