Reversal of Hyperreactivity to Bradykinin in Renal Hypertensive Rats

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Increased blood pressure responsiveness to bradykinin in comparison with other vasodilator agents was demonstrated in rats with long-term one-kidney and two-kidney, one clip hypertension. In the present study, we analyzed the reactivity to intra-aortically injected bradykinin in unanesthetized one-kidney, one clip hypertensive rats during the control period and 1, 5, and 8 hours after reversal of hypertension after removal of the renal artery constriction. One and 5 hours after unclipping the renal artery, the mean blood pressure decreased markedly (from 195±7 to 124±8 and 145±9 mm Hg, respectively), whereas the hyperreactivity to bradykinin reverted only slightly, and the responses to nitroprusside remained unchanged. In another group of hypertensive rats examined 8 hours after unclipping (pressure decreased from 192±4 to 143±8 mm Hg), the hyperreactivity to bradykinin had partially reverted. Significantly larger doses of bradykinin were necessary to produce the same decrease in blood pressure when compared with the control period (16.4±2.0 vs. 7.2±1.2 ng). The same degree of reversal of hyperreactivity to bradykinin was observed when the blood pressure of hypertensive rats was reduced (from 207±8 to 143±5 mm Hg) during 1 hour by hydralazine injection. Complete reversibility of bradykinin hyperreactivity was produced by nitroprusside infusion (from 201±13 to 142±10 mm Hg). Pronounced enhancement of the blood pressure reactivity to bradykinin was observed in normotensive rats submitted to acute hypertension (15 minutes) produced by phenylephrine infusion (from 131±2 to 193±3 mm Hg) and a fivefold decrease in bradykinin reactivity was produced after 1 hour of hypotension (from 128±4 to 91±3 mm Hg) by hydralazine infusion. The data suggest that blood pressure responsiveness to bradykinin is markedly affected by the level of pressure, probably because of the degree of vasoconstriction and vasodilation existing in the rats. (Hypertension 1990;15(suppl I):I-140–I-143)

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inins are potent vasodilator peptides that have been implicated in the regulation of local blood flow and water electrolyte excretion.1 Because of these effects, kinins might be involved in blood pressure regulation, and defects in the kallikrein-kinin system might contribute to the development of various forms of hypertension.

Increased blood pressure reactivity to bradykinin (BK) was observed in long-term renal hypertensive rats.2–3 In these studies, hyperreactivity was not observed for vasodilators other than BK. Salgado et al4 found that one-kidney, one clip (1K1C) long-term hypertensive rats exhibited a greater decrease in mean blood pressure than did normotensive rats for any given concentration of blood kinin achieved after BK infusion. The sensitivity to BK returned to the normal range 24 hours after unclipping the renal artery when blood pressure was completely normalized, whereas the responses to other vasodilators remained unaltered.2

To test whether BK response is affected by changes in blood pressure, in the present study, we analyzed the blood pressure responsiveness to intra-arterially injected BK in unanesthetized 1K1C hypertensive rats during the early phase (1–8 hours) of the reversal of hypertension after removal of the renal artery constriction. In addition, we compared the effect of unclipping the renal artery to the effect of vasodilator infusion (hydralazine and sodium nitroprusside) that produced a similar reduction in mean blood pressure of 1K1C hypertensive rats.

Methods

Experiments were performed with male Wistar rats (250–300 g). All surgical procedures were performed on ether-anesthetized rats. To induce 1K1C hypertension, a silver clip (0.25 mm i.d.) was placed around the left renal artery, and right nephrectomy was performed simultaneously. Periodic blood pressure measurements were taken afterward by
the tail plethysmographic method, and only the rats with blood pressure higher than 170 mm Hg were used 8 weeks after surgery.

The day before the experiment, polyethylene catheters (Clay-Adams, Persippany, New Jersey) were inserted into the femoral artery for direct blood pressure measurements, and into the femoral vein and the left carotid for drug injection or infusion. Blood pressure reactivity to BK (synthesized by Dr. A.C.M. Paiva, Escola Paulista de Medicina, São Paulo, Brazil) was analyzed by intra-aortic administration of increasing doses of BK injected as a single bolus (0.1–0.2 ml). The dose-response curves to BK were compared with those produced by sodium nitroprusside (Sigma Chemical Co., St. Louis, Missouri) injected intravenously into 1K1C hypertensive and normotensive age-matched control conscious rats. Changes in blood pressure responsiveness to BK and nitroprusside in the different groups or within one group at different times were analyzed by comparing the doses that produced a 20 mm Hg decrease in mean blood pressure.

**Effect of Unclipping the Renal Artery of One-Kidney, One Clip Rats**

After the control analysis, the clip was removed from the renal artery under ether anesthesia, and the dose-response curves to BK and nitroprusside administration were repeated 1 and 5 hours (n=7) or 8 hours (n=8) later. The same protocol was used in 1K1C (n=14) and normotensive age-matched control (n=17) rats that were subjected to sham surgery (laparotomy under ether anesthesia).

**Effect of Hydralazine and Nitroprusside on One-Kidney, One Clip Rats**

In another group of 1K1C and normotensive age-matched control rats, blood pressure reactivity to BK and nitroprusside administration was investigated during the control period and 1 hour after reversal of hypertension by means of hydralazine injection (20 μg i.v.) into 1K1C (n=8) and normotensive age-matched control (n=6) rats or nitroprusside infusion (3 μg/min, at 0.1 ml/min) into 1K1C rats (n=6).

**Effect of Phenylephrine Infusion on Normal Rats**

Blood pressure reactivity to BK administration was also examined in normotensive age-matched control rats (n=5) during the control period and 15 minutes after increasing mean blood pressure with phenylephrine (Sigma Chemical Co.) infusion (3 μg/min i.v., at 0.1 ml/min).

**Statistics**

Data are expressed as the mean±1 SEM. The changes in mean blood pressure with different doses of BK or nitroprusside were determined by analysis of variance for repeated measures. The differences between groups were evaluated using two- or three-way analysis of variance for repeated measures or

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**FIGURE 1.** Scatterplots showing effect of intra-arterially injected bradykinin and intravenous nitroprusside on mean blood pressure (MBP) of normotensive control (NC) and one-kidney, one clip hypertensive (1K1C) conscious rats. Panel A: Control period. Responses to different doses of bradykinin in 1K1C groups were significantly different from NC group (p<0.001). There was no difference in dose-response curves to nitroprusside. Panel B: Eight hours after unclipping renal artery or sham surgery. Curves differed significantly (p<0.001). *Indicate significant difference (p<0.05) compared with NC group, and ◆ indicate significant difference (p<0.05) compared with 1K1C-Sham-operated rats.

Student's t test as appropriate. Differences were considered significant if the p value was less than 0.05.

**Results**

Rats with long-term 1K1C hypertension (mean blood pressure, 192±4 mm Hg) showed increased blood pressure reactivity to intra-arterially injected BK when compared with the normotensive age-matched control rats, as shown in Figure 1A. The dose needed to produce a 20 mm Hg decrease in pressure in the normotensive age-matched control group was 48.1±8.3 ng, whereas in the 1K1C rats, the dose was significantly smaller (7.2±1.6 ng). No significant difference in the response to nitroprusside administration was observed between normotensive age-matched control and 1K1C rats.

Unclipping the renal artery produced a rapid reversal of hypertension. In the group analyzed 1 and 5 hours after unclipping, mean blood pressure decreased from 195±7 to 124±8 and 145±9 mm Hg, respectively. Sham surgery in the group of 1K1C and normotensive age-matched control rats produced no significant changes in mean blood pressure (from 196±6 to 196±9 and 201±7 mm Hg and from 116±2 to 121±3 and 126±4 mm Hg, in the 1K1C and normotensive age-matched control rats, during control phase and 1 and 5 hours after surgery, respectively). At 5 hours after unclipping, the
hyperresponsiveness to BK administration reverted only slightly (the dose needed to produce a decrease of 20 mm Hg increased from 7.6±0.5 to 11.2±2.5 ng) and the responses to nitroprusside administration remained the same. In another group of rats, blood pressure reactivity to BK and nitroprusside administration was examined before and 8 hours after unclipping when a decrease in mean blood pressure from 192±4 to 143±8 mm Hg occurred. As shown in Figure 1B, the hyperreactivity to BK administration had partially reverted 8 hours after unclipping. Significantly larger doses of BK were necessary to produce a 20 mm Hg decrease in pressure (from 7.2±1.6 to 16.4±2.0 ng). The responses to nitroprusside administration actually increased after normalization of blood pressure. The same degree of reversal of the reactivity to BK administration was observed only 1 hour after maintained normalization of the blood pressure of 1K1C rats by hydralazine injection (from 206±8 to 143±5 mm Hg), as shown in Figure 2A. When mean blood pressure was normalized by nitroprusside infusion during 1 hour (from 201±13 to 142±10 mm Hg), reversal of BK hyperresponsiveness was complete (from 10.4±2.1 to 31.3±3.2 ng BK to produce a 20 mm Hg decrease in mean blood pressure).

A decrease in mean blood pressure in normotensive age-matched control rats similar to that in 1K1C rats (30%) after 1 hour of hydralazine administration (from 128±4 to 91±3 mm Hg) produced a fivefold decrease in BK reactivity (the dose needed to produce a 20 mm Hg decrease in mean blood pressure increased from 38.3±10.7 to 210.5±43.2 ng) as shown in Figure 2B. An increase in mean blood pressure in normotensive age-matched control rats during 15 minutes of phenylephrine infusion (from 131±2 to 193±3 mm Hg) caused a hyperreactivity to BK administration (Figure 2C) similar to that observed in 1K1C rats (the dose needed to produce a 20 mm Hg decrease in mean blood pressure decreased from 32.4±5.8 to 12.9±2.4 ng).

Discussion

The present results confirmed previous findings from our laboratory showing that intra-arterially injected BK is potentiated in long-term renal hypertensive rats. We also found that acute hypertension produced by phenylephrine infusion in normotensive rats similarly increased blood pressure sensitivity to BK administration. Unclipping or vasodilator administration partially reversed hyperreactivity to BK administration in 1K1C hypertensive rats. The most salient feature of our findings was that, 8 hours after unclipping, a significant reversal in BK hyperreactivity was achieved, whereas the reactivity had remained elevated after 1 and 5 hours. This was unexpected because after only 1 hour of hydralazine administration, an alteration in reactivity similar to that observed 8 hours after unclipping was demonstrated. Indeed, the dose needed to produce a standard 20 mm Hg decrease in pressure was 63% and 68% of that required in normal rats after 8 hours of unclipping and after 1 hour of hydralazine administration, respectively. When reversal of hypertension was produced by nitroprusside infusion, reversibility of BK hyperreactivity was complete (100%) within only 1 hour. On the other hand, when mean blood pressure was raised to hypertensive levels by phenylephrine infusion during 15 minutes in normal rats, an increase in blood pressure responsiveness to BK administration similar to that exhibited by long-term 1K1C hypertensive rats during the control period was observed. Also, when mean blood pressure was decreased by means of hydralazine injection in normal rats, a fivefold decrease in reactivity to BK administration was demonstrated.

These data, taken together, suggest that blood pressure responsiveness to BK but not to nitroprusside administration, which was used as control, can be rapidly altered by vasoconstriction or vasodilation. Therefore, the enhanced reactivity of long-
term 1K1C hypertensive rats to BK administration can be partially explained as a consequence of the increased peripheral resistance documented in this model of hypertension. The unexpected result, that blood pressure reactivity to BK administration was not significantly decreased 1 and 5 hours after removing the renal artery constriction although hypertension had been reverted, could be explained by our previous hemodynamic studies in conscious 1K1C hypertensive rats showing that the decrease in mean blood pressure was caused by a decrease in cardiac output with peripheral resistance remaining elevated.

The renomedullary system has been demonstrated to be important for reversal of hypertension after unclipping the renal artery. There is no indication, however, that this system influenced the reversal of the enhanced response to BK administration because when the vasodilators are used to normalize pressure a similar or even larger effect is observed in BK reactivity. On the other hand, changes in the physical state of the cell membrane that occur during vasoconstriction and vasodilation alter the vascular responses to vasopressin in the cerebral circulation of the newborn pig.

Kinins are destroyed very rapidly by a group of enzymes known as kininases found in blood, endothelial cells, and other tissues. Thus, potentiation of BK administration would be expected when the rate of inactivation is diminished. In vivo experiments using the blood pressure assay or measurements of arterial blood kinin concentration during intravenous infusion of different doses of BK have indicated that kinins inactivation is increased in 1K1C hypertensive rats, suggesting that this mechanism of BK potentiation is not involved. Also, kinin effects can be mediated by other hormonal systems such as prostaglandins, endothelium-derived relaxing factors, or both. The hyperreactivity of 1K1C hypertensive rats to BK administration was not affected by indomethacin treatment, suggesting that prostaglandin release was not an important factor for BK potentiation. Many studies have demonstrated that vascular responsiveness to various stimuli is altered in hypertension. The endothelium releases both dilator and constrictor factors in response to various stimuli, and several recent in vitro studies have demonstrated that these endothelial functions are altered in hypertension. Although the vasodilator effect of BK is known to depend on the integrity of the endothelium, there is no report on in vivo alterations in BK reactivity mediated by endothelial cells in hypertension. Because, however, the responses to nitroprusside, which acts by an endothelium-independent mechanism, were not affected by changes in blood pressure, the increase in reactivity to BK administration in hypertensive rats might be due to alterations of the endothelial function. Thus, more information is needed on the in vivo effects of endothelium-dependent and endothelium-independent vasodilators in hypertension.

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References

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