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AT_2 RECEPTORS: BENEFICIAL COUNTER-REGULATORY ROLE IN CARDIOVASCULAR AND RENAL FUNCTION

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Abstract

The renin–angiotensin system (RAS) is a coordinated hormonal cascade intimately involved in cardiovascular and renal control and blood pressure regulation. Angiotensin II (Ang II), the major RAS effector peptide, binds two distinct receptors, the angiotensin type-1 receptor (AT_1R) and the angiotensin type-2 (AT_2R) receptor. The vast majority of the physiological actions of Ang II, almost all of them detrimental, are mediated by AT_1Rs. In contrast, AT_2Rs negatively modulate the actions of AT_1Rs under the majority of circumstances and generally posess beneficial effects. AT_2Rs induce vasodilation in both resistance and capacitance vessels, mediate natriuresis directly and via interactions with dopamine D1 receptors in the renal proximal tubule. AT_2Rs inhibit renin biosynthesis and secretion and protect the kidneys from inflammation and ischemic injury. Our understanding of the exact role of AT_2Rs in physiology and pathophysiology continues to expand; the purpose of this review is to provide an up-to- date summary of the functional role of AT_2Rs at the organ, tissue, cellular and subcellular levels with emphasis on the vascular and renal actions that bear on blood pressure regulation and hypertension.

Introduction

The renin–angiotensin system (RAS) is a coordinated hormonal cascade involved in cardiovascular control with angiotensin II (Ang II) as the main effector peptide regulating blood pressure]1]. Ang II binds two distinct RAS receptors, the angiotensin type-1 receptor (AT_1R) and the angiotensin type-2 (AT_2R) receptor, with high affinity [1,2]. The vast majority of the physiological actions of Ang II are mediated by AT_1Rs, 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 activation of the sympathetic nervous system [1,2]. In contrast, AT_2Rs negatively modulate the actions of AT_1Rs under the majority of circumstances [1–4]. However, our understanding of the exact role of AT_2Rs in physiology and pathophysiology continues to expand. The purpose of this review is to provide an up-to- date summary of these actions.

AT_2R Expression

The AT_2R is a 7-transmembrane G protein-coupled receptor composed of 363 amino acids (molecular weight 41,220 Da) with only 34% sequence homology with the AT_1R [2]. The sequence homology between the two receptors occurs mainly in the transmembrane hydrophobic regions of the molecules which form their 7-transmembrane helical columns [2].

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AT_2Rs are expressed ubiquitously at very high levels in the fetus, but decline precipitously in the neonatal period in most, but not all, tissues [2,5]. Although there is relatively low expression of AT_2Rs compared to AT_1Rs in adult tissues, AT_2Rs are expressed in the adult kidney, adrenal cortex, heart and vasculature, and predominate over AT_1Rs in specific sites such as the uterus, ovary, adrenal medulla and in discrete areas of the brain [5–8].

Cell signaling mechanisms of AT_2Rs

AT_2R signaling mechanisms differ markedly from those of AT_1Rs. As shown in Figure 1, AT_2R activation initiated via binding of Ang II to the receptor on the plasma membrane triggers G protein coupling through Giα2 and Giα3 via the third intracellular loop of the receptor. This signal initiates the activation of phosphotyrosine phosphatases, whose function is to dephosphorylate and thus inactivate mitogen-activated protein (MAP) kinases such as extracellular-regulated kinase (ERK)-1 and ERK-2. Phosphotyrosine phosphatase activation also can occur via a G protein-independent mechanism. In any case, MAP kinase inhibition opposes MAP kinase activation as a result of AT_1R activation. This fundamental difference in cell signaling at the MAP kinase level is thought to form the basis for the counter-regulatory action of AT_2Rs opposing at least some of the actions of AT_1Rs [1–4].

AT_2R stimulation can also activate lipid signaling pathways including increased phospholipase A_2 activity and arachidonic acid release [4]. Long-term activation of AT_2Rs by Ang II can also increase the biosynthesis of ceremides, which in turn can activate stress kinases and caspases to induce apoptosis [4].

A major AT_2R signaling pathway, involved in almost all of the reported physiologic pactions of AT_2Rs, is the bradykinin (BK)-nitric oxide (NO)-cyclic guanosine 3′,5′-monophosphate (cGMP) signaling cascade as described in more detail below.

Vascular Actions of AT_2Rs

There is now overwhelming evidence that AT_2Rs oppose the AT_1R-mediated vasoconstrictor action of Ang II [9–22] AT_2R-mediated vasodilation has been demonstrated in small resistance arteries of the mesenteric, uterine, adrenal, coronary and peripheral circulations in a wide variety of animal models and in humans [9–16,19–22]. Vasodilation due to AT_2R activation has also been demonstrated in large capacitance vessels such as the aorta [17, 18], and in the fetal circulation [20]. AT_2R-induced vasodilation is mediated by activation of the vasodilator cascade composed of BK, NO, and cGMP [23–25]. AT_2Rs increase the production of NO and cGMP either by stimulating increased BK production with a subsequent effect mediated through BK B_2 receptors, or by direct activation of NO production independent of BK [26–28] (Figure 2).

The AT_2R-mediated vasodilator action of Ang II is most easily demonstrated when AT_1Rs have been inhibited using an Ang (AT_1) receptor blocker (ARB) [3,10,11,13,14]. For example, chronic Ang II administration induces hypotension in AT_1R-blocked rats and this response is abolished by co-administration of AT_2R antagonist PD-123319 (PD; Figure 3). This is likely to be related to the predominance of AT_1R over AT_2R expression in blood vessels [29,30]. The vasodilator effects mediated by AT_2Rs are also facilitated when the RAS is activated – for example, by dietary Na^+ restriction, Ang II infusion or in renovascular hypertension [9,10,31]. In all instances, AT_2Rs are upregulated, augmenting the vasodilator response to Ang II [6,9,31]. Other conditions which upregulate AT_2Rs also elicit its vasodilator actions, such as increased pressure load due to aortic banding [17,18]. In such studies, suprarenal abdominal aortic banding results in a marked (300%) increase in vascular AT_2R mRNA. AT_2R blockade with specific AT_2R antagonist PD-123319 (PD) or BK B_2 receptor antagonism with icatibant largely restores diminished Ang II contractile

responses [17,18]. The nine-fold increase in aortic cGMP stimulated by Ang II in pressure-loaded aortas is also abolished by PD or icatibant. These studies underscore the potential importance of AT_2R upregulation in circulatory disorders that that trigger pcounter-regulatory depressor mechanisms mediated by BK and NO.

The vasodilator and hypotensive effects of AT_2R activation are both acute and long-term, and are not associated with desensitization, making the AT_2Rs a potential therapeutic target in hypertension [11,13]. Studies have also demonstrated that the blood pressure-lowering effects of AT_1R blockade with an ARB might be mediated, at least in part, by AT_2R activation [9,10]. Indeed, in a study of resistance arteries in diabetic, hypertensive human subjects, chronic ARB administration upregulated vascular AT_2Rs and facilitated a vasodilator response to Ang II *in vitro* [22]. Similarly, in the presence of AT_1R blockade, direct pharmacological AT_2R activation with Compound 21 (a highly selective non-peptide AT_2R agonist) resulted in depressor responses in spontaneously hypertensive rats [32] (Figure 4). These studies indicate the potential therapeutic value of a non-peptide AT_2R agonist in combination with an ARB in the treatment of hypertension and other cardiovascular disorders.

The role of AT_2Rs in the vasculature has recently expanded to include insight into the contribution of AT_2Rs in the progression of aortic aneurysms. In mouse model of Marfan's syndrome, loss of AT_2R expression accelerates the aberrant growth and rupture of the aorta [33]. Selective AT_1R blockade abrogated aneurysm progression, but only when AT_2R-induced attenuation of extracellular signal-regulated kinase (ERK) phosphorylation remained intact [33]. This study highlights the protective nature of intact AT_2R signaling in aortic aneurysm progression.

Renal Actions of AT_2Rs

AT_2Rs mediate natriuresis

AT_2Rs are expressed throughout the kidney in both vascular and tubular elements and are heavily expressed in renal proximal tubule cells [6,7]. Definitive evidence has now been produced that AT_2Rs mediate natriuresis [34–36]. This was achieved by using the technique of direct renal interstitial microinfusion of pharmacological agents, enabling evaluation of renal function selectively, without the influence of systemic hemodynamic or hormonal changes. Selective intrarenal AT_1R blockade in rats induced a highly significant natriuretic response that was abolished with intrarenal co-infusion of the AT_2R-specific antagonist PD [34]. These results further indicate that, similar to vasodilation, the beneficial natriuretic response to ARB administration is related to AT_2R activation. Because des-aspartyl^1-Ang II (Ang III) is highly active at AT_2Rs in the brain [37], a study was conducted to investigate whether Ang III might be an agonist for AT_2R-mediated responses in the kidney [34]. When systemic AT_1Rs were blocked, direct intrarenal Ang III infusion induced a highly significant increase in renal Na^+ excretion that was abolished by concurrent AT_2R blockade with PD. Similar to vascular responses, intrarenal Ang III infusion alone demonstrated no effect on renal Na^+ excretion in the absence of systemic AT_1R blockade [34].

Surprisingly, in the presence of systemic AT_1R blockade, intrarenal administration of Ang II, even at substantially higher molar-equivalent concentrations than with Ang III, was unable to induce natriuresis [34]. To explain this observation, it was hypothesized that Ang II needs to be converted to Ang III to interact with AT_2Rs in the kidney. 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) (Figure 5). In the presence of systemic AT_1R blockade, intrarenal infusion of Ang III engendered a natriuretic response, which was markedly augmented by intrarenal coinfusion of the APN inhibitor 2-amino-4-

methylsulfonyl-butane-thiol, methane-thiol (PC-18) [35]. The augmentation in Ang III-induced natriuresis due to APN inhibition was also abolished by intrarenal AT_2R blockade with PD [35] (Figure 6). Intrarenal infusion of Ang II induced a natriuretic response only in the presence of APN inhibition. These results indicate that Ang III is the preferred AT_2R agonist in the regulation of renal Na^+ excretion. These studies are potentially important for disease states such as diabetes mellitus and obesity, wherein AT_2Rs are upregulated in renal proximal tubule cells, inhibit Na^+/K^+ ATPase (NKA) and reduce Na^+ transport [38, 39]. The inhibition of NKA is mediated via a NO/cGMP-dependent pathway [40], and inhibition of NAD(P)H oxidase potentiates AT_2R-mediated natriuresis [41].

Recent *in vivo* studies have demonstrated that renal AT_2Rs mediate natriuresis via a NO/cGMP signaling cascade operating in the renal proximal tubule [42]. *In vitro* studies also have recently shown that renal AT_2Rs decrease AT_1R function in the proximal tubule by the common NO/cGMP pathway and also decrease AT_1R expression at the transcriptional level via the ubiquitous transcription factor Sp1 [43]. AT_2Rs also were shown upon activation with a ligand to heterodimerize with AT_1Rs reducing their expression level via a direct protein-protein interaction at the cell surface [43]. Therefore, renal AT_2Rs may oppose AT_1Rs via several signaling pathways.

Recent studies also have demonstrated that AT_2Rs may induce natriuresis in part through an action in the thick ascending limb of Henle (TAL) (44, 45). Ang II increases NO production in TALs via activation of AT_2Rs. In turn, NO inhibits the $Na^+/K^+/2Cl^-$ co-transporter (NKCC2) and reduces Na^+ reabsosrption in TALs (45). Interestingly, this effect of AT_2Rs is diminished in Dahl salt-sensitive rats, which has been widely employed as a model of salt-sensitive hypertension. In addition, the defect in the ability of TALs to respond to AT_2R activation could be overcome with a higher dose of the AT_2R peptide agonist CGP42112A (45). This observation suggests that AT_2R activation may provide therapeutic benefit for salt-sensitive hypertension. Re-examination of this effect using the newer non-peptide AT_2R agonist Compound 21 would be of interest in confirming these findings.

The renal dopaminergic system is a crucial system in the control of renal Na^+ excretion and blood pressure [46]. Dopamine (DA) is synthesized from filtered L-dihydroxyphenylalanine taken up into proximal tubule cells (Figure 7). DA is exported from the proximal tubule cell largely into the tubule lumen, where it binds to the D_1-like receptor family (D_1 and D_5 subtypes). D_1-like receptor activation inhibits Na^+ reabsorption through an adenylate cyclase–cAMP-dependent mechanism. The importance of the renal dopaminergic system is underscored by the fact that renal D_{1LIKE} receptors account for about 50% of basal Na^+ excretion under normal-high Na^+ intake [46]. Exogenous administration of fenoldopam, a highly selective D_1-like receptor agonist, in animals and humans elicits a major natriuretic response that is based almost exclusively upon inhibition of proximal tubule Na^+ reabsorption [46]. It has been demonstrated that intrarenal fenoldopam administration induces a highly significant natriuresis in the sodium-loaded rat [36]. The natriuretic response is blocked completely by SCH-23390, a highly selective D_1-like receptor antagonist. However, surprisingly, the natriuretic response to fenoldopam is also abolished by concomitant intrarenal administration of AT_2 receptor antagonist PD [36] (Figure 8).

To explore the possible mechanism of AT_2R involvement in D_1-like receptor ($D_{1LIKE}R$)-induced natriuresis, studies were performed to determine intracellular AT_2R trafficking in renal proximal tubule cells [45]. Fenoldopam administration *in vivo* was accompanied by translocation of AT_2Rs from intracellular sites to the apical plasma membranes of renal proximal tubule cells (Figure 9) [47]. Neither total [36] nor basolateral proximal tubule cell AT_2R expression was changed in response to fenoldopam infusion [47], suggesting that apically distributed AT_2Rs participated in the natriuretic response. Previous experiments

have established that D_{1LIKE}Rs translocate to the cell surface in response to D_{1LIKE}R activation in cultured kidney cells, kidney section preparations, and isolated proximal tubules [48–50] and that this response requires an intact microtubule network [50]. Recently, these findings have been extended to the natriuretic mechanism of renal AT_2Rs *in vivo*. Fenoldopam-induced natriuresis and AT_2R translocation to the apical plasma membrane are completely abolished during intrarenal co-infusion of nocodazole, which disrupts the microtubule network but preserves the actin microfilaments of renal proximal tubule cells [51]. Thus, microtubules are not only necessary for D_{1LIKE}R recruitment but also for AT_2R recruitment in response to fenoldopam, suggesting a common pathway for the natriuretic function of these receptors.

Furthermore, since D_{1LIKE}Rs signal through cAMP/protein kinase A to mediate natriuresis, the role of RI cAMP generation in AT_2R-mediated natriuresis was also investigated *in vivo* [47]. 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 AT_2R-mediated natriuresis. Direct agonist stimulation of D_{1LIKE}Rs was not necessary for AT_2R-mediated natriuresis since forskolin+IBMX-induced natriuresis persisted in the presence of D_{1LIKE}R blockade with SCH-23390 [47]. Thus, one mechanism by which AT_2Rs and D_{1LIKE}Rs interact in high Na^+ conditions to mediate natriuresis is related to D_{1LIKE}R-cAMP signaling, which, in turn, provides the stimulus necessary for AT_2R translocation and natriuresis (Figure 10). Because the effect is independent of specific D_{1LIKE}R-induced activation of adenylyl cyclase, activation of other receptors that signal through cAMP/protein kinase A pathway may be advantageous in promoting AT_2R-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 AT_2Rs are known to inhibit Na^+-K^+-ATPase activity [40], and increased renal cAMP may regulate this process.

AT_2Rs suppress renin biosynthesis and secretion

Ang II participates in a so-called ‘short-loop negative feedback’ suppression of renin biosynthesis and secretion through AT_1Rs on renal juxtaglomerular cell plasma membranes [1]. This mechanism functions as a physiological ‘brake’ to prevent potential detrimental actions of high circulating Ang II mediated through AT_1Rs. Studies have demonstrated that, similar to AT_1Rs, AT_2Rs also contribute to renin suppression in response to high Ang II levels [53]. Furthermore, in obese Zucker rats, chronic AT_2R antagonism with PD resulted in a 13mm Hg increase in mean arterial pressure, and a 3-fold increase in renin expression in the kidney cortex [54]. These data suggest that renal AT_2Rs serve a protective role against increases in blood pressure, in part, due to AT_2R-mediated renin suppression.

AT_2Rs protect kidneys from ischemic damage

One of the first studies to examine the role of renal AT_2Rs in ischemic damage subjected AT_2R transgenic (AT_2R-Tg) and wild-type mice to five-sixths (5/6) nephrectomy, a model of ischemic renal injury [55]. In AT_2R-Tg mice, glomerular expression of AT_2Rs was up-regulated by 5/6 nephrectomy. Urinary albumin excretion was decreased by about one-third in AT_2R-Tg mice compared to wild-type mice. AT_2R-Tg mice had a significant reduction in transforming growth factor-β and platelet-derived growth factor. Urinary excretion of NO metabolites was increased 2.5-fold in AT_2R-Tg mice. All of the above-mentioned responses were blocked by AT_2R antagonist, PD. Thus, AT_2R over-expression protected glomeruli from injury in the 5/6 nephrectomy model.

Vazquez *et al*. [56] also studied AT_2R expression and function in the 5/6 nephrectomy model, which developed a time-dependent increase in AT_2R expression at 7, 15, and 30 days after renal ablation. Animals pre-treated with AT_1R antagonist, losartan, showed a further increase in AT_2R expression. AT_2R antagonist PD was associated with down-regulation of AT_2Rs, increased renal damage and increased blood pressure in the 5/6 nephrectomy model. Both of these studies suggest that the AT_2R represents a beneficial counter-regulatory mechanism to protect the kidney from ischemic injury.

AT_2Rs protect kidneys from inflammation

Consistent with the above-mentioned protection from ischemic injury, AT_2R activation also protects the kidneys from inflammatory changes induced by AT_1Rs in renovascular hypertension [57,58]. AT_1R activation is well known to induce inflammation via stimulation of a variety of inflammatory molecules, including interleukin (IL)-6, tumor necrosis factor (TNF)-α, transforming growth factor (TGF)-β and induction of oxidative stress [59]. 2K1C Goldblatt hypertensive animals develop early inflammation in the ischemic kidney with increased renal production of TNF-α, IL-6 and TGF-β and reduction of NO and cGMP in ischemic kidneys followed by progressive fibrosis [57]. Direct pharmacological activation of AT_2Rs with non-peptide selective agonist Compound 21 abolished the inflammatory process associated with reduction of inflammatory markers and improvement in renal NO and cGMP levels [57]. Interestingly, AT_2R activation was coupled to increased AT_2R expression in the ischemic kidneys. AT_1R activation also induces signal transducer and activator of transcription proteins 3 (STAT3), which in turn mediates inflammation. Other studies [58] have shown in cells expressing AT_2Rs but not AT_1Rs (PC12W cells) that stimulation of AT_2Rs reduces STAT 3 phosphorylation and TNF-α production and that this response can be blocked with AT_2R antagonist PD-123319. Taken altogether, these findings suggest that AT_2R activation may become part of a therapeutic strategy to prevent and/or reverse inflammation in cardiovascular and renal disease.

Relative Angiotensin Peptide Affinities for AT_2R

It is increasingly recognized that angiotensin I (Ang I) metabolites, other than Ang II, can exert biological activity. The peptides include the C-terminal breakdown products of Ang II, namely the heptapeptide Ang III and the hexapeptide Ang IV, as well as the N-terminal heptapeptide angiotensin 1–7 (Ang 1–7). In most instances, these peptides induce cardiovascular effects that are opposite to the classical effects of Ang II [34, 60–63]. For example, chronic treatment with Ang 1–7 has been reported to evoke both AT_2R-mediated vaso- and athero-protective effects in apoliproprotein E-deficient mice [60]. Likewise, in the kidney, while Ang II is known to cause sodium reabsorption via actions at the AT_1R, Ang III induces AT_2R-mediated natriuresis [34]. When the degradation of Ang III is inhibited, this effect is enhanced, both in the presence [35] and absence of systemic AT_1R blockade [42]. Further studies using APA (EC-33) and APN (PC-18) inhibitor combinations (Figure 5) have indicated that intrarenal Ang II must be converted to Ang III in order to observe AT_2R-mediated natriuresis [65], suggesting that this heptapeptide is the preferred agonist of AT_2Rs-mediated natriuresis.

In addition to the kidney, two other cardiovascular hormonal systems have been linked to Ang III as the preferred agonist for AT_2Rs, 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 AT_2R-mediated vasodilation [66]. Ang III is also the preferred agonist for AT_2R-mediated aldosterone secretion from the adrenal cortex [67].

A recent systematic *in vitro* study performed in human embryonic kidney cells examined the relative AT_2R-binding affinities and selectivities of a number of endogenous angiotensin

peptides [68]. Affinity is defined by the IC_{50} value of a ligand for an individual receptor, whereas selectivity refers to the ratio of IC_{50} values of a ligand at two different receptors. Thus, a ligand can have a similar affinity. While Ang III demonstrated a similar affinity for AT_2Rs as Ang II, it had at least a 30-fold selectivity for AT_2Rs over AT_1Rs. Moreover, while Ang 1–7 and Ang IV exhibited ~500- and ~100-fold reduced affinity at AT_2Rs compared with Ang II, these peptides were 40- and 200-fold more selective for AT_2Rs than AT_1Rs [68]. Collectively, these AT_2R affinity-profiling results provide further evidence for the functional data pinpointing Ang III as the preferred agonist of AT_2Rs in certain tissues. The precise molecular conformation that renders Ang III a better fit within the AT_2R binding pocket awaits further investigation.

Novel Intracrine (Nuclear and Mitochondrial) AT_2R Actions

While the RAS in the past was regarded as an exclusively circulating hormonal (endocrine) system, recent evidence has identified several local tissue RASs that function independently of the circulating system [1]. These local systems function at the cell-to-different cell (paracrine) and/or cell-to-same cell (autacrine) levels and are now considered crucial regulators in physiology and pathophysiology. In the periphery, local hormonal systems have been identified in the kidney, heart and blood vessels, among several other tissues and organs. At present, the exact role of AT_2Rs in these paracrine and autacrine RAS actions is largely unknown. However, recent studies have identified AT_2Rs as important signal transducers at the subcellular level potentially contributing to important actions of a completely intracellular (intracrine) RAS. Recent interest has been focused on two intracellular organelles, nuclei and mitochondria.

Regarding nuclear Ang receptors, AT_2Rs do not contain a canonical nuclear localization sequence as do AT_1Rs. In spite of this, AT_2Rs are highly expressed in renal cortical nuclei of fetal and adult sheep and in response to Ang II release NO, likely from nuclear endothelial NOS activation [69]. Since soluble gualylyl cyclase has been identified in isolated nuclei it is possible that cGMP production may increase in response to nuclear AT_2R activation. BK also has been shown to induce NO production in isolated nuclei and, therefore may be involved in intranuclear AT_2R signaling [70]. As evidence of a functional role for nuclear AT_2Rs, AT_2R antagonist PD-123319 augmented the generation of reactive oxygen species in response to Ang II in isolated nuclei. As a non-peptide highly selective AT_2R agonist, Compound 21 should cross the plasma and nuclear membranes and have access to the intracellular and nuclear compartments [71]. If the intracellular RAS has a role in cardiovascular and renal pathophysiology, targeting of the nuclear RAS pathway may convey additional benefits to those of extracellular inhibition.

Regarding mitochondrial Ang receptors, neither AT_1Rs nor AT_2Rs have a consensus targeting sequence for mitochondria. However, recent studies have demonstrated that, while AT_1Rs are not expressed, AT_2Rs are heavily localized to the inner mitochondrial membranes where they co-localize with Ang II [72]. Activation of AT_2Rs in isolated mitochondria dose-dependently increased NO production, a response that was abolished with AT_2R antagonist PD-123319 [72]. Importantly, AT_2R activation was accompanied by inhibition of oxygen consumption (respiration) that was reversed by NOS inhibitor L-NAME [72]. Furthermore, during the process of aging there was a significant increase in AT_1R and decrease in AT_2R mitochondrial expression. This balance was reversed with chronic treatment with an AT_1R antagonist, suggesting that AT_1R antagonist and/or AT_2R agonist treatment might hold promise for prevention of the chronic disease burden of aging [72]. Indeed, it has been shown that disruption of AT_1Rs promotes longevity in mice and hypertensive rats, and effect that could be related to increased AT_2R expression and/or activation [73, 74].

Sex Differences in AT_2R Responses

AT_2Rs are encoded on the X-chromosome, suggesting the possibility that females may have increased expression of AT_2Rs as compared to males. Indeed, AT_2R expression was enhanced in female mice accompanied by decreased inflammation, cell proliferation and oxidative stress in response to injury compared to male mice [75]. However in AT_2R-null female mice vascular injury was enhanced. Moreover, treatment with an AT_1R blocker had a more beneficial effect in female than male wild-type mice. These results indicated that sex differences in response to vascular injury could be related at least in part to exaggerated AT_2R expression in females.

Female Sprague-Dawley rats also have enhanced AT_2R-mediated renal vasodilator and tubuloglomerlar feedback responses compared to males [76]. In females, this difference did not translate to renal Na^+ excretion, which was identical in male and female rats; that is, AT_2R activation caused a similar increase in Na^+ excretion via an action at the renal tubule in both male and female Sprague-Dawley rats [77,78]. In addition, AT_2R activation directly inhibits renal tubule Na^+-K^+ATPase activity both in male obese Zucker rats and in male Sprague-Dawley rats [40,79]. Furthermore, AT_2R activation induces natriuresis via a renal tubule mechanism in male Sprague-Dawley rats [41]. Thus, there is ample evidence in the literature that renal tubule AT_2R activation induces natriuresis in both male and female rats.

Summary and Conclusions

In summary, the cardiovascular and renal actions of AT_2Rs generally oppose those mediated by AT_1Rs. At the level of cell signaling, AT_2Rs inhibit MAP kinases ERK-1 and -2 reducing or nullifying AT_1R-mediated increases in ERK activity. The BK-NO-cGMP pathway is now recognized as the extracellular AT_2R signaling pathway relevant to the majority of AT_2R actions and is a candidate intracellular mediator as well. From a functional standpoint, AT_2Rs induce systemic vasodilation and natriuresis, the latter of which appears to be mediated by endogenous Ang III rather than Ang II. AT_2R inhibition of renal tubule Na^+ transport occurs largely in the proximal tubule but also to some extent in the thick ascending limb of Henle. Natriuresis induced by renal dopamine via D_1Rs in the proximal tubule requires cAMP-stimulated translocation and activation of AT_2Rs at the plasma membrane. Characterization of intracellular (intracrine) RASs is currently focusing on the potential role of AT_2Rs as predominant effectors in nuclei and mitochondria.

In conclusion, AT_2Rs play a beneficial counter-regulatory role in cardiovascular and renal function. Reduced AT_2R expression and/or activity may contribute to the initiation/aggravation of disease processes such as hypertension, edema-forming states and inflammation/fibrosis leading to cardiovascular and renal tissue damage. Many of the beneficial effects of AT_1R blockade are attributable at least in part to increased AT_2R expression and activation. In the future, pharmacologic AT_2R activation holds promise to provide a therapeutic advantage or even a preventive strategy alone or combined with concurrent AT_1R blockade. Recent findings suggest the possibility that AT_2R activation may offer limited protection from the aging process and/or its accompanying chronic diseases. Whether the increased longevity observed with AT_1R ablation may be related at least in part to AT_2R activation constitutes an exciting area of future investigation.

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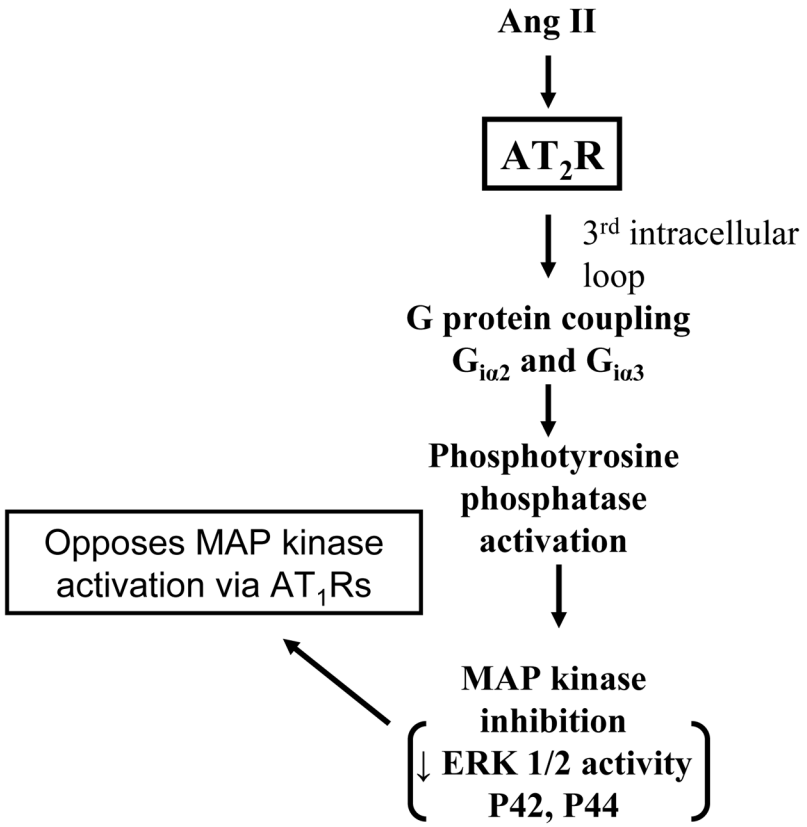

Figure 1.
Schematic representation of the major intracellular signaling pathway of angiotensin type-2 receptors (AT_2Rs). MAP = mitogen-activated protein; ERK = extracellular-regulated kinase; AT_1R = angiotensin type-1 receptor.

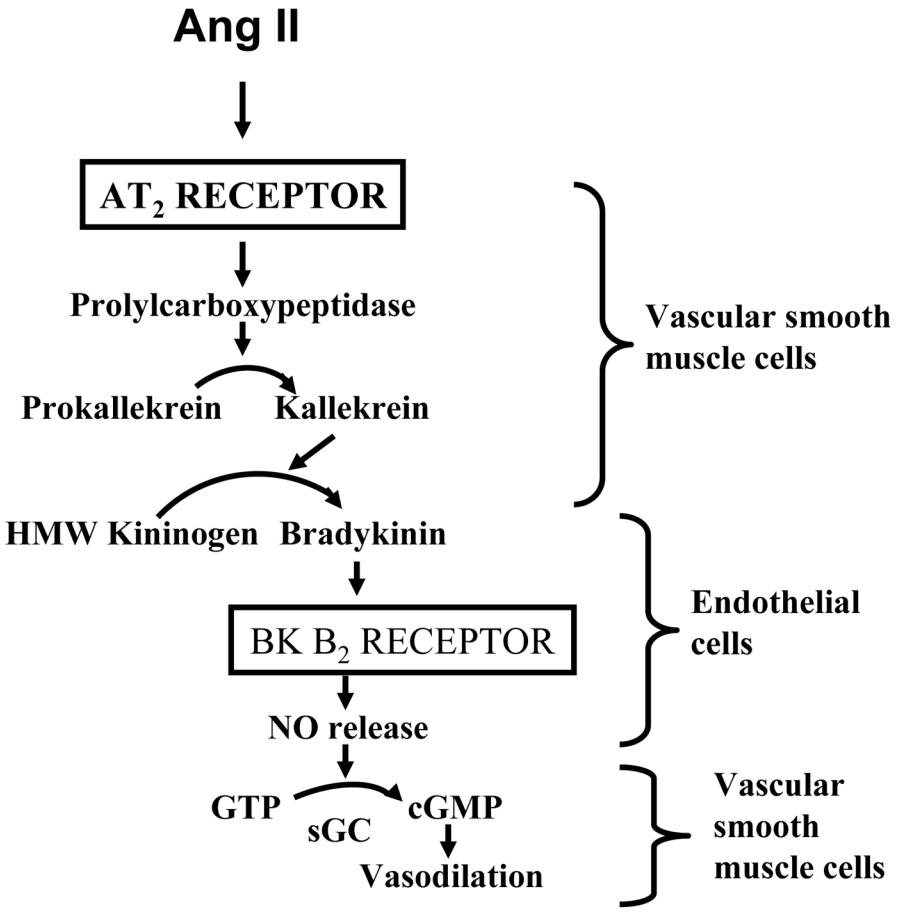

Figure 2.

Schematic representation of the most important extracellular signaling pathway of AT_2Rs: the autacrine/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

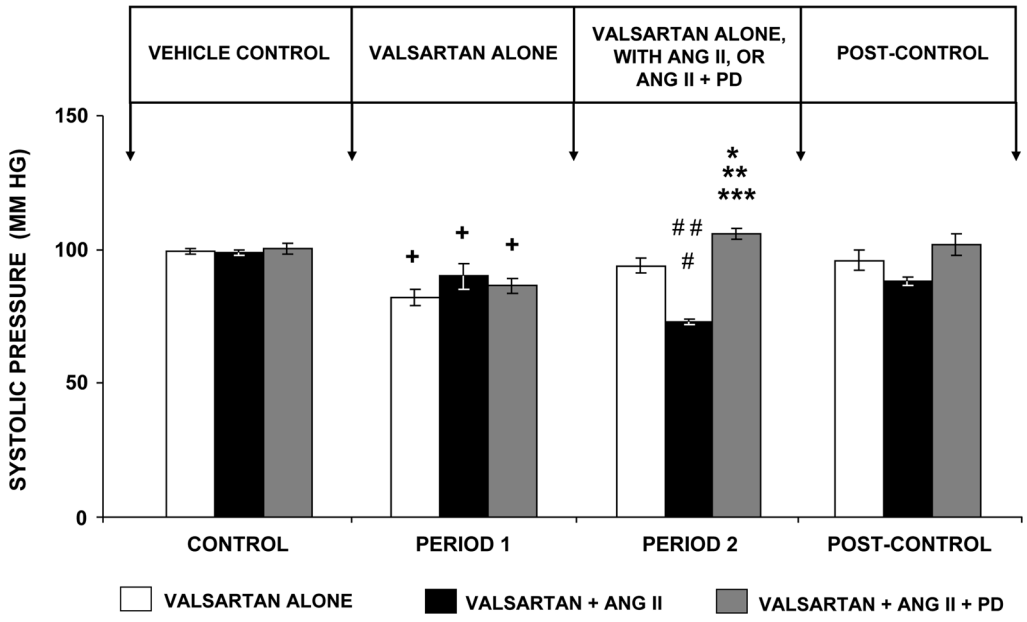

Figure 3.

Systolic blood pressure responses to angiotensin II (Ang II) in the absence or presence of AT_1R antagonist valsartan (VAL) or VAL + AT_2R antagonist PD-123319 (PD). VAL induced hypotension that was augmented by Ang II and the BP reduction was fully blocked by co- administration of PD. Values represent mean ± 1SE. + P<0.001 from control in Period 1; # P<0.0001 from VAL alone in Period 2; ## P< 0.00001 from corresponding control in Period 1; * P<0.0001 from VAL alone in Period 2; ** P<0.0001 from corresponding control in Period 1; *** P<0.00001 from VAL + Ang II in Period 2. From Carey *et al. Hypertension*. 2001;38:1272–1277 with permission.

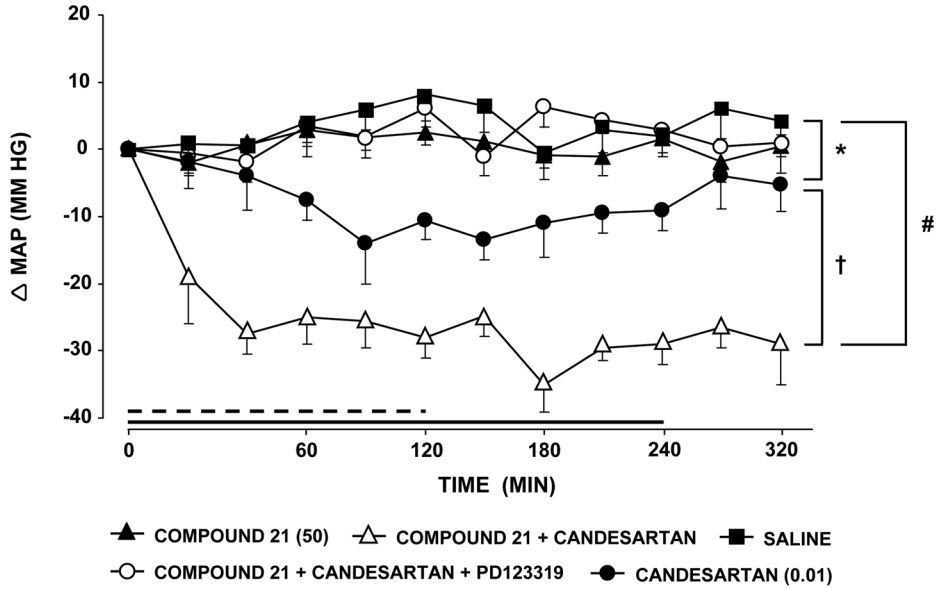

Figure 4.

Effect of AT_2R agonist Compound 21 (50 ng/kg/min IV), low dose AT_1R antagonist candesartan (0.01 mg/kg bolus IV), Compound 21 + candesartan and Compound 21 + candesartan + AT_2R antagonist PD-123319 (PD; 50 μg/kg/min IV) on mean arterial pressure in spontaneously hypertensive rats (SHR). Infusions were for 2 hours (dashed line). Values represent mean ± 1SE. * $P<0.05$ for treatment effect between Compound 21 and candesartan; # $P<0.01$ for overall effect of individual treatment vs. Compound 21; † $P<0.01$ for treatment effect between Compound 21 + candesartan and candesartan alone. Compound 21 augmented the hypotensive response to candesartan which was abolished in the presence of PD. From Bosnyak *et al. Br J Pharmacol.* 2010;159:709–716 with permission.

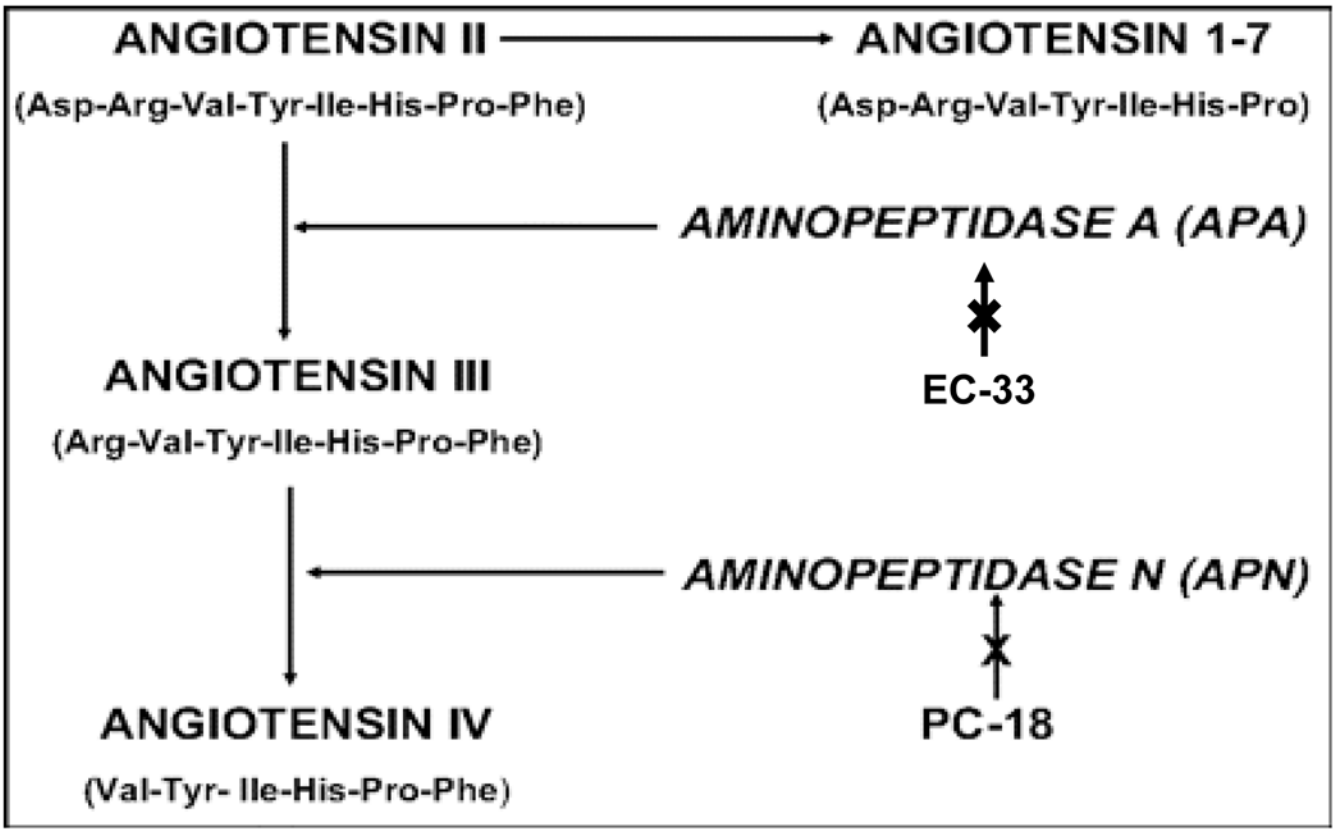

Figure 5.
Schematic diagram depicting the metabolism of angiotensin II via aminopeptidases A (APA) and N (APN). EC-33 is an APA antagonist; PC-18 is an APN antagonist.

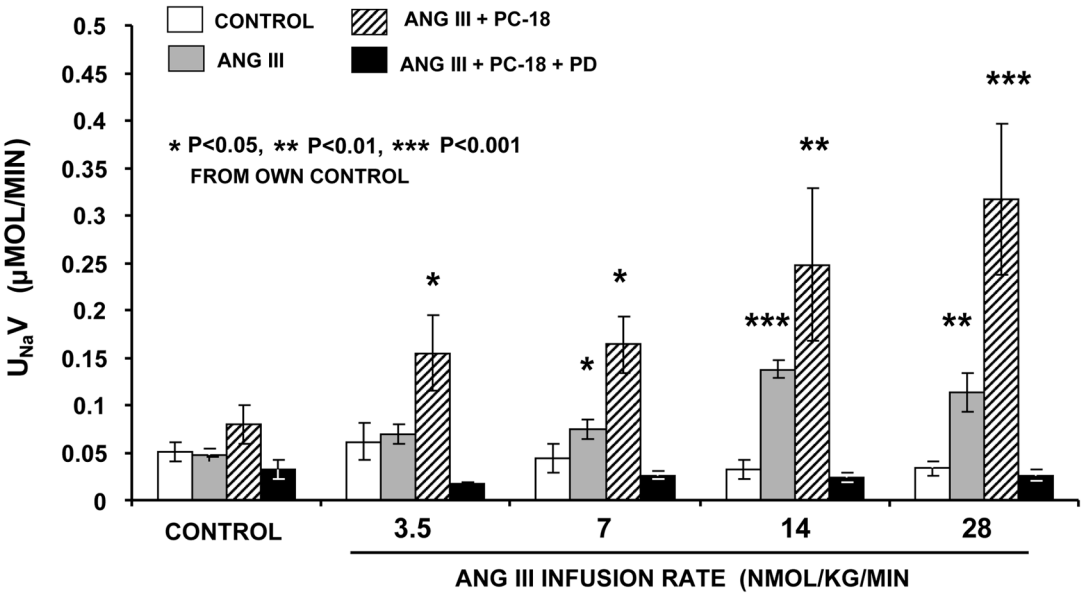

Figure 6.

Increase in urinary sodium excretion ($U_{Na}V$) in reponse to cumulative renal interstitial infusion of angiotensin III (Ang III) alone (3.5, 7, 14 and 28 nmol/kg/min), Ang III + aminopeptidase N inhibitor PC-18 and Ang III + PC-18 + PD in Sprague-Dawley rats (N=7 per group). PC-18 markedly augmented the natriuretic response to Ang III infusion and this response was abolished by PD. Adapted from Padia *et al. Hypertension*. 2007;49[Part 2]: 625–630 with permission.

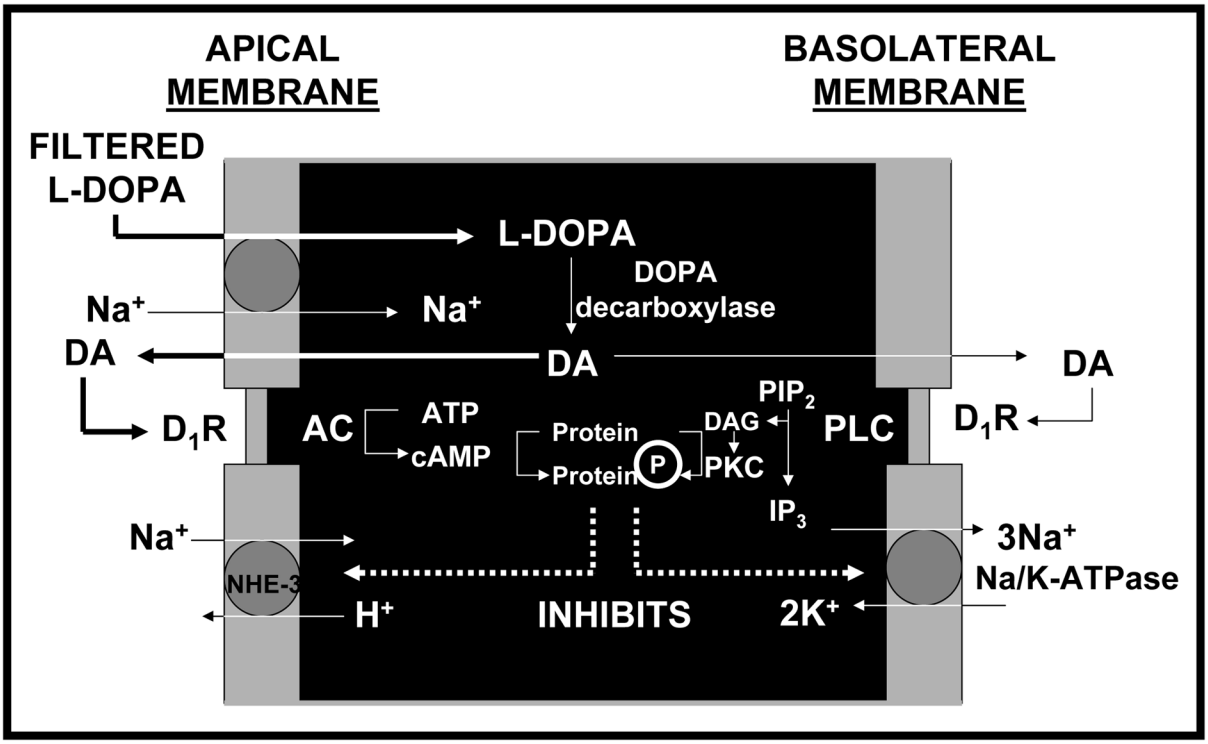

Figure 7.

Schematic diagram of a renal proximal tubule cell depicting the pathways by which dopamine (DA) is formed within the cell, is secreted and acts as an autacrine/paracrine substance to inhibit Na^+ reabsorption. L-DOPA = L- dihydroxyphenylalanine; D1R = dopamine type-1 receptor; ATP = adenosine triphosphate; AC = adenylyl cyclase; cAMP = cyclic 3′,5′ adenosine monophosphate; PKC = protein kinase C; DAG = diacylglycerol; PLC = phospholipase C; NHE-3 = sodium-hydrogen exchanger 3; Na/K-ATPase = sodium/potassium ATPase.

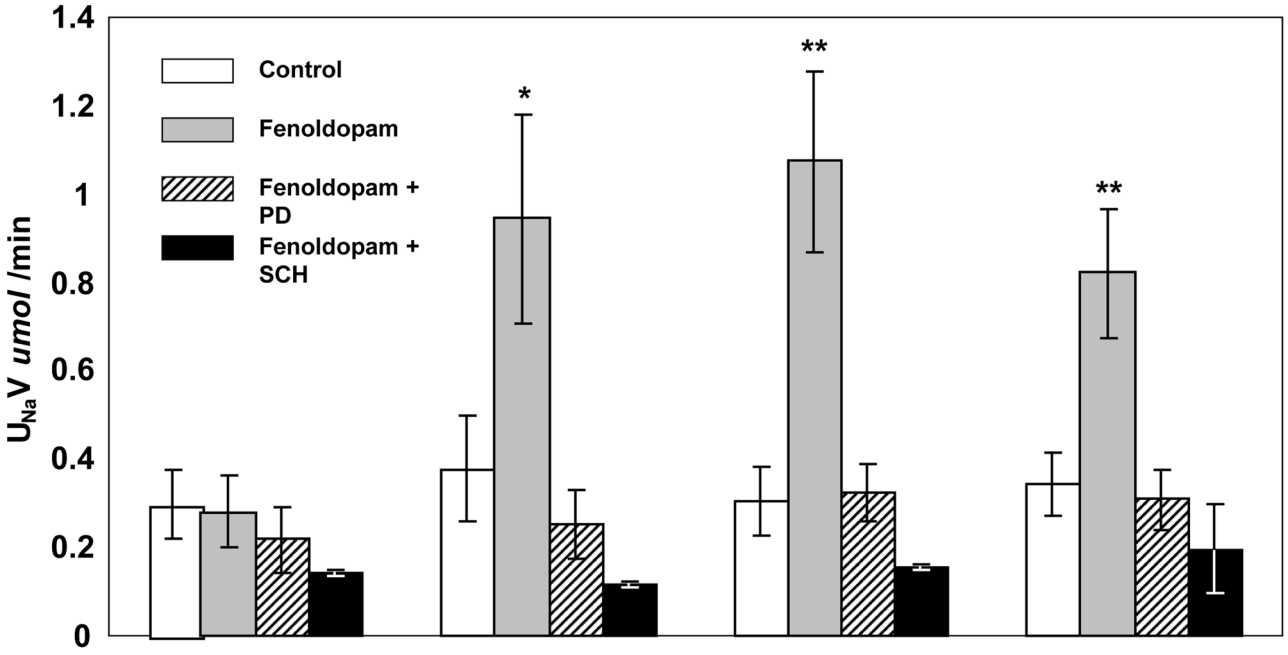

Figure 8.

Direct renal interstitial D_1-like receptor activation induced sustained natriuresis that was abolished by RI co-infusion of AT_2R antagonist PD-123319 (PD) and by RI co-infusion of D_1-like receptor antagonist SCH-23390 (SCH) in Sprague-Dawley rats (N=10 per group). Values represent mean ± 1 SE. * P <0.01 from control; ** P<0.001 from control. From Salomone *et al. Hypertension*. 2007;49:155–161 with permission.

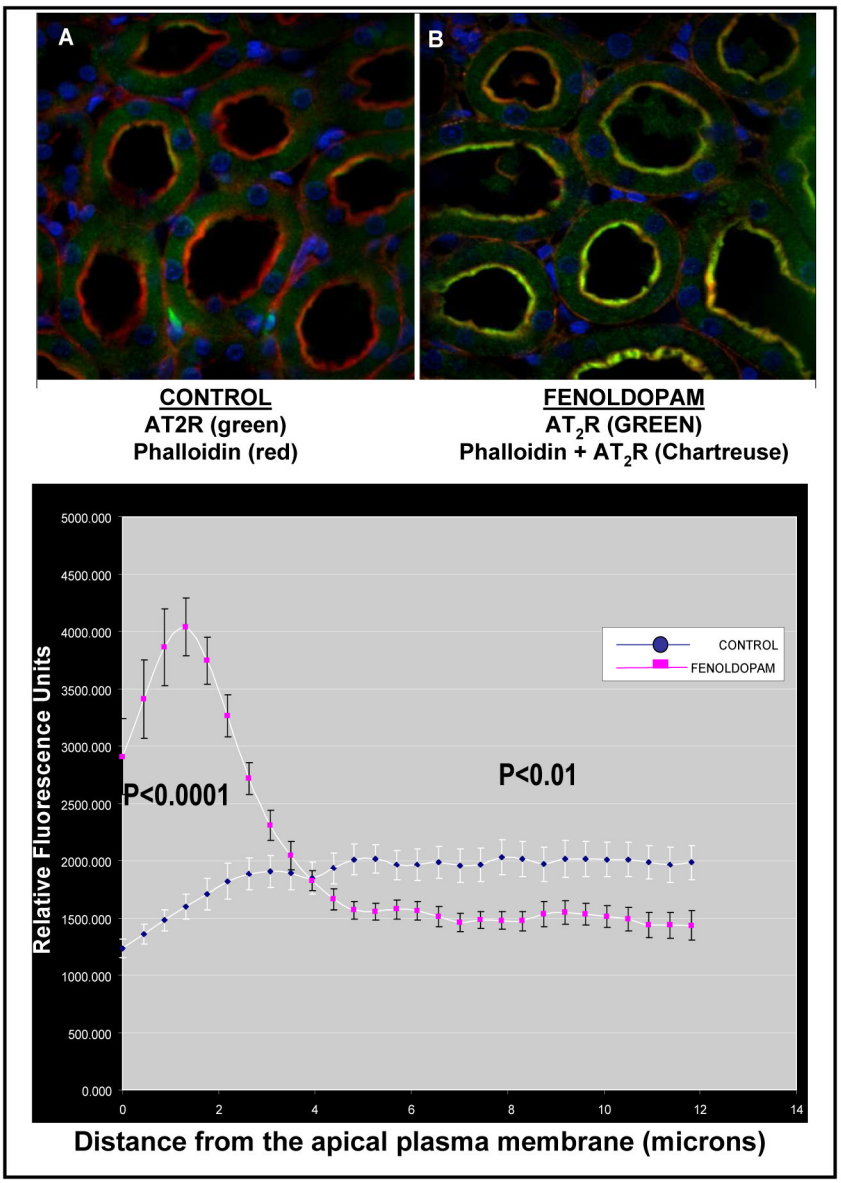

Figure 9.

Confocal micrographs (top panel; 600×) of renal cortical thin sections (8microns) from *in vivo* experiments demonstrating fenoldopam-stimulated translocation of AT_2Rs from intracellular sites to the plasma membrane. Sections are stained with Texas-red labeled phalliodin (red), antibody to the AT_2R (green) and Hoechst nuclear stain (blue). Localization of AT_2Rs to the apical plasma membrane is shown as chartreuse. Quantification of renal proximal tubule cell (RPTC) apical membrane AT_2R fluorescence intensity (bottom panel). For *in vivo* quantification, each data point represents mean±1 SE of 22 independent measurements of RPTCs. Modified from Padia *et al. Hypertension*. 2012;59[Part 2]:437–445 with permission.

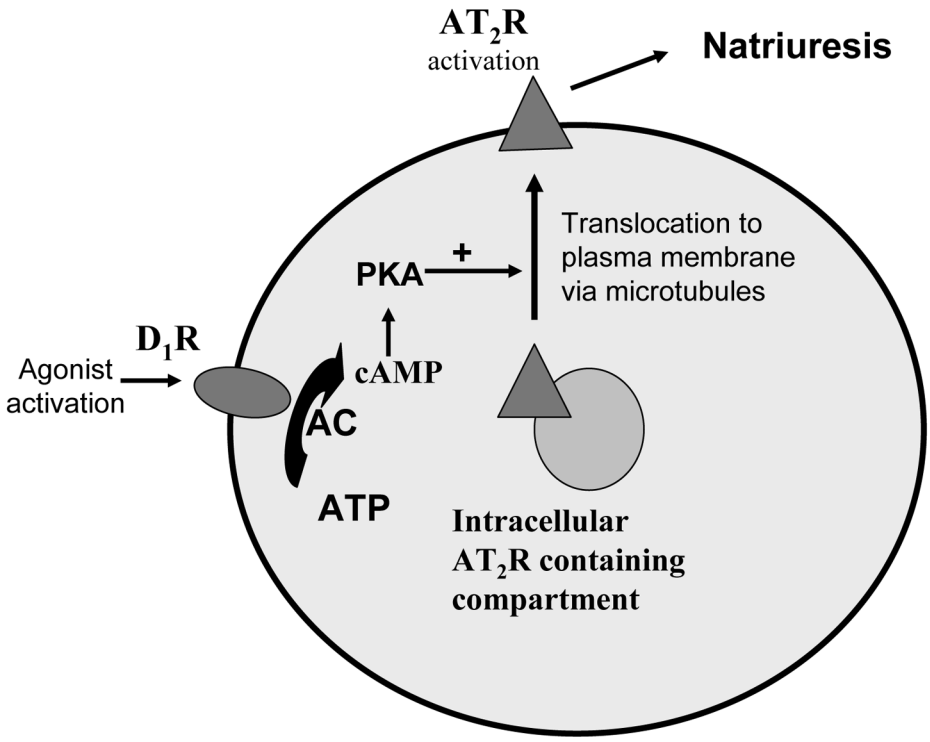

Figure 10.

Schematic diagram showing the intracellular signaling pathway by which angiotensin type-2 receptors (AT_2Rs) and dopamine -1 receptors (D_1Rs) interact in the control of Na^+ transport. D_1Rs activate adenylyl cyclase (AC) generating intracellular cyclic AMP (cAMP), which acts via protein kinase A (PKA) to induce translocation of intracellular AT_2Rs across a microtubule network to the plasma membrane. AT_2R translocation is required for D_1R-induced natriuresis.