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ROLE OF ANGIOTENSIN AT₂ RECEPTORS IN NATRIURESIS: INTRARENAL MECHANISMS AND THERAPEUTIC POTENTIAL

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SUMMARY

1. The renin-angiotensin system is a coordinated hormonal cascade critical for the regulation of blood pressure (BP) and kidney function. Angiotensin II (Ang II), the major angiotensin effector peptide, binds to two major receptors, type-1 (AT₁Rs) and type-2 (AT₂Rs). AT₁Rs engender antinatriuresis and raise BP, whereas AT₂Rs oppose these effects, inducing natriuresis and reducing BP
2. AT₂Rs are highly expressed in the adult kidney, especially in the proximal tubule. In AT₂R-null mice, long-term Ang II infusion results in pressor and antinatriuretic hypersensitivity compared to responses in wild-type animals.
3. The major endogenous receptor ligand for AT₂R-mediated natriuretic responses appears to be des-aspartyl¹-Ang II (Ang III) instead of Ang II. Recent studies have demonstrated that Ang II requires metabolism to Ang III by aminopeptidase A in order to induce natriuresis and that inhibition of aminopeptidase N increases intrarenal Ang III and augments Ang III-induced natriuresis.
4. The renal dopaminergic system is another important natriuretic pathway. Renal proximal tubule D₁-like receptors (D₁LIKERs) control approximately 50% of basal sodium (Na⁺) excretion. We have recently found that natriuresis induced by proximal tubule D₁LIKERs requires AT₂R activation and that D₁LIKE stimulation induces recruitment of AT₂Rs to the apical plasma membrane via a cyclic AMP-dependent mechanism.
5. Initial studies employing potent AT₂R non-peptide agonist Compound 21 demonstrate natriuresis in both the presence and absence of AT₁R blockade indicating the therapeutic potential of this compound in fluid retaining states and hypertension.

Keywords

Renin-angiotensin system; Angiotensin; Receptors; Natriuresis; Dopamine; Dopamine Receptors; Hypertension

INTRODUCTION

The renin-angiotensin system (RAS) is a coordinated hormonal cascade critical for the regulation of blood pressure (BP) and kidney function [1]. Angiotensin II (Ang II), the most important RAS effector peptide, classically acts by binding to two major Ang receptors, type-1 (AT₁Rs) and type-2 (AT₂Rs) [1–3]. Most of the physiological actions of Ang II are transduced via AT₁R activation, including cellular dedifferentiation and proliferation; vasoconstriction; reduction of vascular compliance; cardiac contractility; increased renal tubule sodium (Na⁺) reabsorption; aldosterone, vasopressin and endothelin secretion; salt appetite; thirst; and stimulation of the sympathetic nervous system [1,3]. Knowledge of AT₂R functions and their mechanisms is less well understood than those of AT₁Rs. However, AT₂Rs have been found to oppose Ang II actions, especially vasoconstriction and cellular proliferation, via AT₁Rs under the majority of circumstances [1–4]. Indeed, the only instance to our knowledge in which AT₁Rs and AT₂Rs have been found to act in concert is in the negative feedback suppression of renin secretion directly at the renal juxtaglomerular cell [5].

AT₂Rs are 7-transmembrane G protein-coupled receptors encoded by a single copy gene on the X chromosome and composed of 363 amino acids (molecular weight 41,220 Da) with only 34% amino acid sequence homology with AT₁Rs [2]. The main sequence homology between AT₁Rs and AT₂Rs occurs in the transmembrane hydrophobic regions of the molecules which form their 7-transmembrane helical columns [2].

AT₂R SIGNALING PATHWAYS

AT₂R signaling mechanisms have been found to differ substantially from those of AT₁Rs [1–4,6]. As summarized in Figure 1, **Panel A**, AT₂R activation initiated by binding of Ang II to the receptors on the plasma membrane triggers G protein coupling through G_{iα2} and G_{iα3} via the third intracellular loop of the receptor. This signal activates phosphotyrosine phosphatases, the function of which is to inactivate mitogen-activated protein (MAP) kinases, such as extracellular signal-regulated kinase (ERK)-1 and ERK-2. Phosphotyrosine phosphatase activation can also occur through a G protein-independent mechanism. MAP kinase inhibition by AT₂Rs is thought to be a major signaling mechanism by which AT₂Rs oppose the actions of AT₁Rs, which activate these kinases. AT₂Rs can also activate lipid signaling pathways including increased phospholipase A2 activity and arachidonic acid release, and long-term AT₂R activation can increase the biosynthesis of ceramides, which can activate stress kinases and caspases to induce apoptosis [6].

A major AT₂R signaling pathway, involved in almost all of the reported physiological actions of AT₂Rs is the bradykinin (BK) – nitric oxide (NO) – cyclic GMP (cGMP) signaling cascade shown in Figure 1, **Panel B** [1,3,7–12]. AT₂Rs have consistently been found to increase the production of NO and cGMP either by stimulating an increase in BK production with consequent activation of BK B₂ receptors or by direct activation of NO production independently of BK [12]. This pathway is critical, for example, for AT₂R-induced vasodilation [1,3,4,6,13].

AT₁R/AT₂R INTERACTIONS IN NATRIURESIS

The RAS is a primary regulator of BP, fluid and electrolyte balance and is thought to be a key pathophysiological driving force for the development of hypertension. As most effectively articulated by Guyton and colleagues, the capacity of the kidney to excrete Na⁺ via the pressure-natriuresis mechanism is central in the regulation of BP [14,15]. Pressure-natriuresis is the protective mechanism by which an increase in BP and thereby renal perfusion pressure increases Na⁺ excretion bringing BP back towards control levels. The elegant servo-control studies by Hall and colleagues defined the importance of pressure-natriuresis as the mechanism of escape from the Na⁺-retaining consequences of a variety of hormonal influences, including those of norepinephrine, angiotensin II and aldosterone [16–18]. Under the Guyton hypothesis, there must be a defect in renal Na⁺ handling in order to sustain a chronic increase in BP and the development of hypertension [14,15]. Over the years, this hypothesis has been substantiated by kidney transplantation studies in genetically hypertensive rats showing that hypertension follows the transplanted kidney [19–21].

Recent work by the Coffman laboratory using cross-transplantation studies has confirmed the essential role of renal AT₁Rs in the development of hypertension induced by Ang II [22–24]. Renal AT₁Rs were required to sustain a hypertensive response to continuous Ang II infusion for two weeks in mice [23]. In particular, mice with AT₁Rs specifically deleted from renal proximal tubule cells using Cre-lox technology under the control of a proximal tubule-specific *Pepck* promoter) had a 10 mmHg reduction in baseline BP accompanied by a 36% decrease in proximal tubule Na⁺/fluid reabsorption together with reduced expression of key proximal tubule apical Na⁺ transporter, Na⁺-hydrogen exchanger-3 (NHE-3) [24]. Importantly, these mice were protected from the antinatriuretic and pressor actions of infused Ang II [24]. Sigmund and colleagues also recently demonstrated that AT₁R knockout specifically in the proximal tubule reduced baseline BP by approximately 14 mmHg and that AT₁R over-expression in the proximal tubule increased BP by approximately 15 mm Hg [25,26]. These studies underscore the importance of renal proximal tubule AT₁Rs in the control of BP by means of their effects on Na⁺ reabsorption.

While the effects of AT₁Rs on Na⁺ reabsorption and BP are now relatively well understood, the potential role of AT₂Rs in counteracting these actions is less appreciated. The purpose of this Mini-Review is to provide an up-to-date summary of the renal actions of AT₂Rs with an emphasis of their role in the control of Na⁺ excretion and BP.

ANGIOTENSIN PEPTIDES AND AT₂R-INDUCED NATRIURESIS

In the fetus, AT₂Rs are expressed throughout the kidney, and their expression level decreases markedly during the post-natal period [27–29]. Nevertheless, in adulthood, AT₂Rs continue to be expressed in glomeruli as well as vascular and tubule cells, albeit in smaller quantities [28,29]. However, AT₂Rs are highly expressed in the adult proximal tubule [28,29].

Definitive evidence now exists that renal AT₂Rs mediate natriuresis [30–35]. This hypothesis was initially strongly suggested by AT₂R knockout studies in mice wherein receptor-null mice had markedly exaggerated antinatriuretic responses to systemically

infused Ang II compared to wild-type mice [36]. AT₂R-null mice also had marked pressor hypersensitivity to Ang II, and their pressure-natriuresis mechanism was markedly impaired. The mechanism of the antinatriuresis in AT₂R-null mice was identified as reduction in intrarenal generation of BK, NO and cGMP in these studies [36].

Further investigation of the role of endogenous renal AT₂Rs in natriuresis incorporated the novel technique of direct renal interstitial microinfusion of pharmacological and molecular probes in mice and rats, enabling the direct evaluation of renal function without systemic hemodynamic or hormonal influences. With this technique, selective intrarenal AT₁R blockade with candesartan in rats induced a highly significant natriuresis that was abolished with concurrent intrarenal administration of the AT₂R-specific antagonist PD-123319 (PD) [30]. These results suggested that the natriuretic response to intrarenal angiotensin receptor blockade is dependent upon Ang activation of unblocked AT₂Rs.

ANGIOTENSIN III (Ang III) AND AT₂R-INDUCED NATRIURESIS

We were surprised to find, however, that in the presence of systemic AT₁R blockade, direct intrarenal administration of Ang II, even at very high infusion rates, was unable to induce a natriuretic response [30]. Because the heptapeptide des-aspartyl¹-Ang II (Ang III), an Ang II metabolite, is highly active at AT₂Rs in the brain, we investigated whether Ang III might be an agonist for AT₂R-mediated responses in the kidney [30]. When systemic AT₁Rs were blocked, direct intrarenal Ang III infusion induced a highly significant increase in renal Na⁺ excretion that was abolished by concurrent AT₂R blockade with PD. However, intrarenal Ang III infusion alone demonstrated no effect on renal Na⁺ excretion in the absence of systemic AT₁R blockade [30].

To explain the effectiveness of Ang III, but not Ang II, to induce natriuresis, we hypothesized that Ang II must be converted to Ang III in order to interact with AT₂Rs within the kidney [31]. Ang II is converted to Ang III by aminopeptidase A (APA), and Ang III is converted to the hexapeptide angiotensin IV (Ang IV) by aminopeptidase N (APN). In the presence of systemic AT₁R blockade, intrarenal infusion of Ang III again resulted in a natriuretic response, which was markedly augmented by intrarenal co-administration of the APN inhibitor 2-amino-4-methylsulfonyl-butane-thiol, methane-thiol (PC-18) [31]. The augmentation in Ang III-induced natriuresis due to APN inhibition was also abolished by intrarenal AT₂R blockade with PD [31]. Intrarenal infusion of the canonical Ang receptor ligand Ang II induced a natriuretic response only in the presence of APN inhibition and this response was blocked completely by concomitant intrarenal APA inhibition with 3-amino-4-thio-butyl-sulfonate (EC-33) [32]. These results strongly suggest that Ang III, not Ang II, is the canonical AT₂R ligand in the regulation of renal Na⁺ excretion and that Ang II must be converted to Ang III to mount an AT₂R-dependent natriuretic response (Figure 2).

These studies are potentially important for disease states such as type-2 diabetes mellitus and obesity, wherein AT₂Rs are upregulated in renal proximal tubule cells, inhibit Na⁺/K⁺ ATPase (NKA) and reduce Na⁺ transport [35–37]. The inhibition of NKA appears to be mediated via a NO/cGMP-dependent pathway, and inhibition of NAD(P)H oxidase potentiates AT₂R-mediated natriuresis [37].

The exact mechanism whereby the AT₂R responds to Ang III and not to Ang II in the nephron is currently unknown. However, it is likely to be due to the role of the N-terminal arginine in stabilizing the binding pocket of the AT₂R allowing optimal binding of the remaining c-terminal amino acid residues. In addition to the kidney, two other cardiovascular hormonal systems have been linked to Ang III as the preferred agonist for AT₂Rs, the coronary vascular bed and the adrenal zona glomerulosa. In the coronary microcirculation, Ang III, rather than Ang II, is the preferred ligand to induce AT₂R-mediated vasodilation [38]. Ang III is also the preferred agonist for AT₂R-mediated aldosterone secretion from the adrenal cortex [39]

Recent *in vivo* studies using endogenous Na⁺/lithium clearance techniques, indicate that renal AT₂Rs engender natriuresis mainly by reducing Na⁺ reabsorption in the renal proximal tubule without systemic or intrarenal hemodynamic changes [40]. Endogenous intrarenal Ang III was shown to be the major agonist for the natriuretic response, which could not be induced either by Ang II or Ang (1–7) [40]. Ang III-induced natriuresis was accompanied by an increase in renal cGMP levels and was abolished by soluble guanylyl cyclase inhibitor 1-H-(1,2,4) oxadiazolo-(4,2- α) quinoxalin-1-one (ODQ), strongly suggesting that Ang III-induced natriuresis is mediated by renal production of cGMP [40].

Recent studies also have shown that AT₂Rs may induce natriuresis in part through an action in the thick ascending limb of Henle (TAL) [41,42]. Ang II increases NO production in TALs by activation of AT₂Rs, and NO inhibits the Na⁺/K⁺/2Cl⁻ co-transporter, thereby reducing Na⁺ reabsorption in this nephron segment. However, no effort was made to determine if Ang II conversion to Ang III is required to inhibit Na⁺ reabsorption in these studies. Interestingly, the natriuretic effect of TAL AT₂R activation is decreased in Dahl salt-sensitive rats, a model of salt-sensitive hypertension in humans in which Na⁺ retention occurs predominantly in TAL.

In vitro studies also have recently shown that renal AT₂Rs decrease AT₁R function in the proximal tubule by the common NO/cGMP pathway and also reduce AT₁R expression at the transcriptional level via the ubiquitous transcription factor Sp1 [43]. AT₂Rs also have been shown following ligand activation to dimerize with AT₁Rs, reducing their expression level via a direct protein-protein interaction at the cell surface. Therefore, renal AT₂Rs may oppose AT₁R actions by multiple signaling pathways [43].

In most studies so far reported, Ang III-induced natriuresis has required the concurrent blockade of systemic AT₁Rs. However, we recently demonstrated that the heptapeptide is capable of inducing natriuresis in the absence of systemic AT₁R blockade if intrarenal Ang III levels are increased by APN inhibition [40]. Therefore, in a pure physiological sense, intrarenal Ang III probably does not play a significant role in the regulation of Na⁺ excretion when AT₁Rs are intact because the antinatriuresis by Ang II acting at AT₁Rs with substantially greater renal expression levels would overwhelm the ability of AT₂Rs to induce natriuresis. Thus, AT₂Rs would be expected to induce natriuresis when AT₁Rs are blocked or when APN is blocked in the absence of AT₁R inhibition.

We also conducted a series of experiments to clarify whether endogenous Ang III is capable of inducing natriuresis in the normal rat [40]. In the AT₁R-blocked rat, we found that intrarenal administration of APN antagonist PC-18 alone was able to increase Na⁺ excretion in a sustained fashion and that the natriuresis was abolished by concurrent AT₂R blockade [40]. Therefore, endogenous intrarenal Ang III induces natriuresis via AT₂R activation under certain circumstances.

AT₂R-DOPAMINE RECEPTOR INTERACTIONS IN NATRIURESIS

The renal dopaminergic system is an important regulator of renal Na⁺ excretion and blood pressure. Dopamine (DA) is synthesized from filtered L-dihydroxyphenylalanine (L-DOPA) transported from the nephron lumen into proximal tubule cells [44]. The synthesized DA is secreted from the proximal tubule cell predominantly across the apical plasma membrane into the tubule lumen, where it binds to D₁-like receptors (D₁LIKE_R, D₁ and D₅ subtypes). D₁LIKE_R activation inhibits Na⁺ reabsorption into the proximal tubule cell through an adenylate cyclase - cAMP - signaling mechanism [44]. Systemic administration of fenoldopam, a highly selective D₁LIKE_R agonist, in animals and humans elicits a robust natriuretic response that is based almost exclusively upon inhibition of proximal tubule Na⁺ reabsorption [45–48]. Renal dopaminergic tone accounts for approximately 50–60% of basal Na⁺ excretion, as selective intrarenal administration of SCH-23390 (SCH), a highly selective D₁LIKE_R antagonist, induces antinatriuresis of this magnitude in dose-dependent fashion under conditions of moderate Na⁺ balance [49]. Conversely, intrarenal fenoldopam administration induces a highly significant natriuresis that is blocked completely by SCH in the sodium-loaded rat [48]. Surprisingly, however, we discovered that the natriuretic response to fenoldopam was also abolished by concomitant intrarenal administration of AT₂R antagonist PD [48,50].

To learn the possible mechanism of AT₂R participation in D₁LIKE_R - induced natriuresis, we investigated cellular AT₂R trafficking in renal proximal tubule cells [48,50]. Fenoldopam administration *in vivo* was accompanied by translocation of AT₂Rs from intracellular sites to the apical plasma membranes of renal proximal tubule cells [48,50]. Neither total nor basolateral proximal tubule cell AT₂R expression was changed in response to fenoldopam, suggesting that apically distributed AT₂Rs are involved in the natriuretic response [48]. D₁LIKE_Rs translocate to the cell surface in response to D₁LIKE_R activation in cultured kidney cells, kidney section preparations, and isolated proximal tubules and this response requires an intact microtubule network [51]. Recently, we extended these findings to the natriuretic mechanism of renal AT₂Rs *in vivo* [50]. Fenoldopam-induced natriuresis and AT₂R translocation were completely abolished during intrarenal co-infusion of nocodazole (NOC), which disrupts the microtubule network but preserves the actin microfilament cytoskeleton of renal proximal tubule cells [50]. Thus, microtubules are not only necessary for D₁LIKE_R recruitment but also for AT₂R recruitment in response to fenoldopam, a common trafficking pathway for the natriuretic function of these receptors.

Since D₁LIKE_Rs signal through cAMP/protein kinase A to mediate natriuresis, the role of renal interstitial cAMP generation in AT₂R-mediated natriuresis was investigated *in vivo* [50]. Renal interstitial accumulation of cAMP [via direct activation of adenylyl cyclase with

forskolin and selective inhibition of cAMP degradation with 3-isobutyl-1-methylxanthine (IBMX)] caused a significant and sustained increase in AT₂R-mediated natriuresis [50]. Direct agonist stimulation of D₁LIKERs was not necessary for AT₂R-mediated natriuresis since forskolin+IBMX-induced natriuresis persisted in the presence of D₁LIKER blockade with SCH [50]. Thus, one mechanism by which AT₂Rs and D₁LIKERs interact during high Na⁺ balance states to mediate natriuresis is D₁LIKER-cAMP signaling, which provides the stimulus necessary for AT₂R translocation and natriuresis. Because the effect is independent of specific D₁LIKER-induced activation of adenylyl cyclase, stimulation of other receptors that signal through cAMP/protein kinase A pathway may be advantageous in promoting AT₂R-mediated natriuresis *in vivo*. Indeed, administration of parathyroid hormone, through its cAMP/protein kinase A-dependent but not phospholipase C/protein kinase C-dependent signaling, has been shown to redistribute Na⁺ transporters such as Na⁺-hydrogen exchanger-3 and inhibit Na⁺-K⁺-ATPase activity in a direction favoring natriuresis and diuresis [52]. Renal AT₂Rs are known to inhibit Na⁺-K⁺-ATPase activity, and increased renal cAMP may regulate this process [35,53].

The above-mentioned *in vivo* results in experimental animals have been confirmed by recent *in vitro* studies in a human renal proximal tubule cell line showing that D₁R, but not D₅R, stimulation of AT₂R plasma membrane recruitment is cAMP- and protein phosphatase 2A (PP2A)-dependent [54]. These studies also demonstrated that D₁R stimulation recruits plasma membrane AT₂Rs from very low quantities to levels comparable to those of AT₁Rs and that AT₂R recruitment to the cell surface is required for its various functions [54].

THERAPEUTIC IMPLICATIONS OF RENAL AT₂R-INDUCED NATRIURESIS

From the foregoing discussion, it is apparent that renal AT₂Rs are natriuretic receptors, counter-balancing the Na⁺ retaining actions of Ang II via AT₁Rs. Whereas the primary AT₁R agonist in the kidney is Ang II, the preferred AT₂R agonist governing Na⁺ excretion is the Ang II metabolite Ang III. Either blocking renal AT₁Rs, indirectly activating renal AT₂Rs, or directly activating AT₂Rs with their endogenous ligand Ang III induces natriuresis (Figure 3). Evidence also has been provided that AT₂R recruitment to the plasma membrane and activation are required for renal D₁R-induced natriuresis. Thus, AT₂R activation appears to be common to the natriuretic capacity of two of the most powerful hormonal systems, the intrarenal RAS and the renal dopaminergic system, governing Na⁺ homeostasis in the body. Consequently, targeting AT₂Rs would be predicted to offer a new, potentially complimentary therapeutic approach to AT₁R blockade in disorders of fluid retention and hypertension.

Is there any existing evidence that AT₂R activation might hold therapeutic potential? Compound 21 (C21) is a new, potent, highly selective non-peptide AT₂R agonist [55,56]. Three papers, published in 2012, reported the natriuretic effects of C21 in experimental animals [40,57,58]. Kemp *et al.* published that systemic C21 administration (0.3 µg/kg/min) increased urinary Na⁺ excretion (U_{Na}V) by 12-fold in male uninephrectomized Sprague-Dawley rats [40]. The natriuretic response was significantly augmented by AT₁R blockade and was abolished with concomitant renal interstitial administration of AT₂R antagonist PD [40]. Hilliard *et al.* reported that intravenous C21 infusion (0.3 µg/kg/min) induced an

approximately 3-fold increase $U_{Na}V$ and fractional excretion of Na^+ (FE_{Na}) that was abolished by intravenous co-infusion of AT_2R antagonist PD in normotensive male and female rats [57]. Ali and Hussain also reported that intravenous C21 (5.0 $\mu g/kg/min$; a 16-fold higher dose) increased $U_{Na}V$, FE_{Na} and fractional excretion of lithium (FE_{Li}) and that this response was neutralized with systemic PD coinfusion in obese Zucker rats [58]. These three studies encourage further research on the therapeutic potential of pharmacologic AT_2R activation, possibly in combination with AT_1R blockade, in the treatment of disorders associated with fluid retention and hypertension.

ABBREVIATIONS

Ang II	angiotensin II
Ang III	des-aspartyl ¹ -angiotensin II
APA	aminopeptidase A
APN	aminopeptidase N
AT_1R	angiotensin type-1 receptor
AT_2R	angiotensin type-2 receptor
BK	bradykinin
BP	blood pressure
cAMP	cyclic adenosine monophosphate
cGMP	cyclic guanosine monophosphate
DA	dopamine
$D_{1LIKE}R$	dopamine D_1 -like receptor
ERK	extracellular signal-regulated kinase
FE_{Li}	fractional excretion of lithium
FE_{Na}	fractional excretion of sodium
IBMX	3-isobutyl-1-methylxanthine
MAP	mitogen-activated protein
L-DOPA	L-dihydroxyphenylalanine
Na^+	sodium
NHE-3	sodium-hydrogen exchanger-3
NKA	sodium/potassium ATPase
NO	nitric oxide
ODQ	soluble guanylyl cyclase inhibitor
PD	PD-123319
PP2A	protein phosphatase 2A

RAS	renin-angiotensin system
SCH	Schering-23390, D ₁ -like receptor antagonist
TAL	thick ascending limb of Henle
U_{Na}V	urinary sodium excretion

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Figure 1.

A. Schematic representation of the major intracellular signaling pathway of AT₂Rs. MAP = mitogen-activated protein; ERK = extracellular-regulated kinase; AT₁R = angiotensin type-1 receptor. **B.** Schematic representation of the most important extracellular signaling pathway of AT₂Rs: the autocrine/paracrine bradykinin (BK)- nitric oxide (NO)-cyclic GMP (cGMP) cascade. Recent studies also suggest that NO and cGMP may function as signaling molecules intracellularly in nuclei and mitochondria. Ang II = angiotensin II; HMW = high molecular weight; GTP = guanosine triphosphate; sGC = soluble guanylyl cyclase.

Figure 2.

Schematic diagram depicting the necessity of conversion of Ang II to Ang III for the induction of a natriuretic response via AT₂R activation. PC-18, a selective aminopeptidase N inhibitor; EC-33, a selective aminopeptidase A inhibitor.

Figure 3.

Schematic diagram of the renal proximal tubule summarizing the Interrelationships among AT₁Rs, AT₂Rs and D₁Rs in the control of renal Na⁺ reabsorption. AT₁R antagonists inhibit Na⁺ reabsorption leading to natriuresis via activation of unblocked AT₂Rs. D₁LIKE R activation with selective agonist fenoldopam (FEN) induces natriuresis via AT₂R activation. Ang III, the preferred endogenous AT₂R agonist, activates AT₂Rs engendering natriuresis. Compound 21 (C-21), a highly selective non-peptide AT₂R agonist, activates AT₂Rs inducing natriuresis. For diagrammatic purposes, the receptors are shown on the basolateral membranes of tubule cells; in fact, these receptors are distributed on both the apical and basolateral membranes of these cells.