Facilitation of Noradrenergic Transmission by Angiotensin in Hypertensive Rats

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We examined the angiotensin-induced potentiation of noradrenergic transmission in the isolated mesenteric arteries of one-kidney, one clip (1K1C) hypertensive rats. The angiotensin converting enzyme activity measured in plasma did not change during the development of hypertension, whereas the activity measured in the aortic tissue was significantly augmented 28 days after the renal artery was clipped. Although the pressor responses to nerve stimulation were basically unaltered, a significant increase in the sensitivity to norepinephrine developed during hypertension. The 1K1C preparations presented an increased sensitivity to the facilitatory effect of angiotensin II on the response to periarterial nerve stimulation. The facilitatory effect of angiotensin II on both nerve stimulation and exogenous norepinephrine was blocked by saralasin. Angiotensin I induced similar facilitatory action on noradrenergic transmission that was inhibited by saralasin. When a high concentration of angiotensin I was used, the facilitatory effect was significantly higher in mesenteric arteries from 1K1C rats than in controls. Captopril reduced the facilitatory effect of angiotensin I in 1K1C preparations, whereas the responses of the normotensive control rats were unaffected by captopril. These findings are consistent with angiotensin I acting directly on angiotensin II receptors or with angiotensin I being converted to angiotensin II by an alternative pathway not involving angiotensin converting enzyme. (Hypertension 1992;19[suppl II]:II-30-II-35)

Angiotensin II (Ang II) influences vascular tone by several mechanisms, including a direct vasoconstrictor action and an ability to enhance noradrenergic neuroeffector function. Several mechanisms have been suggested to explain the effect of Ang II on the sympathetic neurotransmission, including an increase in the rate of norepinephrine synthesis, an increase in neuronal release of norepinephrine in response to nerve stimulation, inhibition of neuronal reuptake, and increased vascular smooth muscle reactivity to norepinephrine.

There is substantial evidence that components of the renin-angiotensin system are present in extrarenal tissues, including blood vessels. It is possible that locally generated Ang II modulates the vascular sympathetic neurotransmission. One-kidney, one clip (1K1C) hypertension is considered to be independent of the circulating renin-angiotensin system; however, we have found that the in vivo extent of angiotensin I (Ang I) conversion was augmented in 1K1C hypertensive rats. The angiotensin converting enzyme (ACE) activity measured in plasma and lung homogenate of 1K1C hypertensive rats was not different from that of normotensive rats up to 12 weeks after the renal artery was clipped, whereas the aortic tissue ACE activity was found to be elevated 2-12 weeks after clipping when compared with that of normotensive age-matched rats. Therefore, in view of an increased ACE activity in vascular tissue, an enhancement in local formation of Ang II might occur in the 1K1C model of hypertension, which would result in vasoconstriction by a direct action and indirectly through noradrenergic nerve on vascular smooth muscle. The hypothesis that increased vascular formation of Ang II would induce a greater facilitatory effect on sympathetic neurotransmission was tested by investigating the effect of Ang II and Ang I, the natural substrate of ACE, on the sympathetic neurotransmission in the isolated mesenteric arteries of 1K1C hypertensive rats. The perfused in vitro preparation was used to avoid the effect of circulating components of the renin-angiotensin system and the systemic actions of renin-angiotensin system inhibitors.

Methods

To induce 1K1C hypertension, a silver clip (internal gap, 0.25 mm) was placed around the left renal
artery and right nephrectomy was performed in male Wistar rats (140–180 g). Right nephrectomized (1K) rats were used as controls. The 1K1C and 1K rats were used 1, 7, and 28 days after surgery. A control group for each period was used, because we learned that tissue ACE activity (mesentery and lungs) can increase during the normal development of the intact rat (unpublished results from our laboratory).

The day before the experiments, the rats were anesthetized with ether, and a polyethylene catheter (PE-50, Clay Adams, Parsippany, N.J.) was inserted into the ascending aorta via the left carotid artery for direct blood pressure measurements. On the day of the experiments, blood pressure of unanesthetized rats was recorded with an HP 7754 A recorder via an HP 1280 C pressure transducer (Hewlett-Packard Co., Palo Alto, Calif.).

In one group of 1K1C rats 1, 7, and 28 days after clipping and in 1K age-matched controls, blood was collected by heart puncture into a heparinized syringe, with rats under ether anesthesia. Then, after exsanguination, the whole aorta was immediately removed and cleaned of adhering connective tissue. The aorta was minced and homogenized in 10 vol of 0.05 M sodium borate buffer (pH 7.4) containing 0.32 M sucrose. ACE activity of plasma and aorta homogenate was determined by a fluorimetric method, using Hip-His-Leu as substrate. Protein concentration in aorta homogenates was determined by the method of Lowry et al., using bovine serum albumin for the standard curve.

The mesenteric vascular bed was removed as described by McGregor from another group of 1K1C rats 1, 7, and 28 days after clipping and from 1K age-matched controls, with rats under ether anesthesia. The isolated mesenteric vascular bed was placed in a 10-ml water-jacketed organ bath maintained at 37°C and perfused with a modified Krebs' solution at a constant flow rate of 4 ml/min. The perfusion solution (millimolar concentration: NaCl 120.0, CaCl2 3.0, KCl 4.7, MgCl2 1.43, NaHCO3 25.0, KH2PO4 1.17, glucose 11.0, and EDTA 0.03), pH 7.4, was aerated with a mixture of 95% O2-5% CO2 and maintained at 37°C. Perfusion pressure was continuously monitored with a pressure transducer connected to a side arm of the mesenteric artery perfusion cannula. After a 30-minute period of stabilization, the perfused mesenteric arteries were subjected to either periarterial nerve stimulation (PNS) or injection of exogenous norepinephrine. The PNS was delivered at 5-minute intervals via bipolar stainless steel ring electrodes placed around the superior mesenteric artery. Rectangular pulses of 5-msec duration and supramaximal voltage (34 V) were applied for 20 seconds at variable frequencies (7–30 Hz). Norepinephrine (64–3,200 ng) was injected as a bolus directly into the perfusion solution proximal to the arterial cannula in a volume of 30–60 μl at 5-minute intervals. Injection of these volumes of Krebs' solution alone resulted in no change in perfusion pressure.

The effect of Ang II or Ang I on the pressor responses to PNS and norepinephrine was examined in perfused mesenteric arteries of 1K1C rats 28 days after clipping and from 1K age-matched controls. The PNS and norepinephrine concentrations used in this protocol were such as to produce a pressor response of 20–30 mm Hg during a control period in the absence of angiotensins. Ang II or Ang I was added to the perfusion solution after the control responses to PNS and norepinephrine at the following subpressor concentrations: Ang II: 1.0, 5.0, and 10.0 ng/ml; Ang I: 1.0, 5.0, 10.0, 25.0, and 50.0 ng/ml. These peptide concentrations produced no change in basal perfusion pressure throughout the experimental period. To test the effect of an Ang II receptor antagonist on the potentiating effect of Ang II and Ang I on PNS or norepinephrine responses, saralasin was added to the perfusion solution at a final concentration of 50.0 ng/ml. The effect of ACE inhibition on the potentiating effect of Ang I was obtained by adding captopril (E.R. Squibb & Sons, Inc., Princeton, NJ) to the perfusion solution (1.0 μg/ml). The saralasin concentration used completely blocked the pressor response (35–45 mm Hg) elicited by 115 pmol Ang I or 10 pmol Ang II; the captopril concentration used produced the maximal reduction (80%) of the pressor response (35–45 mm Hg) elicited by 115 pmol Ang I in mesenteric arteries (data not shown).

The effect of cocaine (Sigma Chemical Co., St. Louis, Mo.) on the potentiator effect of Ang II (10 ng/ml) after PNS and norepinephrine was evaluated in perfused mesenteric arteries from 1K rats. The responses to PNS and norepinephrine were determined during a control period, in the presence of cocaine (120 μg/ml), and in the presence of cocaine plus Ang II.

All peptides (Ang II, Ang I, saralasin, Hip-His-Leu, and His-Leu) were synthesized by Dr. A.C.M. Paiva (Escola Paulista de Medicina, São Paulo, Brazil), and concentrations were determined by amino acid analysis. Analysis of Ang I samples by high-performance liquid chromatography revealed no contamination by Ang II.

Results are expressed as mean±SEM. Statistical analysis was performed by analysis of variance for repeated measurements, one-way analysis of variance, and paired and unpaired t test, as appropriate. Differences between potentiation ratios were analyzed by the Wilcoxon rank-sum test followed by the Mann-Whitney test.

Mean blood pressure values of the 1K and 1K1C rats studied 1, 7, and 28 days after surgery in all protocols were 104±3 versus 115±2 mm Hg, 109±2 versus 127±4 mm Hg, and 112±2 versus 174±2 mm Hg, respectively. The blood pressure of 1K1C rats was significantly (p<0.01) greater than that of 1K rats at all periods studied. A small but significant decrease in body weight was observed in 1K1C rats 28 days after clipping (280±3 versus 293±3 g in 1K).
FIGURE 1. Line graphs show vasoconstrictor responses to periarterial nerve stimulation (7–30 Hz) (left panels) and to exogenous norepinephrine (64–3,200 ng) (right panels) in mesenteric arteries from one-kidney (1K) and one-kidney, one clip (1K1C) rats 1, 7, and 28 days after renal artery was clipped. *p<0.05, **p<0.01 by analysis of variance for repeated measurements.

No difference in body weight was detected between 1K1C and 1K rats the other times.

No change in ACE activity was observed in plasma of 1K1C rats during the development of hypertension (73.6±7.1, n=12; 88.8±14.2, n=10; and 94.9±14.7, n=8, nmol His-Leu/min/ml, at 1, 7, and 28 days after clipping, respectively), and activity was not different from that found in 1K rats (59.4±8.4, n=13; 83.8±12.7, n=12; and 78.5±8.9, n=10, nmol His-Leu/min/ml, at 1, 7, and 28 days, respectively). However, the aortic ACE activity in the 1K1C group (n=9) was significantly (p<0.01) greater than that found in 1K rats (n=9) 28 days after clipping (67.5±4.9 versus 45.4±3.1 nmol His-Leu/min/mg of protein). At 1 and 7 days after clipping, no difference was found between aortic ACE activity of 1K1C (n=7) and 1K (n=8) rats (22.8±5.3 versus 27.1±3.1 and 40.5±4.5 versus 38.7±2.3 nmol His-Leu/min/mg of protein, respectively), although within each group, a significant (p<0.01) increase in aortic ACE activity from day 1 to day 7 was observed, and no significant changes from day 7 to day 28 in the 1K group.

As shown in Figure 1, no difference between the pressor responses to PNS of 1K1C (n=9) and 1K (n=11) isolated mesenteric arteries was found at all periods studied, whereas the pressor responses to norepinephrine were significantly augmented in preparations from 1K1C (n=7) compared with those from 1K (n=9) rats 7 and 28 days after clipping.

Ang II (1.0 ng/ml) induced an enhancement (approximately 20%) of the pressor responses to PNS only in preparations from 1K1C rats (n=5), whereas no change in the responses to PNS was observed in the 1K group (n=5) or in the responses to norepinephrine in either group (n=7) (Figure 2). When higher concentrations of Ang II were used (5.0 and 10.0 ng/ml), similar potentiation ratios of the pressor responses to both PNS and norepinephrine were found in the 1K1C and 1K groups.

In the presence of 1.0 ng/ml Ang I, the responses of 1K1C and 1K rats to PNS and norepinephrine did not change. When higher concentrations of Ang I (5–50 ng/ml) were used, the responses to PNS and norepinephrine were potentiated in both groups. No significant difference in the potentiation ratios of PNS and norepinephrine responses of the 1K and 1K1C groups was observed, except with 50 ng/ml Ang I (Figure 3), when the potentiation ratio of the responses to PNS of 1K1C preparations was significantly increased when compared with that found in 1K rats (3.8±0.6 versus 2.5±0.1). The potentiation of the responses to norepinephrine induced by the different Ang I concentrations in 1K1C preparations was similar to that in 1K preparations. In the range of
Nerve stimulation

Control  Ang II (1 ng/ml)

100  80  60  40  20  0  120  100  60  40  20  0

Changes in perfusion pressure (mmHg)

Norepinephrine

Control  Ang II (1 ng/ml)

100  80  60  40  20  0  120  100  60  40  20  0

Changes in perfusion pressure (mmHg)

FIGURE 2. Bar graphs show vasoconstrictor responses to periartrial nerve stimulation (left panels) and exogenous norepinephrine (right panels) in mesenteric arteries from one-kidney (1K) and one-kidney, one clip (1K1C) rats 28 days after renal artery was clipped, during a control period and in the presence of angiotensin II (Ang II: 1 ng/ml, top panels; 5 ng/ml, middle panels; and 10 ng/ml, bottom panels). *p<0.05, **p<0.01 by paired Student's t test.

1.0–10.0 ng/ml, Ang I and Ang II seemed to be equipotent in potentiating the pressor response to PNS and norepinephrine in both 1K and 1K1C groups, because no difference was found between the potentiating ratio curves for Ang I and Ang II.

Saralasin and captopril by themselves did not affect either basal perfusion pressure or PNS and norepinephrine responses in 1K and 1K1C preparations. Saralasin markedly reduced the Ang II (10 ng/ml, n=7) and Ang I (50 ng/ml, n=7) enhancement of the PNS and norepinephrine responses in both groups (Figure 3). In the presence of captopril, the potentiation of the responses to PNS and norepinephrine induced by Ang I (50 ng/ml) observed in the 1K (n=5) preparations was not affected, whereas in the 1K1C (n=7) preparations, the potentiating effect of Ang I was markedly reduced (p<0.01; Figure 3).

Discussion

The present study demonstrates that, whereas the responsiveness of the mesenteric arteries to PNS is basically unaltered, a significant increase in sensitivity to exogenous norepinephrine develops in these vessels during 1K1C hypertension. Increased sensitivity to norepinephrine in hypertension has been consistently reported and may be the result of structural changes causing an increase in the wall-to-lumen ratio or of factors beyond the vasculature membrane leading to possible changes in the excitation-contraction coupling mechanism.11,12 When combined with the increased sensitivity to norepinephrine, the unaltered responses to PNS observed in 1K1C vessels may indicate that a decrease in sympathetic function occurs during the development of 1K1C hypertension. Nevertheless, it cannot be ruled out that norepinephrine released by nerve stimulation and norepinephrine administered into the lumen of the arteries might act on different types of adrenergic receptors.13

It is well known that exogenous Ang II can enhance noradrenergic neurotransmission; however, a role for locally generated Ang II has yet to be elucidated. In the present study, the 1K1C hypertensive rats showed increased ACE activity in aortic tissue 28 days after clipping. From current studies using isolated mesenteric arteries, we found that both the pressor response elicited by Ang I and the ACE activity in mesenteric arteries of 1K1C hypertensive rats (4 weeks) were significantly increased (unpublished observations from our laboratory). The hypothesis that an increased vascular formation of Ang II would induce a greater facilitatory effect on sympathetic neurotransmission was tested in isolated mesenteric arteries of 1K1C rats. An increase in sensitivity to the potentiating effect of Ang II on the PNS was observed in 1K1C preparations, because the smallest concentration of Ang II (1.0 ng/ml) potentiated the PNS responses only in the 1K1C preparations, without affecting the responses to exogenous norepinephrine. Higher concentrations of Ang II (5.0
and 10.0 ng/ml) potentiated the responses to PNS to a greater extent than the responses to norepinephrine. The Ang II enhancement of the pressor responses to PNS and norepinephrine seems to be mediated both by facilitation of norepinephrine release from nerve terminals and by an enhanced sensitivity to norepinephrine. The involvement of an Ang II-mediated decrease in norepinephrine neuronal uptake can be excluded, because cocaine did not prevent the facilitatory effect of Ang II on PNS and norepinephrine responses.

The Ang I–induced potentiation of the pressor responses to PNS and exogenous norepinephrine was similar to that found with Ang II. In fact, the potentiation ratios of the pressor responses to PNS and norepinephrine induced by Ang II and Ang I were similar in the range of 1–10 ng/ml of both peptides, indicating that these peptides are equipotent in terms of their facilitatory effect on noradrenergic function. However, a higher concentration of Ang I (50 ng/ml) elicited a significantly greater potentiation of the PNS responses of 1KIC than of 1K preparations. These results are consistent with an increased ACE activity in vascular tissue found in 1KIC hypertensive rats.

The demonstration that saralasin, an Ang II receptor antagonist, significantly reduced the facilitatory effect of both Ang II and Ang I on PNS and norepinephrine responses strongly suggests that the action of Ang I on noradrenergic transmission is preceded by its conversion to Ang II. Nevertheless, the effect of an ACE inhibitor, captopril, on the 1K mesenteric arteries does not support the view that Ang I conversion by ACE is involved. The failure of captopril to inhibit the effect of Ang I may not be due to contamination of our Ang I by Ang II, as indicated by high-performance liquid chromatographic analysis of the Ang I used in the present experiment.

The inability of an ACE inhibitor to reduce the facilitatory effect of Ang I on noradrenergic transmission in mesenteric arteries from normotensive rats, observed in the present study, contrasts with two other studies that used the same vascular preparations.14,15 In those studies, the Ang I enhancement of noradrenergic transmission was reduced by ACE inhibition. In the mesenteric arteries from 1KIC hypertensive rats, in which the vascular ACE activity was found to be increased, captopril significantly reduced the facilitatory effect of Ang I on noradrenergic transmission. On the other hand, these findings are consistent with those reported for the rat isolated kidney16 and rat isolated caudal artery,15 in which the enhancement of noradrenergic transmission produced by tetradecapeptide renin substrate was unaltered by inhibition of ACE. Thus, the finding of the present study, that in mesenteric arteries from normotensive rats Ang I induces a facilitatory effect on noradrenergic transmission that is blocked by saralasin and unaffected by captopril, is consistent with Ang I directly acting on Ang II receptors or with Ang I being converted to Ang II or to [des-Phe^8]Ang II, Ang-(1–7), another

**Figure 3.** Bar graphs show potentiation ratios of the pressor response to periarterial nerve stimulation (top panel) and exogenous norepinephrine (bottom panel) induced by angiotensin II (Ang II) and angiotensin I (Ang I) in the absence and presence of saralasin (50 ng/ml) and captopril (1 μg/ml) in mesenteric arteries from one-kidney (1K) and one-kidney, one clip (1KIC) rats 28 days after renal artery was clipped. *p<0.05, **p<0.01 between 1K and 1KIC within a period by Wilcoxon rank-sum test followed by Mann-Whitney test.
circulating bioactive angiotensin peptide,\textsuperscript{16} by an alternative pathway not involving ACE.

The present study could not demonstrate the hypothesis that an increased formation of endogenous Ang II in 1K1C isolated arteries due to an augmented ACE activity should play an important role in modulating noradrenergic transmission. Saralasin alone, at a concentration that blocked the facilitatory action of exogenous Ang II on noradrenergic function, did not change the pressor responses to either PNS or norepinephrine. However, there is accumulating evidence indicating that vascular Ang II production results from the action of renin on plasma-derived angiotensinogen, together with the interaction of locally synthesized components.\textsuperscript{4} Because in the present study isolated arterial preparations were used, the lack of a plasma-derived component, such as angiotensinogen, cannot exclude that an increased vascular formation of Ang II may be important in regulating the noradrenergic function of 1K1C rats in vivo. Indeed, in the in situ blood-perfused rat mesentery of two-kidney, one clip hypertensive rats, the responses to PNS and norepinephrine were greater than in normotensive controls, and Ang II antagonist markedly suppressed vascular responses to PNS, without affecting responses to norepinephrine.\textsuperscript{19}

In summary, isolated mesenteric arteries from 1K1C hypertensive rats, although presenting normal responses to PNS, have an increased sensitivity to the Ang II–induced potentiation of PNS responses. The Ang I–induced potentiation of PNS responses in mesenteric arteries from normotensive rats was not blocked by captopril, whereas in preparations from 1K1C hypertensive rats, in which vascular ACE activity was found to be increased, captopril significantly suppressed the Ang I–induced potentiation of noradrenergic transmission. These findings suggest that Ang I may be converted to Ang II by an alternate pathway not involving ACE and that ACE inhibitors may be effective in attenuating the potentiating effect of endogenous Ang II on noradrenergic transmission only when ACE activity is increased.

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