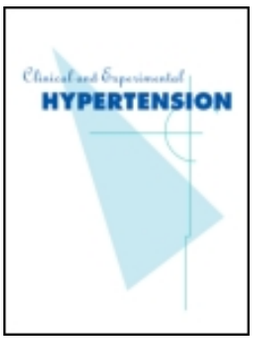

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Differential Regulation of Angiotensin Peptides in Plasma and Kidney: Effects of Adrenalectomy and Estrogen Treatment

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DIFFERENTIAL REGULATION OF ANGIOTENSIN PEPTIDES IN PLASMA AND KIDNEY: EFFECTS OF ADRENALECTOMY AND ESTROGEN TREATMENT

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ABSTRACT

Eight angiotensin peptides [angiotensin-(1-7), angiotensin II, angiotensin-(1-9), angiotensin I, angiotensin-(2-7), angiotensin-(2-8), angiotensin-(2-9), and angiotensin-(2-10)] were measured in plasma and kidney of adrenalectomized rats and estrogen-treated rats. In comparison with sham-operated rats, adrenalectomy increased plasma renin levels by 50-fold and reduced plasma angiotensinogen levels by 67%. Adrenalectomy increased plasma angiotensin peptide levels by 9- to 30-fold, but the increases in renal angiotensin peptide levels were much less than those seen for plasma. In comparison with vehicle-treated rats, estrogen treatment increased plasma angiotensinogen levels by 3-fold and reduced plasma renin levels by 41%. Estrogen treatment decreased plasma angiotensin peptide levels, whereas renal angiotensin peptide levels increased by as much as 2- to 3-fold. These results confirm the differential regulation of angiotensin peptide levels in plasma and kidney, and provide further support for the essential role of angiotensinogen in modulating plasma and tissue angiotensin peptide levels.

INTRODUCTION

Angiotensin II (Ang II) plays a major role in the regulation of blood pressure, fluid and electrolyte homeostasis. The major pathway of Ang II

formation is by the action of renin (EC 3.4.99.19) to cleave the decapeptide Ang I from angiotensinogen (1,2), with subsequent cleavage of the C-terminal dipeptide from Ang I (3) by angiotensin converting enzyme (ACE, dipeptidyl carboxypeptidase, peptidyldipeptide hydrolase; EC 3.4.15.1).

Whereas the renin angiotensin system was previously considered to be primarily an endocrine system, whereby renin secreted by the kidney acts on plasma angiotensinogen to generate angiotensin peptides in plasma, it is now generally accepted that angiotensin peptide formation also occurs in tissues (4). Angiotensin peptide formation in tissues is important because of the potential for differential regulation of angiotensin peptide levels in plasma and tissues. In previous studies of the effects of the ACE inhibitor perindopril on circulating and tissue angiotensin peptides (5,6) we observed a dissociation between angiotensin peptide responses in plasma and kidney. Whereas perindopril produced marked increases in plasma levels of renin and Ang I, plasma Ang II levels were not suppressed except at the highest doses of perindopril (6) because of the compensatory effects of increased plasma Ang I levels on Ang II formation in plasma (7). In contrast to plasma, perindopril failed to increase renal Ang I levels and renal Ang II levels were suppressed by low doses of perindopril (5,6).

ACE inhibition is associated with marked increases in circulating renin, with consequent consumption of angiotensinogen and accumulation of des-Ang I-angiotensinogen in plasma (8). The fall in plasma levels of angiotensinogen has an important rate-limiting effect on Ang I production, in that the reduced plasma angiotensinogen levels produce a proportionate reduction in the plasma Ang I response to the increased plasma renin levels (6). The failure of renal Ang I levels to increase with perindopril administration led us to propose that renal levels of angiotensinogen may have a similar role in limiting Ang I production in the kidney (5).

In the present study we investigated further the relationship between angiotensin peptide levels in plasma and kidney by studying the effects of both a high renin model (adrenalectomy) and a high angiotensinogen model (estrogen treatment) on components of the renin angiotensin system in plasma and kidney of rats. Using high performance liquid chromatography (HPLC)-based radioimmunoassays (RIA) we quantified levels of 8 angiotensin peptides in plasma and kidney: Ang-(1-7), Ang II, Ang-(1-9), Ang I, Ang-(2-7), Ang-(2-8), Ang-(2-9), and Ang-(2-10).

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METHODS

Male Sprague Dawley rats (250-300 g), maintained in a room with 12-hour light/dark cycle (lights on 6:00 AM to 6:00 PM), were fed a diet of GR 2+ pellets (Clarke King & Co., Melbourne, Australia) and received tap water to drink. These studies were performed in accordance with the guidelines of the St. Vincent's Hospital Animal Experimentation Ethics Committee.

Rats were adrenalectomized using ether anesthesia, and were maintained on tap water for 7 days with free access to food. Control rats were subjected to sham operation. Whereas sham-operated rats gained weight post-surgery, adrenalectomized rats lost weight. Estrogen-treated rats were administered 17α-ethynylestradiol (Sigma Chemical Co., St. Louis, MO) in peanut oil at a dose of 1 mg/kg/day by daily subcutaneous injection for 7 days. Control rats were given daily injections of vehicle. After 7 days, the rats were killed at 10:00 AM by decapitation without prior anesthetic. Trunk blood was collected for measurement of plasma levels of renin, angiotensinogen, ACE, and angiotensin peptides, and the left kidney was removed for measurement of renal levels of angiotensin peptides.

Extraction and Radioimmunoassay of Angiotensin Peptides From Rat Plasma and Kidney

Trunk blood (5-6 mL) was rapidly collected into tubes containing 0.5 mL inhibitor solution (100 μmol/L H-77, 50 mmol/L 1,10-phenanthroline, 125 mmol/L ethylenediaminetetra-acetate, 2 g/l neomycin sulfate and 2% ethanol in water) at 4°C (5). The blood was centrifuged and the plasma (~3 mL) immediately extracted with Sep-Pak C_{18} cartridges (Waters, Milford, MA, USA). The methods of extraction, acetylation, HPLC and RIA of angiotensin peptides, and correction of data for recovery are described in detail elsewhere (9). Angiotensin peptides were acetylated before HPLC and, after reconstitution in assay buffer, each HPLC fraction was assayed in duplicate with two different RIA. Antibody 41, raised against N-acetyl-AspArgValTyrIleHisProPheLys, measured AcAng-(1-7), AcAng II, AcAng-(1-9) and AcAng I. Antibody 52, raised against N-acetyl-ArgValTyrIleHisProPheLys, measured AcAng-(2-7), AcAng-(2-8), AcAng-(2-9), and AcAng-(2-10).

The left kidney (1.2 - 1.7 g wet weight) was removed immediately after collection of blood and homogenized in 20 mL 4 mol/L guanidine thiocyanate,

1% (vol/vol) trifluoroacetic acid in water (5). Tissue homogenates were sonicated briefly and then centrifuged at 5000 *g* for 20 min. Half (10 mL) of the supernatant from each homogenate was extracted on a Sep-Pak C_{18} cartridge and the extract evaporated to dryness under vacuum. Each extract was then dissolved in 1 mL 1 mol/L HCl and extracted twice with 1 mL diethyl ether. The extracts were then evaporated to dryness again, acetylated, and run on HPLC before measurement of angiotensin peptides by RIA as described for plasma.

Measurement of Renin, Angiotensinogen and ACE in Plasma

For measurement of plasma renin, angiotensinogen and ACE, trunk blood was collected into heparinized tubes at room temperature. After centrifugation at 2000 g for 10 min, 0.9 mL aliquots of plasma were transferred to tubes containing 0.1 mL 300 mmol/L sodium phosphate, 25 mmol/L N-ethylmaleimide, 100 mmol/L benzamidine, pH 7.4 (in order to prevent cryoactivation of inactive renin (10)), then rapidly frozen in dry ice and stored at -30°C until assay. The concentrations of active renin, angiotensinogen and ACE were measured as described previously (5). Plasma ACE activity was measured by spectrophotometric assay using 2 mmol/L 3-(2-furylacryloyl)-L-phenylalanyl-glycyl-glycine as substrate (11).

Data Analysis

Comparisons between control and experimental groups were made by unpaired t test. Logarithmic transformation of the data was performed where appropriate to obtain similar variances amongst groups.

RESULTS

Effects of Adrenalectomy

Adrenalectomy increased plasma renin levels by 50-fold, with a corresponding reduction in plasma angiotensinogen levels to 33% of the levels in sham-operated rats (Table 1). Adrenalectomy also resulted in a 24% decrease in plasma ACE levels. Adrenalectomy increased plasma levels of all 8 angiotensin peptides measured, with a 21-fold increase in Ang II levels and a 12-fold increase in Ang I levels (Table 2). Adrenalectomy also increased angiotensin peptide levels in kidney, but for each peptide the increase was less

TABLE 1

Plasma levels of renin, angiotensinogen and ACE in sham-operated and adrenalectomized rats.

	Sham	Adrenalectomized
Renin (pmol Ang I/mL/h)	27 ± 2	1372 ± 378**
Angiotensinogen (pmol/mL)	493 ± 27	161 ± 46**
ACE (U/l)	257 ± 12	196 ± 20*

Values are mean ± SEM, n = 6.

*, $P < 0.025$; **, $P < 0.001$, in comparison with sham-operated rats.

TABLE 2

Angiotensin peptide levels in plasma and kidney of sham-operated and adrenalectomized rats.

	Plasma (fmol/mL)		Kidney (fmol/g)	
	Sham	Adrenalectomized	Sham	Adrenalectomized
Ang-(1-7)	2.6 ± 0.9	31.2 ± 3.0**	13 ± 5	24 ± 8
Ang II	15 ± 3	308 ± 57**	73 ± 16	507 ± 135**
Ang-(1-9)	0.7 ± 0.2	6.1 ± 1.9*	22 ± 5	74 ± 47
Ang I	52 ± 10	641 ± 97**	39 ± 3	91 ± 24*
Ang-(2-7)	3 ± 1	45 ± 8**	15 ± 6	12 ± 4
Ang-(2-8)	9 ± 2	284 ± 33**	6 ± 2	32 ± 8**
Ang-(2-9)	0.6 ± 0.1	14.3 ± 2.9**	10.6 ± 5.3	4.5 ± 1.0
Ang-(2-10)	16 ± 6	310 ± 51**	14 ± 2	6 ± 2*

Values are mean ± SEM, n = 6.

*, $P < 0.02$; **, $P < 0.001$, in comparison with sham-operated rats.

than that shown for plasma. For example, renal Ang II levels increased by 7-fold and renal Ang I levels increased by 2.3-fold. Despite a 19-fold increase in plasma Ang-(2-10) levels, renal Ang-(2-10) levels showed a statistically significant decrease in adrenalectomized rats (Table 2). The Ang II/Ang I ratio was unchanged in plasma, but was increased in kidney of adrenalectomized rats (Ang II/Ang I ratio: 1.8 ± 0.3, mean ± SEM, n = 6, in sham-operated rats; 5.7 ± 0.8 in adrenalectomized rats, $P < 0.01$).

Effects of Estrogen Treatment

Estrogen treatment increased plasma angiotensinogen levels by 3-fold, accompanied by a 42% decrease in plasma renin levels and a 41% decrease in plasma ACE levels (Table 3). Estrogen treatment resulted in 30-60% decreases in the plasma levels of angiotensin peptides, which were statistically significant for Ang-(1-9), Ang I, and Ang-(2-10) (Table 4). By contrast, estrogen treatment produced statistically significant increases of 2- to 3-fold in the renal levels of Ang-(1-7), Ang I, Ang-(2-8), and Ang-(2-10). Other renal angiotensin peptides showed no change in levels except for a statistically significant decrease in renal Ang-(2-7) levels in estrogen-treated rats. The Ang II/Ang I ratio was unchanged in plasma, but was reduced in kidney of estrogen-treated rats (Ang II/Ang I ratio: 4.4 ± 0.8, mean ± SEM, n = 6, in vehicle-treated rats; 1.7 ± 0.4 in estrogen-treated rats, $P < 0.01$).

DISCUSSION

This study used two different strategies to reveal the differential regulation of angiotensin peptide levels in plasma and kidney. The first strategy was a high renin model in which adrenalectomy increased plasma renin levels by 50-fold, accompanied by a 67% decrease in plasma angiotensinogen levels. The second strategy was a high angiotensinogen model in which estrogen treatment increased plasma angiotensinogen levels 3-fold, accompanied by a 42% decrease in plasma renin levels. With the high renin model we showed that the renal angiotensin peptide response to the high renin state of adrenalectomy was markedly blunted in comparison with that of plasma angiotensin peptides. With the second model we showed that increased plasma (and presumably renal) angiotensinogen levels were associated with increased renal levels of Ang-(1-7), Ang I, Ang-(2-8), and Ang-(2-10), despite reduced plasma levels of renin and angiotensin peptides.

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TABLE 3

Plasma levels of renin, angiotensinogen and ACE in vehicle- and estrogen-treated rats.

	Vehicle	Estrogen
Renin (pmol Ang I/mL/h)	19 ± 1	11 ± 2**
Angiotensinogen (pmol/mL)	654 ± 33	2008 ± 125**
ACE (U/l)	215 ± 5	127 ± 16*

Values are mean ± SEM, n = 6.

*, $P < 0.01$; **, $P < 0.001$, in comparison with vehicle-treated rats.

TABLE 4

Angiotensin peptide levels in plasma and kidney of vehicle- and estrogen-treated rats.

	Plasma (fmol/mL)		Kidney (fmol/g)	
	Vehicle	Estrogen	Vehicle	Estrogen
Ang-(1-7)	2.1 ± 0.5	1.2 ± 0.4	18 ± 4	36 ± 6*
Ang II	42 ± 7	29 ± 9	229 ± 19	221 ± 24
Ang-(1-9)	2.0 ± 0.6	0.2 ± 0.0**	36 ± 6	48 ± 3
Ang I	65 ± 6	36 ± 11*	61 ± 12	143 ± 15**
Ang-(2-7)	2.3 ± 0.2	1.4 ± 0.6	18 ± 6	6 ± 2*
Ang-(2-8)	26 ± 4	17 ± 4	8 ± 3	21 ± 4*
Ang-(2-9)	1.6 ± 0.5	0.5 ± 0.0	14 ± 4	19 ± 6
Ang-(2-10)	26 ± 3	11 ± 2**	10 ± 6	28 ± 5*

Values are mean ± SEM, n = 6 for plasma of vehicle-treated rats and n = 5 for plasma of estrogen-treated rats, and n = 6 for kidney of both vehicle- and estrogen-treated rats.

*, $P < 0.05$; **, $P < 0.01$, in comparison with vehicle-treated rats.

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Mendelsohn et al. (12) have previously shown that adrenalectomy causes a fall in ACE levels in lung, in agreement with our finding of reduced plasma ACE levels in adrenalectomized rats. However, in the present experiments the plasma Ang II/Ang I ratio was unaltered by adrenalectomy. Moreover, in contrast to the fall in plasma ACE levels, the renal Ang II/Ang I ratio was increased, suggesting an increase in renal ACE levels mediating increased Ang I conversion to Ang II in kidney of adrenalectomized rats. Despite this increase in renal Ang II/Ang I ratio, renal Ang II levels failed to increase to the same extent as plasma Ang II levels in response to adrenalectomy.

The dissociation between plasma and renal angiotensin peptide responses to adrenalectomy was in agreement with our previous findings for ACE inhibition (5,6) and for rats treated with the angiotensin type 1 (AT_1) receptor antagonist losartan (13). Each of these models produces marked stimulation of renin secretion and reduction in plasma angiotensinogen levels. Clauser et al. (14) have shown that the reduced plasma angiotensinogen levels in adrenalectomized rats are due to consumption by the high plasma renin levels. We have shown that the increased renin secretion associated with ACE inhibition also reduces renal angiotensinogen levels (5). Since the kidney is the source of the high plasma renin levels in each of these models, we proposed that renal angiotensinogen is more susceptible to consumption than plasma angiotensinogen, and therefore angiotensinogen is predicted to be more rate-limiting in kidney than in plasma (5). Consequently, in high renin states renal angiotensin peptide levels fail to increase to the same extent as plasma angiotensin peptide levels because renal angiotensinogen is more rapidly consumed by the increased amount of renin secreted by the kidney.

The significance of the dissociation between plasma and renal angiotensin peptide levels in high renin states is illustrated by our finding that the threshold dose of perindopril for increase in plasma renin levels was the same as that required for suppression of renal Ang II levels, providing evidence that renal Ang II levels, and not plasma Ang II levels, mediate the negative feedback regulation of renin secretion by Ang II (6). Moreover, the renal angiotensin peptide responses to ACE inhibition and AT_1 receptor antagonism are major determinants of the renal actions of these compounds, reviewed elsewhere (15).

The limitation of angiotensin peptide formation in the kidney in high renin states has two consequences. Firstly, the kidney is relatively protected from

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the very large increase in angiotensin peptide levels which might otherwise occur if renal angiotensinogen levels were not so rate-limiting. Secondly, the limitation in angiotensin peptide formation in the kidney also limits the inhibitory effects of renal Ang II on renin secretion, thus enabling higher renin secretion than might otherwise occur.

Estrogens are potent stimulants of plasma angiotensinogen levels (16). It is of interest that despite a 3-fold increase in plasma angiotensinogen levels in estrogen-treated rats, plasma angiotensin peptide levels decreased in parallel with the 42% decrease in plasma renin levels. Given that plasma angiotensinogen levels of control rats are lower than the Michaelis constant (K_m) for the renin-angiotensinogen reaction (17), one would predict that plasma angiotensin peptide levels would be relatively unaltered by the opposite changes in plasma levels of renin and angiotensinogen in estrogen-treated rats. The present data suggest, however, that normal and elevated plasma angiotensinogen levels approach the level required for zero-order kinetics, such that plasma angiotensin peptide levels are more dependent on plasma levels of renin than angiotensinogen.

In contrast to the fall in plasma angiotensin peptide levels, renal angiotensin peptide levels were increased in estrogen-treated rats. As discussed above, we propose that renal angiotensinogen levels are more rate-limiting to angiotensin peptide formation than are plasma angiotensinogen levels. Thus, the increase in renal angiotensin peptide levels in estrogen-treated rats is consistent with their greater dependence on angiotensinogen levels than plasma angiotensin peptide levels. Although the renal levels of Ang I and its metabolites Ang-(1-7), Ang-(2-10), and Ang-(2-8) increased 2- to 3-fold, renal Ang II levels were not altered by estrogen treatment. These different responses of renal Ang II and Ang I may be due to a decrease in Ang I conversion to Ang II in the kidney, possibly due to a fall in renal ACE levels, similar to that observed in plasma of estrogen-treated rats. In support of this possibility, the renal Ang II/Ang I ratio was reduced in estrogen-treated rats. It is also possible that the different responses of renal Ang II and Ang I levels represent compartmentalization of these peptides within the kidney. As discussed above, renal Ang II levels appear to be more important than plasma Ang II levels in the regulation of renin secretion, and the fall in plasma renin levels in estrogen-treated rats is suggestive of an increase in renal Ang II levels, at least in that renal compartment which regulates renin secretion.

Further evidence that the critical interaction between renal renin and renal angiotensinogen determines renal angiotensin peptide levels is provided by our study of the transgenic TGR(mRen-2)27 rat, in which there is over-expression of the mouse renin gene in tissues other than the kidney (18). Whereas in normal rats the kidney is the source of renin, with consequent depletion of renal angiotensinogen levels, renal production of renin is suppressed in the transgenic rat and renin is delivered to the kidney via the circulation. As a result, renal angiotensinogen levels are likely to be less susceptible to depletion, and this is the one high renin model where we find the increase in renal Ang II levels to be similar to the increase in plasma Ang II levels (18).

CONCLUSION

This study confirmed the differential regulation of angiotensin peptide levels in plasma and kidney, and provided further support for the essential role of angiotensinogen in modulating plasma and tissue angiotensin peptide levels.

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