Effects of Angiotensin II and Phenylephrine on Urinary Endothelin in Normal Female Volunteers

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Endothelin (ET) is a 21–amino acid peptide produced and secreted mainly by endothelial cells. Small amounts of ET are found in plasma, whereas large amounts are present in the urine. Despite the abundance of ET in the kidneys and urine, little is known about its regulation and clinical significance. The present study was designed to examine the effects of angiotensin II (Ang II) and phenylephrine (Phe) on the excretion of ET in normal female volunteers. Ang II and Phe were infused for 1 hour each and titrated to increase the mean arterial pressure by 20 mm Hg. There was a 60-minute recovery period before the second drug, and the order of the drugs was randomized. Infusion of Phe induced mild diuresis and natriuresis, which were associated with a significant increase in the excretion of ET. In addition, Phe significantly increased plasma atrial natriuretic factor (ANF). In contrast, infusion of equipressor doses of Ang II decreased urinary sodium excretion and did not significantly alter the excretion of ET. Moreover, Ang II induced only a small and nonsignificant increase in plasma ANF. These results demonstrate that (1) physiological doses of Ang II do not affect excretion of either ET or ANF; (2) Phe markedly increased the excretion of ET and ANF, independently of its effect on blood pressure; and (3) neither agent changed plasma ET, but Phe increased plasma ANF.

ENDOTHELIN (ET) is a 21–amino acid peptide produced and released by endothelial cells.[1] Intravenous infusion of ET induces potent and long-lasting vasoconstriction, especially of the renal vasculature.[1,2] In addition, there is growing evidence that physiological doses of ET produce a remarkable diuresis and natriuresis in the rat. In vitro studies[3,4] demonstrate that ET inhibits Na^+-K^+–adenosine triphosphatase in inner medullary collecting duct cells.[5] These findings suggest that ET plays an important role in renal fluid and sodium homeostasis (for review, see Simonson[6]).

Although plasma levels of ET are extremely low, large amounts are found in urine.[7] Plasma ET is derived mainly from the endothelial lining of blood vessels.[1] However, the source of urinary ET has not been delineated. Recently, we[8,9] and others[10] found that only a small portion ($<0.3\%$) of intravenously administered ^{125}I-ET appears in the urine. Free iodine accounts for more than 90% of the recovered radioactivity. Pretreatment of rats with a neutral endopeptidase inhibitor increases the amount of radioactivity excreted in urine, and most of this increase is due to intact ^{125}I-ET. This indicates that most filtered plasma ET is cleaved in the kidney by neutral endopeptidase, which is abundant in the proximal tubule. Thus, urinary ET is mainly of renal origin.

Several studies have shown that different types of renal cells synthesize and release considerable amounts of ET. Recently, Kohan and Fiedorek[11] reported that inner medullary collecting duct cells synthesize more ET than any other tubular segment, equal to that produced by endothelial cells. An additional source of ET is renal epithelial[12] and mesangial[13] cells, suggesting that urinary ET is produced by several different types of renal cells rather than through glomerular filtration. Despite much interest in ET physiology and pathophysiology, little attention has been paid to ET excretion and its regulation. Many in vitro studies demonstrate that angiotensin II (Ang II) and other agents that activate phospholipase C increase the expression of preproendothelin mRNA and ET production by endothelial cells.[14,15] Moreover, in vivo studies show that ET-1 potentiates the vasoconstrictive response to Ang II in rats.[16] Therefore, we designed the present study to examine the effects of Ang II and a different pressor agent, phenylephrine (Phe), on plasma and urinary levels of ET in normal subjects.

SUBJECTS AND METHODS

Fourteen normal women taking no medications and with no known health problems were recruited for this study. Their ages ranged from 18 to 31 years. The study was approved by the Institutional Review Board of the National Heart, Lung, and Blood Institute. Initial screening included (1) discussing the protocol with the subject and obtaining informed consent, (2) a directed medical history and physical examination, and (3) basic laboratory tests and an electrocardiogram to rule out conditions that would exclude the subject from participation in the study. Subjects with hypertension (blood pressure $\geq$ 145/95 mmHg), diabetes mellitus (fasting blood sugar > 110 mg/dL), renal insufficiency (creatinine > 1.5 mg/dL), congestive heart failure, or coronary artery disease, and pregnant or lactating women were excluded. On the day of the study, subjects came to the special study room early in the morning, after an overnight fast and abstinence from tobacco, alcohol, and any medication, with a 24-hour urine collection. Two intravenous lines were inserted for withdrawal of blood and infusions, respectively. An automated blood pressure cuff measured and recorded the patients' blood pressure and heart rate every 3 minutes. Each subject received an intravenous infusion of 5% dextrose and water at a rate of 30 mL/h for 1 hour. Then an infusion of either Ang II in protocol 1 (initial dose, 3 ng/kg/min) or Phe in protocol 2 (initial dose, 1 μg/kg/min) was started. Protocol 2 was performed after protocol 1 was completed, to determine if any of the effects observed were due to the order in which the drugs were administered. The dose of each drug was titrated to increase the mean arterial blood pressure by 20 mm Hg for a total of 1 hour. At the end of the hour, the drug infusion was stopped and the subject received 5% dextrose and water at the rate of 30 mL/h for the next hour. Finally, an infusion of the second drug, ie, Phe in protocol 1

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or Ang II in protocol 2, was administered for 1 hour. Each subject was asked to empty her bladder at the start and after 30 and 60 minutes of each infusion. Blood samples were drawn at the same times.

Analytical Methods

ET and atrial natriuretic factor (ANF) levels were measured with the Endothelin-1,2 [^{125}I] and (3-[^{125}I]iodotyrosyl$_{28}$)ANF kits, respectively, and Amerlex-M magnetic separation, all from Amersham (Arlington Heights, IL). The urinary sodium level was measured with the Beckman Synchron EL-ISE Electrolyte System (Beckman, Irvine, CA) via an ion-sensitive electrode. Osmolality was determined by the freezing-point–depression method using the Advanced DigiMatic Osmometer model 3DII (Advanced Instruments, Needham Heights, MA). The creatinine level was measured via a modification of the Jaffe reaction using the Roche-Cobas-Mira analyzer (Roche Diagnostic Systems, Somerville, NJ).

RESULTS

Protocol 1

Infusion of Ang II produced the desired increase in the mean arterial pressure of 20 to 25 mm Hg. Urine flow increased modestly in the first period and decrease below the baseline value in the second period (both P = NS). Urinary sodium decreased 47% (P = .004) in the first period and 72% (P = .0001) in the second period, whereas urine osmolarity increased 39% and 69%, respectively (both P = NS). The glomerular filtration rate (GFR) did not change significantly. Both urinary ET and ANF were unchanged in the first period and decreased in the second (all P = NS). Plasma ET was 3.0 ± 0.8 pg/mL in the control period, and no significant differences were found at the 30- and 60-minute time points.

Phe induced the desired increase in the mean arterial pressure and a modest diuresis (P = NS) and natriuresis (P = .004) accompanied by significant increases in the GFR (P = .003) and a modest increase in urine osmolality (P = NS). In contrast to Ang II, Phe increased urinary excretion of ET by 344% (P = .0002) and ANF by 134% (P = .004) (Table 1). Similar to Ang II, Phe did not significantly change the plasma levels of ET. Plasma ANF increased gradually from 18 ± 2 to 27 ± 3 and 30 ± 4 pg/mL, respectively (P = .05; Table 2).

Protocol 2

Phe administered before Ang II induced 52% increases in both the urinary flow (P = .05) and GFR (P = .004), whereas urine osmolality was not changed and sodium excretion increased modestly (P = NS). Similar to the first protocol, Phe increased the excretion of both ET (P = .009) and ANF (P = .02) by 49%. Infusion of Ang II after recovery from Phe reduced urinary flow by 59% (P = .03) and sodium excretion by 65% (P = .0005), with increases in both the GFR and urine osmolality (both P = NS). Urinary excretion of ET and ANF increased by 38% (P = NS) and 56% (P = .05), respectively. Neither Phe nor Ang II significantly changed plasma levels of either ET or ANF (Table 2).

DISCUSSION

The present study demonstrates that (1) Ang II does not significantly affect the excretion of ET; (2) Phe stimulates the excretion of both ET and ANF; and (3) neither Ang II nor Phe has an effect on plasma levels of ET or ANF.

Several earlier studies reported that Ang II enhanced the release of ET both in vivo and in vitro. Emori et al[14] reported that both Ang II and arginine vasopressin stimulated ET secretion from cultured bovine endothelial cells in a dose-dependent manner. Likewise, Dohi et al[15] reported that Ang II enhanced the transcription and secretion of ET by endothelial cells derived from mesenteric arteries of spontaneously hypertensive rats. In both studies, the effect of Ang II occurred only after several hours of stimulation,

Table 1. Effects of Ang II and Phe on Renal Function and Urinary Excretion of ET and ANF

Treatment	UV (mL/min)	UNaV (Meq/min)	GFR (mL/min)	UOsmV (Osm/min)	UETV (pg/min)	UANFV (pg/min)
Protocol 1						
C1	1.1 ± 0.4	0.16 ± 0.02	143 ± 7	18.7 ± 2.2	115 ± 23	81 ± 16
C2	1.7 ± 0.5	0.17 ± 0.02	133 ± 11	15.5 ± 3.2	131 ± 21	96 ± 11
Ang II	2.2 ± 0.8	0.09 ± 0.01*	146 ± 17	21.6 ± 4.3	118 ± 26	97 ± 16
Ang II	1.1 ± 0.6	0.03 ± 0.01*	89 ± 17	26.2 ± 2.0	57 ± 18	55 ± 16
R1	1.6 ± 0.7	0.06 ± 0.01	82 ± 5	13.3 ± 2.0	72 ± 19	67 ± 11
R2	3.7 ± 1.5	0.07 ± 0.02	82 ± 5	11.9 ± 2.4	70 ± 21	68 ± 12
Phe	7.9 ± 2.0	0.19 ± 0.03*	156 ± 11*	15.2 ± 4.2	311 ± 32*	159 ± 18*
Phe	2.4 ± 0.9	0.15 ± 0.04	106 ± 12	21.4 ± 4.4	130 ± 29	79 ± 12
Protocol 2						
C1	6.4 ± 1.9	0.25 ± .03	186 ± 11	14.3 ± 3.6	205 ± 23	72 ± 8
C2	10.8 ± 1.5	0.21 ± .03	116 ± 6	7.2 ± 1.4	199 ± 20	72 ± 8
Phe	16.4 ± 1.9*	0.28 ± .04	176 ± 13*	7.0 ± 1.4	297 ± 20*	107 ± 9*
Phe	9.0 ± 1.8	0.19 ± .02	125 ± 13	6.3 ± 1.8	176 ± 25	59 ± 10
R1	6.2 ± 1.5	0.20 ± .03	101 ± 12	8.1 ± 1.6	190 ± 57	46 ± 6
R2	10.3 ± 1.6	0.17 ± .02	117 ± 7.0	5.6 ± 1.2	182 ± 19	57 ± 8
Ang II	10.1 ± 2.6	0.14 ± .01	176 ± 20	7.9 ± 2.2	251 ± 38	89 ± 14*
Ang II	4.2 ± 0.9*	0.06 ± .01*	134 ± 19	10.41 ± 3.1	127 ± 30	47 ± 6

NOTE. All data are expressed as the mean ± SEM.

Abbreviations: UV, urine flow rate; UNaV, urine sodium excretion rate; UOsmV, urine osmols excretion rate; UETV, urine ET excretion rate; UANFV, urine ANF excretion rate; C, control; R, recovery.

*P < .05 compared with immediate control.

Table 2. Effects of Ang II and Phe Infusions on Plasma Levels of ET and ANF

Treatment	Plasma ET (pg/mL)	Plasma ANF (pg/mL)
Protocol 1		
C	3.0 ± 0.8	14.5 ± 1.2
Ang II	2.5 ± 0.4	19.7 ± 2.8
Ang II	2.8 ± 0.5	19.6 ± 2.1
R	2.4 ± 0.7	17.5 ± 1.7
Phe	2.1 ± 0.4	26.6 ± 3.1
Phe	2.1 ± 0.5	29.2 ± 3.8
Protocol 2		
C	5.4 ± 0.4	13.2 ± 1.9
Phe	5.1 ± 0.5	18.5 ± 3.6
Phe	5.3 ± 0.4	15.3 ± 1.6
R	5.8 ± 0.4	16.4 ± 2.3
Ang II	5.4 ± 0.4	16.8 ± 3.1
Ang II	5.7 ± 0.5	17.7 ± 2.6

NOTE. All data are expressed as the mean ± SEM.

suggesting that the effect requires ET production rather than secretion. In addition, these investigators used supraphysiological doses of Ang II. In the present study, infusion of Ang II in normal women did not enhance the excretion of ET. Differences between the previous reports and the present study may be attributed to several factors: (1) Ang II was added for several hours in vitro, and it was infused for only 1 hour in vivo; (2) high doses of Ang II (10^{-7} mol/L) were added in vitro, whereas only low physiological doses of Ang II (mean, 12.7 ng/kg/min) were infused in humans. As additional support for this explanation, we found that infusion of supraphysiological doses of Ang II (500 ng/kg/min) in rats profoundly increased the excretion of ET, whereas physiological doses of Ang II had no effect on urinary ET. Emmeluth and Bie[17] reported that arginine vasopressin, but not Ang II increased plasma concentrations of ET. We emphasize that ET secretion by endothelial cells is polarized toward the underlying smooth muscle cells,[18] and therefore, measurement of plasma levels of ET does not reflect local production of the peptide. In addition, vascular endothelial cells contain enzymes that degrade the peptide and prevent secretion of the intact peptide into the circulation.[19]

In contrast to Ang II, Phe in equipressor doses significantly increased the excretion of both ET and ANF in the first infusion period, but not in the second. This suggests that it is not the increase in blood pressure but adrenergic stimulation that increases ET secretion. Furthermore, the lack of a Phe effect on ET excretion in the second period indicates that Phe is likely to increase the secretion of ET rather than its synthesis. The parallel increase in ET and ANF could represent an independent pathway of secretion, but may be due to an interaction between the peptides. For instance, several studies both in vivo[20] and in vitro[21-23] show that ET is a potent stimulator of ANF production. The present study, with its short infusion periods, suggests that the stimulatory effect of Phe on ET excretion is due to increased release of the peptide rather than to increased production. Possible support for this idea comes from our demonstration that Phe induced significant increases in urinary flow and sodium excretion. Increases in urinary flow and consequent reductions in the osmolality of the inner renal medulla strongly stimulate ET production.[24,25] Therefore, the observed increase in urinary ET may not represent a specific effect of Phe, but could be secondary to a "renal washout" produced by the drug.

Since filtered plasma ET is subject to degradation by neutral endopeptidase EC 3.4.24.11,[8,9] it is generally believed that urinary ET is of renal origin. Cultured endothelial, mesangial, and epithelial cells[11-13] are capable of producing significant amounts of ET. Kohan et al[11,26] reported that inner medullary collecting duct cells are the main source of renal ET production. In addition, high concentrations of ET and its mRNA are found in these cells.[27,28] Therefore, urinary ET is produced by several different types of renal cells, is not of circulatory origin, and is a good indicator of renal ET production. The kidneys are rich in sympathetic nerve fibers and adrenergic receptors that play important roles in kidney function. Our finding that Phe strongly increases renal ET output suggests that ET may be a mediator for some α-adrenergic receptors in the kidney. This hypothesis takes on added significance because of the recent demonstration that ET acts in an autocrine manner to modify sodium excretion.[29,30] In addition, our finding that Phe is more potent than Ang II in increasing urinary ET, despite their equal hypertensive effects on human subjects, indicates that increases in blood pressure per se are not the primary stimulus for ET release in the kidney.

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