The Subtype-2 (AT\textsubscript{2}) Angiotensin Receptor Regulates Renal Cyclic Guanosine 3',5'-Monophosphate and AT\textsubscript{1} Receptor-mediated Prostaglandin E\textsubscript{2} Production in Conscious Rats

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Abstract

The renal effects of angiotensin II (AII) are attributed to AT\textsubscript{1} receptors. In contrast, the function of renal AT\textsubscript{2} receptors is unknown. Using a microdialysis technique, we monitored changes in renal interstitial fluid (RIF) prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) and cyclic guanosine 3', 5'-monophosphate (cGMP) in response to dietary sodium (Na) depletion alone, or Na depletion or normal Na diet combined with the AT\textsubscript{1} receptor blocker, Losartan, the AT\textsubscript{2} receptor blocker, PD 123319 (PD), or angiotensin II, individually or combined in conscious rats. Na depletion significantly increased PGE\textsubscript{2} and cGMP. During Na depletion, Losartan decreased PGE\textsubscript{2} and did not change cGMP. In contrast, PD significantly increased PGE\textsubscript{2} and decreased cGMP. Combined administration of Losartan and PD decreased PGE\textsubscript{2} and cGMP.

During normal Na diet, RIF PGE\textsubscript{2} and cGMP increased in response to angiotensin II. Neither Losartan nor PD, individually or combined, changed RIF PGE\textsubscript{2} or cGMP. Combined administration of angiotensin II and Losartan or PD produced a significant decrease in response of PGE\textsubscript{2} and cGMP to angiotensin II, respectively.

These data demonstrate that activation of the renin-angiotensin system during Na depletion increases renal interstitial PGE\textsubscript{2} and cGMP. The AT\textsubscript{2} receptor mediates renal production of PGE\textsubscript{2}. The AT\textsubscript{2} receptor mediates cGMP. AT\textsubscript{2} blockade potentiates angiotensin-induced PGE\textsubscript{2} production at the AT\textsubscript{1} receptor. (J. Clin. Invest. 1996. 97:1978–1982.)

Key words: extracellular space • kidney • sodium • Losartan • PD 123319

Introduction

The renin–angiotensin system plays an important role in body fluid volume, electrolyte balance, and arterial pressure (1). The mechanisms whereby these actions occur remain incompletely understood. The majority of studies suggest that the renal actions of angiotensin II (AII) are mediated by angiotensin AT\textsubscript{1} receptors (2). However, AT\textsubscript{2} receptors also are present in the kidney (3) and have been reported recently to regulate pressure natriuresis in rats (4). The physiologic actions of AII at the AT\textsubscript{1} receptor have been difficult to elicit, at least in part because AT\textsubscript{2} receptors have a low degree of expression compared to that of AT\textsubscript{1} receptors (5, 6).

We conducted the present study to investigate changes in renal interstitial fluid (RIF) cyclic GMP (cGMP) and prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) by renal interstitial fluid (RIF) microdialysis during sodium depletion, a condition which is known to stimulate the renin-angiotensin system (RAS), and during normal sodium diet, in response to angiotensin AT\textsubscript{1} and AT\textsubscript{2} receptor blockade in conscious rats. In this study, we utilized a novel microdialysis technique since it has several advantages over the traditional measurements conducted in blood or urine. First, repeated blood sampling in small animals may cause unwanted hemodynamic changes. Second, RIF sampling provides the ability to monitor in vivo chemical change at almost any site in an organ or tissue. Measurement of circulating hormones/autocoids may not reflect the local changes within that organ. Third, the concentration of chemical substances in the circulation may differ from that in the interstitium, which is closer to target receptors (7). Fourth, substances (e.g., kinins) can be formed and degraded in urine and do not reflect their concentrations within the target organ (8). Fifth, the molecular weight cutoff of the microdialysis membrane can function as an initial low-resolution step in discriminating between small and large molecules and help to exclude undesirable substances (degrading enzymes and carrier proteins). The isolation of free (unbound) materials can facilitate their bioanalytical measurement in a small volume without a need for complicated extraction procedures.

Methods

In vivo renal microdialysis technique. For the determination of renal interstitial fluid cGMP and PGE\textsubscript{2}, we constructed a microdialysis probe as previously described (7–9). Each end of single 0.5-cm-long hollow fiber dialysis tubing (0.1 mm inner diameter; molecular mass cutoff, 5000 D: Hospal, Meyzieu, France) was inserted into a manu-

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1. Abbreviations used in this paper: AII, angiotensin II; cGMP, cyclic guanosine 3',5'-monophosphate; PGE\textsubscript{2}, prostaglandin E\textsubscript{2}; RIF, renal interstitial fluid.
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to Losartan. There were no significant changes in V or \( U_{Na}V \) in response to PD administration.

**RIF cGMP and PGE\(_2\) responses to dietary sodium depletion, PD or Losartan in conscious rats (n = 6).** A progressive reduction in 24-h \( U_{Na}V \) was observed during a low sodium intake. 24-h urinary sodium excretion decreased from 605\(\pm\)34 \(\mu\)mol/d during a normal sodium diet to 328\(\pm\)22, 73\(\pm\)11, 35\(\pm\)13, 30\(\pm\)11, and 30\(\pm\)10 \(\mu\)mol/d during days 1 through 5 of the low sodium intake, respectively. RIF PGE\(_2\) and cGMP levels (Fig. 2) increased significantly and progressively during dietary sodium depletion. RIF PGE\(_2\) (Fig. 2 A) and cGMP (Fig. 2 B) increased by \(\sim 3.1\)- and 2.7-fold respectively, by the fifth day of sodium depletion. At the end of the fifth day of sodium depletion, RIF PGE\(_2\) decreased significantly in response to Losartan administration (\(P < 0.001\)), while RIF PGE\(_2\) increased during PD infusion (\(P < 0.001\)). RIF cGMP (Fig. 2 B) did not change in response to Losartan, but decreased to levels observed during normal sodium intake in response to PD (\(P < 0.001\)). Combined infusion of Losartan and PD significantly decreased both RIF cGMP and PGE\(_2\) to the levels observed during normal sodium diet (\(P < 0.001\)).

**RIF cGMP and PGE\(_2\) responses to AII, Losartan or PD during normal sodium intake in conscious rats (n = 6).** RIF PGE\(_2\) (Fig. 3 A) and cGMP (Fig. 3 B) increased during AII infusion from 2.9\(\pm\)0.07 to 6.1\(\pm\)0.1 pg/min and 1.2\(\pm\)0.06 to 1.6\(\pm\)0.1 pmol/min, respectively (\(P < 0.001\)). Losartan or PD, individually or combined, did not change RIF PGE\(_2\) (Fig. 3 A) or RIF cGMP (Fig. 3 B). Combined administration of AII and Losartan decreased the RIF PGE\(_2\) response to AII from 6.2\(\pm\)0.1 to 3.8\(\pm\)0.08 pg/min, but did not change the cGMP response to AII. Combined AII and PD (Fig. 3 B) blocked the cGMP response to AII (0.9\(\pm\)0.08 vs. 1.6\(\pm\)0.1 pmol/min) (\(P < 0.01\)) and significantly increased the PGE\(_2\) (Fig. 3 A) response to AII from 3.0\(\pm\)0.1 to 10.2\(\pm\)0.4 pg/min (\(P < 0.001\)). Simultaneous administration of AII, Losartan and PD blunted both the RIF cGMP and PGE\(_2\) responses to AII (\(P < 0.001\)).

**Discussion**

This study demonstrates clearly, to our knowledge for the first time, a physiological function for the renal subtype-2 angiotensin (AT\(_2\)) receptor in the rat. The AT\(_2\) receptor antagonist, PD 123319 (PD), blocked the increase in RIF cyclic guanosine 3', 5'-monophosphate (GMP) engendered by dietary sodium depletion or by AII administration during normal sodium intake. Our data suggest that the renal AT\(_2\) receptor is tonically...
stimulated to effect a release of cyclic GMP in response to a physiologic stimulus, sodium depletion.

We have shown that sodium depletion is associated with a substantial increase in RIF AII concentrations (9) and it is highly likely that the progressive increase in RIF cyclic GMP was related to augmented AII formation. This thesis is strengthened by our observations that exogenous AII increased RIF cyclic GMP during normal sodium intake. Cyclic GMP mediates the effects of nitric oxide, the endothelium-derived vascular relaxing factor, and has been shown to be released from the renal vascular smooth muscle cell (14). AII releases nitric oxide (15), which activates guanylyl cyclase, and releases cyclic GMP into the RIF. This action of AII is mediated at the AT₂ receptor, according to our data. It is highly unlikely that cyclic GMP is modulated via the AT₁ receptor because the AT₂ antagonist, Losartan, did not affect cyclic GMP and the combination of Losartan and PD resulted in a similar reduction of cyclic GMP as did PD alone.

The infusion rate of PD employed in the present study has been shown to be specific for the AT₂ receptor and not to interact with the AT₁ receptor (2, 4, 12). Other studies using markedly higher doses of PD have demonstrated increased urine volume or free water formation (16, 17) in anesthetized animals, but PD may have influenced other (non-AT₂) angiotensin receptors in these studies. Recently, using a similar infusion rate of PD as in the present study, AT₁ receptors were found to regulate pressure natriuresis in anesthetized rats (4). The results of the present study provide a potential mechanism for this observation.

The present study also demonstrates unequivocally that AII stimulates prostaglandin E₂ (PGE₂) (A) and cyclic guanosine 3', 5'-monophosphate (cGMP) (B) during i.v. infusion of vehicle (V), Losartan (L) (10 mg/kg), PD 123319 (PD) (50 μg/kg per min), or angiotensin II (AII) (30 ng/kg/min), individually or combined in conscious rats (n = 6) on normal dietary sodium intake. Experimental data are shown in solid bars and time control data in open bars. *P < 0.001 compared with vehicle (V) or time control. **P < 0.001 compared with angiotensin II (AII) alone.

Figure 3. Renal interstitial fluid prostaglandin-E₂ (PGE₂) (A) and cyclic guanosine 3', 5'-monophosphate (cGMP) (B) during i.v. infusion of vehicle (V), Losartan (L) (10 mg/kg), PD 123319 (PD) (50 μg/kg per min), or angiotensin II (AII) (30 ng/kg/min), individually or combined in conscious rats (n = 6) on normal dietary sodium intake. Experimental data are shown in solid bars and time control data in open bars. *P < 0.001 compared with vehicle (V) or time control. **P < 0.001 compared with angiotensin II (AII) alone.
hanced by sodium depletion has not been studied to our knowledge.

The question of whether the changes in RIF cyclic GMP stimulated by AII are significant in the control of renal hemodynamic/tubular function awaits further study. Our data in anesthetized sodium depleted rats, reported here, show an increase in sodium and water excretion with Losartan but not with PD. However, since the expression of renal AT$_1$ receptors (compared to AT$_2$) is low, the high levels of angiotensin II formed during anesthesia (24) should stimulate AT$_1$ receptors and overwhelm the effects of AT$_2$ receptors on sodium excretion. Thus, the effects of PD on sodium excretion may be different in conscious animals. In the present study, it was technically impossible to measure urinary sodium excretion every 30 min in conscious rats, but we were able to monitor changes in RIF PGE$_2$ and cGMP in response to PD. It is possible that intrarenal vasodilation initiated by PD and increased PGE$_2$ formation is counter balanced by decreased nitric oxide/cyclic GMP. Further studies are needed in a conscious animal model in which renal function can be carefully monitored.

In conclusion, we have shown that activation of the renin-angiotensin system during sodium depletion in rats increases intrarenal fluid levels of PGE$_2$ and cGMP which are mediated by AT$_1$ and AT$_2$ receptors, respectively. Furthermore, the AT$_2$ receptor modulates PGE$_2$ production by angiotensin II at the AT$_1$ receptor.

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