In Vivo Metabolism of Angiotensin I by Neutral Endopeptidase (EC 3.4.24.11) in Spontaneously Hypertensive Rats

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We investigated the processing enzymes involved in the formation of circulating angiotensin-(1-7) after intravenous administration of angiotensin I to conscious spontaneously hypertensive and Wistar-Kyoto rats. Immunoreactive products, including angiotensin I, angiotensin II, and angiotensin-(1-7), were measured in arterial blood by three specific radioimmunoassays. Angiotensin I infusion (2 nmol) induced a rapid increase in immunoreactive angiotensin II and angiotensin-(1-7). Pretreatment with the angiotensin converting enzyme inhibitor enalaprilat (2 mg/kg) eliminated angiotensin II formation and augmented circulating levels of angiotensin I and angiotensin-(1-7) in spontaneously hypertensive and Wistar-Kyoto rats. The elevated levels of angiotensin-(1-7) in enalaprilat-treated rats were blocked by concurrent treatment with the neutral endopeptidase (EC 3.4.24.11) inhibitor SCH 39,370 (15 mg/kg) in both strains. Administration of SCH 39,370 alone decreased angiotensin-(1-7) levels in spontaneously hypertensive rats not given any of the enzyme inhibitors. In addition, levels of angiotensin I were higher after administration of SCH 39,370 in hypertensive rats. These novel findings reveal that neutral endopeptidase EC 3.4.24.11 participates in the conversion of angiotensin I to angiotensin-(1-7) and in the metabolism of angiotensin II in the circulation of both spontaneously hypertensive and Wistar-Kyoto rats. Our results suggest that neutral endopeptidase EC 3.4.24.11 is a major enzymatic constituent of the circulating renin-angiotensin system. (Hypertension 1992;19:692-696)

Key Words • angiotensin I • angiotensin II • angiotensin converting enzyme • enalaprilat • enzyme inhibitors • peptide peptidohydrolases • spontaneously hypertensive rats

Angiotensin-(1-7) [Ang-(1-7)] is the first member of the angiotensin peptide family to cause cells to secrete hormones and release autacoids without eliciting accompanying changes in blood pressure, water intake, and aldosterone secretion. Incubation of hypothalamic explants with Ang-(1-7) stimulates a dose-dependent release of vasopressin. Addition of Ang-(1-7) to human astrocytes and vascular endothelial cells promotes production of prostanoids by activation of a Ca2+-independent second messenger system. Angiotensin I (Ang I) forms Ang-(1-7) in brain homogenates, vascular endothelium, and neuronal cells in culture. These effects of Ang-(1-7) have prompted us to suggest a regulatory role for this peptide as a paracrine hormone.

Inhibition of angiotensin converting enzyme (ACE) augments the concentration of Ang-(1-7) in plasma. These data suggest that alternate enzymatic pathways exist for the processing of biologically active angiotensin peptides. In searching for peptides that hydrolyze the Pro7-Phe8 bond of Ang I or angiotensin II, we found that prolyl endopeptidase (EC 3.4.21.26) is an Ang-(1-7)-forming enzyme in neuroblastoma glioma cells and brain tissue. However, inhibition of prolyl endopeptidase did not prevent the generation of Ang-(1-7) in other tissues. Therefore, we have now investigated whether neutral endopeptidase EC 3.4.24.11 (NEP 24.11) is involved in the metabolism of Ang I into Ang-(1-7). Studies were done in conscious spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) controls in which Ang I was injected intravenously in the absence and presence of specific inhibitors of ACE, prolyl endopeptidase, and NEP 24.11. Studies were done in this species because we found that circulating levels of Ang-(1-7) were significantly increased in the plasma of rats with spontaneous hypertension.

Methods

Experimental Protocol

All experiments were carried out in strict adherence to the principles sponsored by the American Physiological Society. Male 16-week-old SHR (319±4 g) and WKY (315±4 g) controls (Charles River Breeding Laboratories, Wilmington, Mass.) were housed in rooms maintained on a 12-hour light/dark cycle. Animals ate a solid diet of rat chow (Purina, Bedford, Ohio)
and had free access to water. Cannulas (PE-50 and PE-10, Clay Adams Becton Dickinson, Parsippany, N.J.) were implanted into a femoral artery and vein by using aseptic conditions in rats anesthetized with 1% halothane 24 hours beforehand. The free ends of the cannulas were tunneled under the skin and externalized at the back of the neck.

Conscious rats were divided into five groups. Group 1 (n=4 SHR and 4 WKY controls) received an intravenous injection of 0.9% NaCl. Group 2 (n=4 SHR and 4 WKY controls) was given an intravenous injection of enalaprilat (MK-422, 2 mg/kg). Group 3 (n=4 SHR and 4 WKY controls) was injected with N-[N-1-(S)-car-}

Peptide Extraction and Analyses

Samples were centrifuged at 5,000g for 20 minutes; the supernatant was stored for 12 hours at -20°C. After a second centrifugation step (10,000g for 20 minutes) the supernatant was diluted 1:1 (vol/vol) with 1% heptfluorobutyric acid (HFBA), stored for 4–6 hours at 4°C, and centrifuged again at 15,000g. This supernatant was diluted 1:4 with 0.2% HFBA and Z-prolyl-prolinal (5 mg/kg), a specific inhibitor of prolyl endopeptidase. Either saline (vehicle) or the enzyme inhibitors were administered 20 minutes before an intravenous injection of 0.2 ml Ang I (2 nmol) in heparinized saline. Serial samples of arterial blood (0.2 ml) were rapidly collected into ice-chilled tubes containing 5 ml of 80% ethanol/0.1N HCl every 15–30 seconds before and after injection of Ang I. The amount of blood removed in each sample was replaced with an equal volume of saline. The dead space (0.08 ml) of the femoral artery catheter was cleared of any remaining saline or blood before the samples were obtained.

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giiotensins from analysis of plasma samples by using the combination of high-performance liquid chromatography followed by radioimmunoassay.

Pretreatment with MK-422 significantly elevated baseline levels of Ang I in both WKY (1.4±0.4 pmol/ml) and SHR (0.9±0.1 pmol/ml) and caused a significant upward shift of the time-concentration clearance of Ang I in both strains of rats (Figure 1). This was associated with a marked inhibition of Ang II formation, averaging 1% of values found in untreated WKY and SHR. In contrast, peak and time-concentration levels of Ang-(1-7) were significantly increased in rats given MK-422 as compared with vehicle (Figure 3). Peak levels of Ang-(1-7) averaged 1.8±0.2 pmol/ml in WKY and 1.9±0.1 pmol/ml in SHR (p>0.05). These data represent a twofold and threefold increase over values found in control rats (0.6±0.1 pmol/ml and 0.8±0.1 pmol/ml, p<0.05). In addition, these values are 10% and 13% of peak Ang I levels in WKY and SHR, respectively.

Inhibition of NEP 24.11 produced a pattern of Ang I and Ang II metabolism that did not differ from that obtained in control rats (Figures 1 and 2). Peak levels of Ang I were higher in SHR (6.2±0.4 pmol/ml) compared with WKY rats (4.3±0.4 pmol/ml, p<0.01). Baseline levels of Ang II were significantly higher after SCH 39,370 (WKY rats, 0.04±0.02 versus 0.12±0.04 pmol/ml, p<0.05; SHR, 0.02±0.02 versus 0.18±0.04 pmol/ml, p<0.05). Likewise, peak levels of Ang II were significantly higher than in control rats (p<0.05). Ang II averaged 21.8±5.4 pmol/ml in WKY and 27.2±3.8 pmol/ml in SHR (p>0.05). Figure 3 shows that SCH

Figure 1. Time-concentration curves of angiotensin I in the blood of Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) produced by intravenous injection of 2 nmol angiotensin I. Values are mean±SEM. Ir, immunoreactive; MK422, enalaprilat; SCH, the neutral endopeptidase 24.11 inhibitor SCH 39,370; ZPP, Z-prolyl-prolinal, a specific inhibitor of prolyl endopeptidase.

Figure 2. Time-concentration curves of angiotensin II in the blood of Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR). Values are mean±SEM. Ir, immunoreactive; MK422, enalaprilat; SCH, the neutral endopeptidase 24.11 inhibitor SCH 39,370; ZPP, Z-prolyl-prolinal, a specific inhibitor of prolyl endopeptidase.
39,370 inhibited the formation of Ang-(1–7). Differences in time concentrations of Ang-(1–7) between vehicle and SCH 39,370 groups were statistically significant in SHR (p<0.01) but not in WKY rats (p>0.05).

Combined blockade of ACE and NEP 24.11 decreased the rate of Ang I metabolism, prevented Ang II formation, and caused marked reductions in Ang-(1–7) levels in both WKY and SHR as compared with MK-422 treatment alone (Figures 1–3). Peak levels of Ang-(1–7) averaged 0.15±0.04 pmol/ml in WKY and 0.13±0.03 pmol/ml in SHR (p>0.05). These values represented 1.1% and 0.9% of peak Ang I levels in control WKY and SHR, respectively. In rats pretreated with a combination of MK-422 and an inhibitor of prolyl endopeptidase, the injection of Ang I produced a pattern of metabolism bearing similarities to that found in rats given MK-422 alone. However, this combination of inhibitors produced levels of Ang I (Figure 1) and Ang-(1–7) (Figure 3) that were significantly below those found in WKY and SHR given MK-422 only (p<0.05). Production of Ang II remained at levels that were close to or at the detectable level of the assay.

Discussion

The injection of Ang I into the circulation of WKY and SHR causes increased formation of Ang II and Ang-(1–7). Repeated sampling during the first 60 seconds after the injection of Ang I showed that levels of Ang-(1–7) were consistently greater in SHR compared with WKY controls. Conversion of Ang I into Ang-(1–7) was mediated by NEP 24.11, indicating an active participation of this enzyme in the degradation of Ang II. The dual role of NEP 24.11 acting both as a processing and degrading enzyme may regulate plasma levels of angiotensin peptides. To our knowledge, this is the first in vivo demonstration of how an enzyme can affect levels of angiotensin peptides by cleaving the Pro7-Phe8 bond of Ang I and inactivating Ang II by hydrolysis at the Tyr4-Ile5 bond of the peptide.

The pattern of the metabolism of injected Ang I under the various conditions reveals that a dynamic equilibrium exists among angiotensin-forming enzymes. Strictly speaking, we see the process as a "yin-yang" mechanism. The proportional rate of conversion of Ang I into Ang-(1–7) was augmented when the formation of Ang II was blocked by inhibition of ACE. At the peak of the time–concentration curve, Ang-(1–7) levels in MK-422–treated WKY rats increased 307% compared with vehicle-treated rats. Corresponding increases in SHR averaged 225%. At the same time the levels of Ang II were reduced by 99% in both WKY and SHR. Inhibition of NEP 24.11 reduced peak levels of Ang-(1–7) by 51% in WKY and 74% in SHR. Combined blockade of both ACE and NEP 24.11 produced peak levels of Ang-(1–7) that averaged 73% and 84% below vehicle-treated WKY and SHR, respectively. Neither SCH 39,370 alone nor in combination with MK-422 blocked the formation of Ang-(1–7) in its entirety. In preliminary experiments, larger doses of SCH 39,370 caused no further inhibition of Ang-(1–7) production. In addition, we found no evidence for a contribution by prolyl endopeptidase. Therefore, another enzyme(s) may contribute to the formation of the heptapeptide in the circulation of WKY and SHR. These findings show that there are multiple metabolic pathways for Ang I.

NEP 24.11 degrades both atrial natriuretic peptide and kinins.14-15 Our findings suggest that this endopeptidase also influences the metabolism of Ang I by decreasing hydrolysis of Ang II and facilitating conversion of Ang I into Ang-(1–7). Inhibition of Ang II degradation appears to be an important action of SCH 39,370. In our experiments, SCH 39,370 caused both baseline and peak plasma levels of Ang II to double in both WKY and SHR. Gafford et al16 have reported that NEP 24.11 hydrolyzes Ang II at the Tyr4-Ile5 to yield Ang-(1–4). Therefore, the observation that SCH 39,370 did not reduce blood pressure in SHR may reflect the opposing effects that NEP 24.11 has on the various peptidergic systems that regulate vascular resistance.15
SCH 39,370 has antihypertensive effects in deoxycorticosterone acetate-salt hypertensive rats. Because this form of hypertension is associated with suppression of the peripheral renin-angiotensin system, the vasodilator effects of the inhibitor may not be masked by the counterbalancing actions of NEP 24.11 on Ang I metabolism. We also confirmed that conversion of Ang I into Ang-(1-7) is not dependent on an intermediate production of Ang II. In MK-422-treated animals, inhibition of Ang II production was associated with increased levels of Ang-(1-7).

Our studies also showed that metabolism of Ang I into Ang-(1-7) is regulated by several distinct enzymatic pathways in both the blood and tissues. In both canine hypothalamic homogenates and NG108 cells, prolyl endopeptidase accounted for 40% of the Ang-(1-7)-generating activity. NEP 24.11 was not involved in the metabolism of Ang I in these tissues since production of Ang-(1-7) was not inhibited in the presence of phosphoramidon. Both this and previous studies agree with our suggestion that metabolic pathways contributing to the generation of angiotensin peptides are tissue specific. Diversification of peptide processing arises from different enzymes contributing to alternate pathways for angiotensin metabolism.

Although in a qualitative fashion the metabolism of Ang I over time in WKY and SHR was similar, our findings revealed interesting differences. Production of Ang-(1-7) was augmented in SHR after injection of Ang I. These data are in agreement with a preliminary finding by Kohara et al., who found increased plasma levels of Ang-(1-7) in tissues since production of Ang-(1-7) was not inhibited in the presence of phosphoramidon. Both this and previous studies agree with our suggestion that metabolic pathways contributing to the generation of angiotensin peptides are tissue specific. Diversification of peptide processing arises from different enzymes contributing to alternate pathways for angiotensin metabolism.

In summary, NEP 24.11 has a major role in the in vivo metabolism of Ang I in the circulation of WKY and SHR. This endopeptidase acts on Ang I to form Ang-(1-7) and contributes to the degradation of Ang II.

References
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