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Interaction between endothelin and angiotensin II in the up-regulation of vasopressin messenger RNA in the inner medullary collecting duct of the rat

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Abstract

Recent studies in our laboratory have demonstrated that angiotensin (ANG) II and endothelin (ET) 1 up-regulate the expression of arginine vasopressin V_2 receptor in the inner medullary collecting duct (IMCD) of the rat. The present studies were performed to explore the interaction between ANG II and ET-1 in up-regulating the expression of arginine vasopressin V_2 receptor in the IMCD of the rat. Two sets of studies were done. In the first set of studies, rat IMCD tissue was isolated and incubated with ANG II in combination with ET_A or ET_B antagonist. In the second set of experiments, rat IMCD tissue was incubated with ET-1 with ANG receptor antagonist saralasin. Tissue samples were then analyzed by means of quantitative reverse transcriptase polymerase chain reaction and Western blotting. The ANG II treatment resulted in increased V_2 messenger RNA (mRNA) from control level of 138 ± 12 amol/μg of total RNA to 385 ± 63 amol/μg of total RNA ($P < .01$). The ANG II/ET_A treatment resulted in no significant decrease in V_2 mRNA expression (319 ± 59 amol/μg of total RNA), whereas ET-1/ET_B antagonist and ET-1/ET_A/ET_B antagonist treatments resulted in reducing V_2 mRNA to control levels of 214 ± 25 and 176 ± 22 amol/μg of total RNA, respectively. The ET-1 treatment increased V_2 mRNA expression from control level of 221 ± 25 amol/μg of total RNA to 383 ± 43 amol/μg of total RNA ($P < .02$). The ET-1–induced increase in V_2 mRNA expression was significantly reduced to control level (210 ± 36 amol/μg of total RNA) after saralasin treatment. Western blotting revealed that changes in protein expression in the different treatment conditions were comparable with changes in V_2 mRNA expression. These data suggested that the up-regulation of V_2 receptor induced by ANG II and ET-1 is mediated by both vasoconstricting hormones. These 2 systems interact in up-regulating the expression of V_2 receptors in the kidney.

1. Introduction

The angiotensin (ANG) and endothelin (ET) systems are the vasoactive hormones that have been shown to up-regulate the expression of vasopressin (AVP) V_2 messenger RNA (mRNA) in the inner medullary collecting duct (IMCD) of the kidney [1-3]. The interaction between these 2 potent vasopressor systems in up-regulating the expressions of AVP V_2 mRNA in the kidney has not been examined.

Studies have suggested a significant interaction existed between ET and ANG. Early in vitro studies showed that ANG II–induced preproET-1 mRNA in endothelial cells derived from the rat heart, aorta, and mesenteric arteries [4]. Subsequent in vitro studies also demonstrated that ANG II stimulated ET-1 production in vascular endothelial cells derived from the umbilical cord [5] and glomerular endothelial and mesangial cells [6]. Furthermore, angiotensin-converting enzyme (ACE) inhibitors can modulate the in vivo and in vitro effects of ET. The ACE inhibitor quinapril down-regulated the expression and synthesis of renal ET-1 in a normotensive model of immune-complex nephritis [7]; and in cultured mesangial cells, ACE inhibitors decreased ET-1–induced cell proliferation and matrix synthesis [8,9]. In contrast, in pulmonary artery endothelial cells, ET-1 stimulated the conversion of ANG I to ANG II [10]. Consistent with these results are studies indicating that the hypertensive effects of ANG II can be attenuated or abolished by ET_A receptor antagonist [6,11,12], demonstrating that the activation of the ET system by ANG plays a role in mediating the hypertensive actions of ANG II [13]. Thus,

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collectively, these data suggest that ET-1 and ANG II can affect the expression of ET and ANG in the kidney.

Previous studies showed that ANG and ET up-regulated the expression of AVP V_2 mRNA in the IMCD of the rat, indicating that these vasoactive peptides play an important role in mediating the expression of AVP V_2 mRNA in the kidney [1-3]. Endothelin and ANG antagonists attenuated the ET-1– and ANG II–induced increased expression of AVP V_2 mRNA in the IMCD [1-3]. The interaction of ET-1 and ANG II in mediating the up-regulation of AVP V_2 mRNA in the IMCD has yet to be elucidated. The goal of the present study was to determine if the interaction of ET-1 and ANG II is associated with the up-regulation of AVP V_2 mRNA in the IMCD of the rat.

2. Materials and methods

2.1. Isolation of IMCD

Male Wistar rats weighing 250 to 300 g purchased from UBC Animal Center (Vancouver, British Columbia, Canada) were anesthetized by an intraperitoneal injection of sodium phenobarbital (50 mg/kg; MTC Pharmaceuticals, Cambridge, Ontario, Canada). The kidneys were removed and longitudinally bisected in ice-cold phosphate-buffered saline. The IMCD was isolated from the papillary tissues by a method that was previously described [14]. The IMCD was cultured in RPMI-1640 medium with 5% bovine calf serum (HyClone, Logan, UT) and incubated for 18 hours at 37°C and 5% CO_2.

2.2. Competitive reverse transcriptase polymerase chain reaction

Competitive reverse transcriptase polymerase chain reaction (RT-PCR) was used to quantify changes in the expression of AVP V_2 mRNA in the IMCD. The RT reactions were done with a constant amount of target RNA with the corresponding RNA competitor using a single set of primers to amplify both target and competitor. The 355–base pair competitor was synthesized using the sense primer 5′-AGC AAC AGC AGC CAG GAG GAC-3′ and antisense primer 5′-GGC CCA GCA ATC AA CAC CCG CCA GGA TCA TGT AGG AGG-3′. The sense primer 5′-AGC AAC AGC CAG GAG GAA-3′ and antisense primer 5′-GGC CCA GCA ATC AAACAC CC-3′ result in a 522–base pair target product. All PCR reactions were performed using a PerkinElmer GeneAMP system (PerkinElmer, Norwalk, CT). The PCR products were analyzed using a 1.5% agarose gel (Invitrogen, Burlington, Ontario, Canada) followed by staining with ethidium bromide. The bands were scanned and quantified by computer densitometry (Multi-Image Light Cabinet; Alpha Innotech, San Leandro, CA).

2.3. Western blot

Lysates were prepared and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis. The Semi-Dry Transfer Cell (Bio-Rad, Hercules, CA) was used to transfer the protein samples onto the nitrocellular membrane. All incubations were done at room temperature with gentle agitation. Membranes were blocked in phosphate buffer containing 0.1% Tween-20 and 5% nonfat dry milk for 1 hour. After blocking, membranes were incubated in the primary and secondary antibodies for 1 hour each and were washed with 0.1% Tween phosphate-buffered saline, with agitation after each incubation. Finally, membranes were detected using enhanced chemiluminescence according to the manufacturer's protocol (Amersham, Baie d'Urfe, Quebec, Canada). Anti-rat V_2 purchased from Alpha Diagnostic International (San Antonio, TX) detects a major band at 62 kd in the rats' IMCD. This polyclonal antibody was raised against the synthetic peptide corresponding to the intracellular loop between the fifth and sixth transmembrane domain of rat V_2 receptor [15]. The bands were quantified by computer densitometry. The membrane was stripped and reprobed with β-actin to ensure equal loading of protein.

2.4. Experimental protocol

Previous studies found that IMCD incubated for 18 hours in RPMI-1640 medium containing 5% fetal calf serum at 37°C containing 5% CO_2 gave the best results [3]. Thus, all studies were carried out with 18 hours of incubation. In all of the studies performed, IMCD from one kidney of each animal was treated with an agonist and/or antagonist; and the contralateral kidney served as time control. Studies were done to determine the role of the ANG II receptor antagonist saralasin ([Sar^1, Ile^8]-ANG II; Sigma Aldrich, Oakville, Ontario, Canada) in attenuating the expression of ET-1–induced up-regulation of AVP V_2 mRNA in the IMCD. In these experiments, IMCD were incubated with saralasin (1×10^{-6} mol/L) for 2 hours at 37°C before overnight incubation with ET-1 (1×10^{-7} mol/L). Converse studies were also done to determine if ET_A-specific receptor antagonist BQ123 (10^{-6} M) (Bachem, Torrance, CA), ET_B-specific receptor antagonist BQ788 (1×10^{-6} mol/L) (Bachem), and both BQ123 and BQ788 could mitigate the ANG II–induced up-regulation of AVP V_2 mRNA in the IMCD.

2.5. Statistics

The value of n represents the number of rat kidneys for each treatment conditions. Data are shown as mean ± SEM, and Student t test was used for statistical comparison. P values less than .05 were considered statistically significant.

3. Results

3.1. AVP V_2 receptor expression after incubations with ANG II and ET_A and ET_B receptor antagonists

The effects of ANG II in combination with ET_A- and ET_B-specific receptor antagonists on V_2 receptor and receptor mRNA expression are shown in Fig. 1. Eighteen hours' incubation of rat IMCD cells with 1×10^{-7} mol/L ANG II

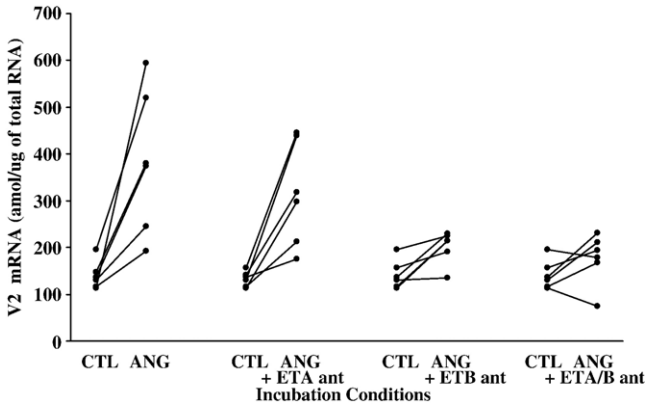

Fig. 1. The V_2 receptor mRNA expression in IMCD treated with ANG II in combination with ET_A, ET_B, and ET_A/ET_B receptor specific antagonists.

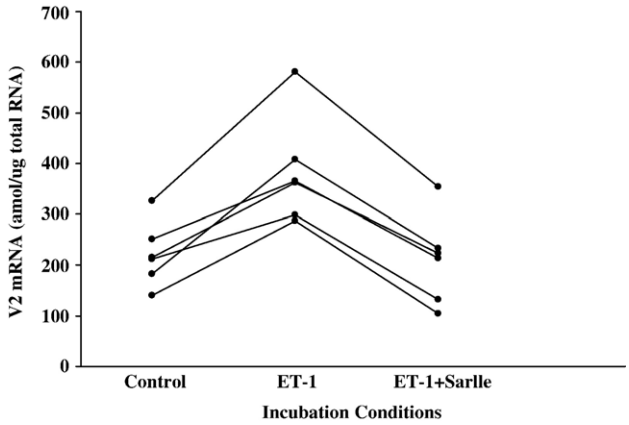

Fig. 3. The V_2 receptor mRNA expression in IMCD treated with ET-1 in combination with saralasin, an ANG receptor specific antagonist.

resulted in a significant increase in V_2 receptor mRNA expression from a control value of 138 ± 12 amol/μg of total RNA to 385 ± 63 amol/μg of total RNA (n = 6, $P < .01$) as determined by RT-PCR (Fig. 1). The ET_A- and ET_B-specific receptor antagonists were added with ANG II in different combinations to determine if ET receptors are mediating the ANG II signaling. Incubating IMCD with a combination of ANG II and ET_A receptor antagonist resulted in no change in V_2 receptor mRNA expression (319 ± 59 amol/μg of total RNA) compared with IMCD just treated with ANG II (n = 6, not significant) (Fig. 1). Western blotting also showed no significant change in V_2 receptor protein expression compared with samples treated solely with ANG II (Fig. 2). In contrast, when IMCD was incubated with ANG II and ET_B receptor antagonist, the level of V_2 receptor mRNA expression fell from 385 ± 63 amol/μg of total RNA to 214 ± 25 amol/μg of total RNA (n = 6, $P < .02$) (Fig. 1). There was also a 34% reduction in protein expression (0.54 ± 0.06) compared with samples treated with ANG II (0.82 ± 0.15) (n = 6, $P < .05$) (Fig. 2). Using both ET_A and ET_B receptor antagonists in conjunction with ANG II, V_2 receptor mRNA expression levels were found to be not different from that of controls (176 ± 22 amol/μg of total RNA) (n = 6, not significant) (Fig. 1). Dual-antagonist treatment reduced relative V_2 receptor protein expression from 0.82 ± 0.15 to 0.43 ± 0.06 (n = 5, $P < .03$) (Fig. 2).

3.2. Effect of saralasin on ET-induced changes in AVP V_2 receptor mRNA and protein expression

Saralasin is a specific ANG II receptor antagonist used in this study to investigate the role of ANG II in mediating the increase in AVP V_2 receptor expression by ET. Endothelin-1 resulted in an increase in AVP V_2 receptor mRNA expression from a control level of 221 ± 25 amol/μg of total RNA to 383 ± 43 amol/μg of total RNA (n = 6, $P < .02$) (Fig. 3). Saralasin treatment inhibited ET-1 from inducing an increase in AVP V_2 receptor mRNA expression. The AVP V_2 receptor mRNA expression with saralasin was found to be 210 ± 36 amol/μg of total RNA, which was comparable with the

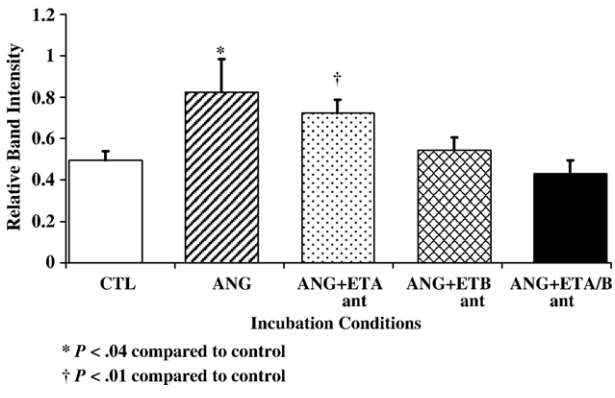

Fig. 2. The AVP V_2 receptor protein expression after the treatments with ANG II and with ANG II in combination with ET_A, ET_B, and ET_A/ET_B receptor specific antagonists. Significance of ANG II vs control, $P < .04$. Significance of ET_A receptor specific antagonist vs control, $P < .01$.

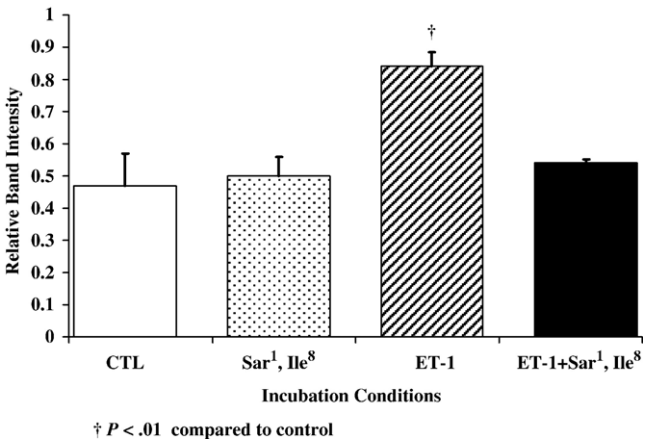

Fig. 4. The AVP V_2 receptor protein expression in IMCD treated with ET-1 and with ET-1 in combination with saralasin, an ANG receptor specific antagonist. Significance of ET-1 vs control, $P < .01$.

control level (n = 6, not significant) (Fig. 3). Western blotting results were comparable with RT-PCR data. Overnight incubation with ET-1 increased V_2 receptor protein expression from control level of 0.47 ± 0.13 to 0.84 ± 0.19 (n = 6, $P < .01$) (Fig. 4). The V_2 receptor protein expression demonstrated no change when incubated solely with saralasin (0.50 ± 0.11, n = 4, not significant) (Fig. 4). However, the ET-1–induced increase in the expression of V_2 protein was brought back to control level in the presence of saralasin (0.54 ± 0.10, n = 6, not significant) (Fig. 4).

4. Discussion

We have previously shown that AVP V_2 receptors were up-regulated in the IMCD of cardiomyopathic hamsters [2,16]. These results suggested that the increase in V_2 receptors in the kidney could contribute to fluid retention seen in congestive heart failure. Subsequent in vitro studies showed that the up-regulation of V_2 receptors in the IMCD could be mediated by ANG II and ET [3], thus suggesting that the elevation of circulating levels of ANG II and ET in cardiomyopathic hamster plays a role in the up-regulation of AVP V_2 receptors in the kidneys of these animals. This notion was supported by studies with enalapril, an ACE inhibitor, or bosentan, a nonspecific ET receptor antagonist that reduced the up-regulation of AVP V_2 receptors in the kidney of cardiomyopathic hamsters to normal levels [2,16]. Previous studies have shown a close relationship between ET and ANG II [4-10], and the present report provides support that such interactions are involved in up-regulating the expression of AVP V_2 receptors.

Studies have demonstrated that hypertension and renal vasoconstriction induced by chronic administration of ANG II can be blocked by ET antagonists, indicating that ET mediates the action of ANG II [17,18]. This observation is confirmed by more recent clearance studies showing that vasoconstriction, diuresis, and natriuresis from ANG II infusion are mediated by ET [19]. Furthermore, ANG promotes ET production [4-6]. In view of these results, it is possible that the ANG II–induced up-regulation of AVP V_2 in the IMCD [3] is in part mediated by ET. We incubated IMCD with ANG in the presence and absence of ET antagonists to test this hypothesis. Our results showed that the ANG II–induced up-regulation of AVP V_2 receptors was abolished by ET_B receptor antagonist but was not blunted by ET_A receptor antagonist. These data suggested that the ET_B receptor mediates the ANG II–induced up-regulation of AVP V_2 receptor in the kidney. This observation is not surprising because both ET receptors ET_A and ET_B are known to be present in the IMCD and the rest of the kidney; however, the ET_B receptor that exists in the IMCD is about 4 times greater than that of ET_A [3,20,21]. On the other hand, our results also indicated that the ET-1–induced up-regulation of AVP V_2 receptors was attenuated by saralasin, an ANG receptor antagonist. This interrelationship between ANG and ET was demonstrated before in both in vivo and in vitro studies. The increase in preproET-1 mRNA levels and ET-1 protein in several structures of nephritic rats was down-regulated by ACE inhibitor [7]. Furthermore, in cultured mesangial cells, ACE inhibitor decreased cell proliferation and matrix synthesis induced by ET-1 [8]. It seems that these 2 vasoconstricting systems interact with each other to mediate the up-regulation of AVP V_2 receptors. Furthermore, the present experiments were conducted in isolated IMCD under cultured condition; it is unlikely that these effects are mediated by hemodynamic changes induced by these vasoactive peptides.

The mechanism by which ANG II and ET interact to induce up-regulation of AVP V_2 receptors in the present study is not identified. A previous report shows that ANG II up-regulation of the expression of AVP V_2 receptors in the IMCD of the kidney can be inhibited by ANG II receptor antagonist [Sar^1, Ile^8]-ANG II [3], and the present study suggests that ET_B antagonist can also dampen this up-regulation. That the presence of ET_B antagonist blunted the ANG II–induced up-regulation of AVP V_2 receptors in the present report argues in favor of ANG II also acting indirectly to promote its effect on AVP V_2 receptors by stimulating ET synthesis. It is possible that this up-regulation of AVP V_2 receptors can be mediated directly or indirectly by ANG II. It is known that ANG II regulates ET-1 release by cultured endothelial cells through an angiotensin I receptor–dependent pathway [5]. The present study cannot delineate the contribution of direct and indirect effect of ANG II in up-regulating the expression of AVP V_2 receptors in the kidney in vivo. Conversely, the ANG receptor antagonist also blunted the ET-1–induced up-regulation of AVP V_2 receptors, suggesting that ET-1 also mediates its effect by promoting the action of ANG II. Early reports have shown that ET-1 can increase the formation of ANG II by raising plasma renin activity and/or by enhancing ACE activity [10,22]. Studies have also shown that the ET-1 increases in the expression of AVP V_2 receptors in the kidney are mediated by ET_B receptor [2]. Therefore, it seems that that ET also has a direct and indirect effect in up-regulating the expression of AVP V_2 receptors in the kidney. These 2 vasoactive substances signal through multiple common intracellular pathways [23,24], and it would seem that ET and ANG might interact with each other in the intracellular cascades in promoting the increased expression of AVP V_2 receptors in the IMCD of the kidney. The natures of these interactions remain to be determined.

The physiological significance of the interaction between ET and ANG receptors in up-regulating the expression of AVP V_2 in the IMCD was not evaluated in the present study. As we have discussed, studies have shown that ANG can increase synthesis of ET; and furthermore, ANG receptors regulate ET receptors by interaction and modulation of receptor expression [25]. The addition of ANG or ET into the cultured medium activates both receptors either directly or indirectly. A possible explanation for the observed results is

that activation of both ANG and ET receptors by ANG or ET is required in promoting up-regulation of AVP V_2 receptors in the IMCD. The blocking of the ANG or ET receptors by antagonists prevented the up-regulation of AVP V_2 receptors induced by these hormones. This possibility remains to be examined. Studies with Na/H antiporter activities with OKP cells showed that the BQ788 or saralasin blocked this antiport activation [26]. It is likely that the BQ788 or saralasin used in the present study can block the up-regulation of AVP V_2 receptors.

In conclusion, our results suggested that ANG II and ET receptor antagonists can blunt ET-1– and ANG II–induced up-regulation of AVP V_2 receptors in IMCDs, respectively. The data presented here provide evidence that the up-regulation of AVP V_2 receptors by ANG II or ET-1 was in fact mediated by both vasoconstricting hormones. These 2 systems interact in up-regulating the AVP V_2 receptors in the kidney.

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