Published in final edited form as:

Int J Biochem Cell Biol. 2016 August ; 77(Pt B): 240–250. doi:10.1016/j.biocel.2016.04.016.

Angiotensin II Receptors and Peritoneal Dialysis-Induced Peritoneal Fibrosis

Thomas A. Morinelli^{a,*}, Louis M. Luttrell^{b,c}, Erik G. Strungs^b, and Michael E. Ullian^{a,c}

Thomas A. Morinelli: morinelt@muscc.edu

^aDivision of Nephrology, Department of Medicine, Medical University of South Carolina, Charleston, SC 29425

^bDivision of Endocrinology, Department of Medicine, Medical University of South Carolina, Charleston, SC 29425

^cResearch Service of the Ralph H. Johnson Veterans Affairs Medical Center, Charleston, SC 29401

Abstract

The vasoactive hormone angiotensin II initiates its major hemodynamic effects through interaction with AT1 receptors, a member of the class of G protein-coupled receptors. Acting through its AT1R, angiotensin II regulates blood pressure and renal salt and water balance. Recent evidence points to additional pathological influences of activation of AT1R, in particular inflammation, fibrosis and atherosclerosis. The transcription factor nuclear factor κ B, a key mediator in inflammation and atherosclerosis, can be activated by angiotensin II through a mechanism that may involve arrestin-dependent AT1 receptor internalization.

Peritoneal dialysis is a therapeutic modality for treating patients with end-stage kidney disease. The effectiveness of peritoneal dialysis at removing waste from the circulation is compromised over time as a consequence of peritoneal dialysis-induced peritoneal fibrosis. The non-physiological dialysis solution used in peritoneal dialysis, i.e. highly concentrated, hyperosmotic glucose, acidic pH as well as large volumes infused into the peritoneal cavity, contributes to the development of fibrosis. Numerous trials have been conducted altering certain components of the peritoneal dialysis fluid in hopes of preventing or delaying the fibrotic response with limited success.

We hypothesize that structural activation of AT1R by hyperosmotic peritoneal dialysis fluid activates the internalization process and subsequent signaling through the transcription factor nuclear factor κ B, resulting in the generation of pro-fibrotic/pro-inflammatory mediators producing peritoneal fibrosis.

*Corresponding author: Division of Nephrology, Department of Medicine, Medical University of South Carolina, Charleston, SC 29425, USA.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Angiotensin II; arrestin; AT1R; fibrosis; peritoneal mesothelial cells

1. Introduction

The heptahelical G protein-coupled receptors (GPCRs) are the largest and most diverse superfamily of cell surface receptors, with nearly 800 human genes encoding full-length GPCRs (1, 2). Their evolutionary diversity permits GPCRs to detect an extraordinary array of extracellular stimuli, from neurotransmitters and peptide hormones to odorants and photons of light. GPCRs function in neurotransmission, neuroendocrine control of physiologic homeostasis and reproduction, regulation of hemodynamics and intermediary metabolism, and control the growth, proliferation, differentiation, and death of cells. Not surprisingly then, GPCRs are the single most common target of drugs in clinical use (3).

Nearly all GPCRs act as ligand-activated guanine nucleotide exchange factors (GEFs) for heterotrimeric G proteins. Activated GPCRs catalyze GTP for GDP exchange on heterotrimeric G protein $G\alpha$ subunits, promoting dissociation of the GTP-bound $G\alpha$ subunit from the $G\beta\gamma$ subunit heterodimer. In turn, free $G\alpha$ -GTP and $G\beta\gamma$ subunits regulate the activity of enzymatic effectors, such as adenylate cyclases, phospholipase-C isoforms, and ion channels, generating small molecule second messengers that control the activity of key enzymes involved in intermediary metabolism. Additionally, GPCRs are capable of generating signals that are independent of their intrinsic GEF activity by 'coupling' to adapter or scaffold proteins that link the receptor to novel, non-G protein-regulated, effectors (4). Among these non-G protein effectors are the arrestins, cytosolic proteins that mediate GPCR desensitization and internalization by binding ligand-activated GPCRs, uncoupling them from their cognate G proteins, and targeting them to clathrin-coated pits. The arrestins also function as ligand-regulated scaffolds bringing catalytically-active cargo proteins into GPCR-based 'signalsome' complexes that regulate non-receptor tyrosine kinase activity, mitogen-activated protein (MAP) kinase cascades, protein ubiquitination/deubiquitination, pro-survival Akt signaling, nuclear factor κB (NF- κB) signaling, and cytoskeletal rearrangement/cell motility (5). It is increasingly recognized that these non-canonical forms of GPCR signaling contribute to both physiologic and pathophysiologic processes, among them cell proliferation, non-proliferative cell growth, survival and apoptosis, cell migration, chemotaxis and secretory function (6).

Not only is downstream GPCR signaling far more diverse than originally envisioned, the process of receptor activation is itself subject to extensive, and often tissue-specific, modulation. Rather than behaving as simple binary switches whose transition between 'on' and 'off' states is determined by the local concentration of agonist, GPCRs adopt multiple conformationally discrete 'active' states that vary in the efficiency with which they promote receptor coupling to different downstream effectors (7). Moreover, anything that comes into contact with the receptor, whether a ligand, another protein, or lipid in the membrane, may influence the receptor's conformational ensemble in a way that impacts signaling. It is clear that differences in orthosteric ligand structure can introduce 'bias' into receptor coupling, so

as to favor coupling to some downstream effectors over others. Protein-protein interactions that modify signaling include the formation of GPCR dimers, the interaction with receptor activity-modifying proteins, and the binding of PDZ domain-containing and non-PDZ domain scaffold proteins to intracellular receptor domains (8). Such interactions can modify GPCR pharmacology and trafficking, localize receptors to specific subcellular domains, limit signaling to pre-determined pathways, and poise downstream effectors for efficient activation. Yet another modifier of GPCR signaling are small-molecule ‘allosteric modulators’ (7). When considered in the drug discovery context, allosteric modulators are synthetic molecules that change GPCR ligand affinity, efficacy, or both by binding the receptor at a site separate from the orthosteric ligand. Still, a number of endogenous compounds, including a variety of ions, lipids, amino acids, peptides, and physical stimuli display different degrees of receptor-specific modulatory effects (9).

In this review, we discuss the involvement of one GPCR, the angiotensin II (AngII) AT1 receptor (AT1R), in peritoneal membrane fibrosis, a common complication of peritoneal dialysis (PD), and consider how ligand-dependent and ligand-independent AT1R signaling may contribute to its pathogenesis.

2. Peritoneal Dialysis

2.1 Peritoneal membrane fibrosis

End-stage renal failure (ESRD) is an important health issue, with approximately 450,000 Americans on renal replacement therapy in 2012 (2014 United States Renal Data Systems). The annual cost of medical care for dialysis patients in this country exceeds \$15 billion. Hemodialysis (HD) and PD are the current choices for dialysis, while patients wait for renal transplantation. PD is a unique biological situation, in that peritoneal mesothelial cells (PMC) in a single cell layer that lines the peritoneal mesothelium are exposed to elevated concentrations of glucose and glucose metabolites (e.g. advanced glycosylation end products) in the PD fluid, physical derangements (PMC shrinkage caused by hyperosmotic stress of the PD fluid and stretch of PMC caused by increased intraperitoneal volume), and acidic pH (5.5) of the PD fluid (for storage purposes). PD has distinct advantages (continuous nature, less hemodynamic instability, ability to dialyze at home, lack of needle sticks, increased ability to travel, patient independence) over HD. However, only about 10% of dialysis patients perform PD. Long-term PD is limited by the development of peritoneal fibrosis that begins soon after PD is initiated and progresses steadily. Disabling peritoneal membrane dysfunction eventually ensues, characterized by development of very rapid small molecule transport and ultrafiltration failure if marked angiogenesis accompanies the fibrosis or by development of failure to clear solutes. Figure 1 depicts the morphological changes seen in the submesothelial layer of the peritoneal cavity after chronic PD. Many nephrologists are reluctant to utilize PD for more than a few years, because of the progression of peritoneal fibrosis. In extreme cases, encapsulating peritoneal sclerosis occurs, a terminal event characterized by strictures, calcification, bowel obstruction, enteric fistulae, and malnutrition. ESRD patients who are not eligible (medically, immunologically, socially, or emotionally) for transplantation of a renal allograft are often maintained on HD for over 30 years, but PD for more than 10 years is exceedingly rare.

2.2 AngII's involvement in peritoneal membrane fibrosis in PD

AngII is known to mediate fibrosis in a number of tissues (vasculature, heart, kidney), and pharmacological inhibitors of the AngII system (AT1R blockers, angiotensin converting enzyme inhibitors) are commonly used to minimize this process. A number of investigators have hypothesized that fibrosis of the peritoneal membrane during chronic PD, similar to fibrosis in other organs, involves AngII acting through AT1R, and a significant amount of published data support this hypothesis. Elements of the renin-angiotensin system (RAS), including angiotensinogen, angiotensin converting enzyme (ACE), AT1Rs, have been detected in cultured human PMC (10-12). Consistently, AngII is present in the PD fluid of stable PD patients (12). AngII receptors have been detected by RT-PCR, immunoblotting, immunocytochemistry, and radio-ligand binding (13). AT1R are the dominant AngII receptor subtype (rather than AT2 receptors) in PMC (13).

AngII contributes to irritant injury of the peritoneal membrane in intact animals. The protective effects of AngII system inhibition against caustic injury of the peritoneum have been examined in murine models. Fibrosis of the peritoneum was created in 20 – 30 g mice with daily 0.3 ml intraperitoneal injections of a solution containing 0.1% chlorhexidine gluconate and 15% ethanol for 4 weeks (14). Fibrosis scores (combination of fibrotic matrix, large collagen fibers, and fibroblast proliferation) increased 10-fold over the 4-week experimental period. An ACE inhibitor in the drinking water reduced the increases in fibrosis scores and submesothelial thickness caused by the intraperitoneal chlorhexidine/ethanol injections by about 50%. In 160 – 180 g rats, a similar model was established: daily intraperitoneal injections with 2 ml of saline (control) or a 0.1% chlorhexidine gluconate-15% ethanol solution (experimental) for 3 weeks (15). The ACE inhibitor enalapril in the drinking water produced 50% reductions in the increase in urea transport status, reduced ultrafiltration, and increased peritoneal nucleated cell number. Since experimental irritant injury to the peritoneum in mice and rats can be significantly prevented by an ACE inhibitor in the drinking water, which reduces the formation of AngII, it can be concluded that AngII contributes to the peritoneal irritant injury.

AngII contributes to infectious injury of the peritoneal membrane in intact animals. Although daily exposure to PD fluid during the course of chronic PD causes repeated injury to the peritoneal membrane via some or all of the factors mentioned above (glucose, glucose metabolites, cell shrinkage, cell stretch, acidity), the occasional episode of bacterial peritonitis (approximately once in 2 years) acutely worsens the inflammatory/fibrotic process. The majority of offending agents are skin organisms, usually staphylococcal species. Infectious models have been established in animals, and the role of the RAS in such injury has been investigated (16). Wistar rats were injected once intraperitoneally with 3×10^9 colony forming units of *S aureus* mixed with dextran beads as an enhancing factor and provided drinking water with or without the AngII receptor antagonist irbesartan (100 mg/kg/day) for 8 days. *S aureus* mixed with dextran beads created marked injury, as documented by formation of adhesions and a 20-fold increase in peritoneal thickness. When irbesartan was included in the drinking water, formation of adhesions caused by the bacterial/dextran mixture was reduced by more than 80%, and the increase in thickness was

reduced by more than 90%. This study suggests that AT1R signaling participates in infectious peritoneal injury.

AngII contributes to peritoneal membrane injury in whole-animal models of PD. Models have been established in animals to mimic chronic PD in ESRD patients. There is controversy about the appropriate elements of these models (17) including: degree of impairment in renal function (normal vs chronic kidney disease model), means of intraperitoneal fluid delivery (indwelling catheter vs daily injections), volume delivered (10 – 20 ml), frequency of daily fluid instillation (1 – 3 times), and duration of the model (3 – 12 weeks).

Studies have been performed in rats with normal renal function to determine if medications that interfere with the RAS protect against peritoneal injury. Healthy Wistar rats were injected intraperitoneally with 10 ml of saline or PD fluid containing 3.86% glucose each day for 4 weeks (18). The PD fluid caused a number of peritoneal abnormalities, including loss of ability to ultrafilter, increase in urea transport characteristics, increased protein losses into the peritoneal space, large increase in TGF β 1 in the peritoneal fluid, reduction of CA125 concentration (marker of PMC health) in the peritoneal space, 15-fold increase in thickness of the peritoneal membrane, and an inflammatory response in the peritoneal membrane. When the ACE inhibitor enalapril at 100 mg/l was included in the drinking water for the entire 4-week period, the above-mentioned markers of peritoneal injury caused by PD fluid were significantly reduced. In a subsequent study using the same model, these authors found that the AT1R antagonist valsartan was equal to the ACE inhibitor lisinopril in mitigating peritoneal injury caused by glucose-containing PD fluid (19). In a similar study from another laboratory, PD fluid containing 4.25% glucose was injected into the peritoneal space of rats with normal renal function daily for 3 or 7 weeks, and the ACE inhibitor captopril at 30 mg/kg/d was administered by gavage each day to half of the animals (20). There were trends toward less peritoneal thickness, less cellularity, and less collagen expression in the captopril group, especially in the parietal peritoneum, but statistical significance was not reached. This study was limited by a small number of animals at each time point because of mortality (probably from the gavage procedure).

To activate the RAS more fully, another group employed a 2-kidney, 1-clip renovascular hypertension model in normal Wistar rats, then superimposed peritoneal injury with 10 ml daily intraperitoneal infusions of PD fluid containing 1.35% glucose at pH 3.5 through an indwelling peritoneal catheter for 6 weeks (21). This treatment resulted in peritoneal adhesions, bowel encapsulation, and a 6-fold increase over normal in the thickness of the peritoneal membrane. Subsets of rats receiving the 1.35% glucose solution at pH 3.5 were treated with oral olmesartan (AT1R antagonist, 5 mg/kg) or oral amlodipine (calcium channel blocker, 3 mg/kg) for the 6-week period. Whereas the 25 mm Hg-increase in blood pressure was completely prevented by either antihypertensive, olmesartan but not amlodipine, prevented the development of adhesions and thickened peritoneum. No alteration of renal function was mentioned.

AngII contributes to peritoneal membrane dysfunction in chronic PD patients. In 3 retrospective studies, peritoneal membrane transport characteristics were examined in PD

patients treated with or without antihypertensive medications that interrupt the RAS system (AT1R antagonists, ACE inhibitors) for at least 1 (22) or 2 years (23, 24). These studies were possible because assessment of small molecule transport was performed at least twice in these patients. After a 1-year period (22), the dialysate/plasma creatinine ratio at the 4th hour of the peritoneal equilibration test had increased by 0.15 in the non-RAS (control) group but only by 0.09 in the RAS (experimental) group, $p < 0.05$. Similar results were observed in the patients followed for at least 2 years (23, 24). The dialysate/plasma creatinine ratio at 24 hours increased by 0.04 in the non-RAS group and decreased by 0.02 in the RAS group, $p = 0.05$ (24), and the mass transfer area coefficient for creatinine increased by 1.03 ml/min in the non-RAS group and decreased by 1.5 ml/min in the RAS group, $p < 0.01$ (23). All 3 studies demonstrate that chronic therapy with ACE inhibitors or AT1R blockers (ARBs) mitigates or prevents the increase in small molecule transport characteristics that occurs over time from PD therapy. Intraperitoneal fluid concentrations of fibronectin, TGF- β 1, and vascular endothelial growth factor (VEGF) increased over the 1-year period in the patients treated with non-RAS anti-hypertensives (30%, 100%, and 100%, respectively), and these increases were significantly less in patients treated with RAS antagonists (22).

Only 1 prospective, interventional study has been performed (25). Fifteen stable PD patients were recruited, and each acted as his/her own control. In the initial control period, the patients did not use any medications that interrupted the RAS, and in the 30-day experimental period the AT1R antagonist irbesartan was taken orally at an average daily dose of 145 mg. Blood pressure was no different between study periods. In the patients who were not anuric, proteinuria was reduced by irbesartan therapy, as many other investigators have demonstrated. Similarly, protein losses into the peritoneal fluid were significantly lower at the end of the irbesartan period compared to the end of the control period ($p < 0.001$). Small molecule transport characteristics and inflammatory mediators in the PD fluid were not assessed.

These human studies have been interpreted to suggest that time on PD results in inflammation, angiogenesis, and fibrosis of the peritoneum, leading to an increase in small molecule transport status and resulting loss of ultrafiltration capacity, which can be ameliorated with oral inhibition of the RAS system. These studies are significantly limited. In none of these human studies was peritoneal histology obtained to confirm that routine PD causes peritoneal membrane fibrosis and membrane thickening and that pharmacological agents that interrupt the RAS reduce the fibrosis and thickening, nor were RAS inhibitors administered in the peritoneal fluid, i.e. the site of initiation of peritoneal injury.

3. Angiotensin II (AngII) and Receptors

3.1 Generation of AngII

AngII is a major regulator of blood pressure. Since its initial discovery and identification as 'hypertensin' in 1940, AngII has been one of the most studied regulators of salt/water homeostasis and blood pressure control in the body (26). AngII is the circulating hormonal product of the activation of the renin-angiotensin system (RAS). Alterations in arterial blood pressure affect circulating concentrations of AngII, which in turn affects blood volume and

arterial vasoconstriction. Reductions in blood volume or blood pressure produce activation of the renal enzyme renin, releasing angiotensin I from the substrate angiotensinogen. Subsequent metabolism of AngI by ACE generates the eight amino acid peptide AngII [Ang(1-8)]. Processing of AngI by additional/alternative aminopeptidases or endopeptidases results in the appearance of other AngII fragments including Ang(1-7) and Ang(2-10). The RAS can be found in several organs and tissues including brain, heart, blood vessels and, where it was originally identified, the kidney (26). The contribution of Ang II to cardiovascular diseases is highlighted by the successful use of inhibitors of Ang II generation and of antagonists to AT1Rs in combating hypertension and other forms of cardiovascular disease.

3.2 AngII Receptors

The receptors for AngII are classical cell surface GPCRs (26, 27) Initial classification of receptors interacting with AngII resulted in the naming of two AngII receptors, AT1R and AT2R. Both AT1Rs and AT2Rs are members of the rhodopsin class of 7 transmembrane-spanning GPCRs and both interact specifically with AngII. Their distinctions lie in their interactions with AngII analogues (agonists and antagonists), intracellular signaling, and tissue distribution.

Historically, subtype-selective small molecule agonists/antagonists have been utilized in determining involvement of AT1R or AT2R in specific cell signaling events (*see Table*). Should a pathway be activated by CGP42112, AT2R are the initiating receptors. Should addition of AngII produce a losartan-inhibitable response, AT1Rs are involved; while inhibition by PD123177 denotes AT2R activation. Metabolites of AngII also interact with both AT1Rs and AT2Rs, with Ang(2-10) showing selectivity for AT2R (28). It should be noted that Ang(1-7) is the cognate ligand for the GPCR Mas (29). Although recent studies have shown an important physiological role for Ang(1-7) and its interaction with the Mas receptor it will not be discussed in this review. In the early 1970's it was shown that the aminopeptidase-derived product [des-Asp1]Angiotensin I (DAA-I) was an alternative, minor metabolite of angiotensin I (30). DAA-I has recently generated renewed interest since it has been suggested to be an endogenous biased agonist for AT1Rs (31).

The AT1R of mouse and rat is further subdivided into two isoforms, AT1A and AT1B, with 95% homology between the two proteins, but significant differences in genomic characteristics, regulation of expression, chromosomal location and tissue distribution. The AT1AR is the predominantly expressed isoform, especially in vascular smooth muscle. Both the AT1AR and the AT1BR are required for normal control of blood pressure and renal development (32-34) and play a role in cardiovascular disorders, including hypertension(34-39).

The majority of the physiological, and possibly pathological, events initiated by Ang II are through AT1Rs. Figure 2 summarizes the major intracellular pathways mediating AT1R signaling. Short-term AT1R signaling is mediated through activation of heterotrimeric G proteins, primarily of the Gq/11, G12/13 and Gi/o families. Gq/11 activation initiates a well-characterized signaling pathway involving phospholipase C β , inositol tris-phosphate (IP3), elevations in intracellular free calcium, activation of intracellular kinases including PKC and

p42/44 ERK, and regulation of smooth muscle contractility and vascular tone (40). G12/13 couple the AT1R to RhoA-GEFs leading to cytoskeletal rearrangement, while Gi/o mediate inhibition of adenylyl cyclases and regulation of potassium channels. AT1R activation is rapidly followed by its phosphorylation by G protein-dependent kinases (GRKs) and recruitment of β -arrestins, that mediate AT1R desensitization, clathrin-dependent endocytosis, and subsequent activation of internalization-dependent signaling pathways.

The engagement of both G protein and non-G protein effectors enables AT1Rs to regulate a multitude of downstream pathways. Other transducers of AT1R signals are JAK2-STAT3(41, 42), pp60 c-Src and many of its effectors including Phospholipase-C γ , FAK, Pyk2, CamK II, GIT1 and Cas (43-45) and CARMA3, which promotes activation of NF- κ B (46). AT1Rs promote 'transactivation' of epidermal growth factor (EGF) receptor tyrosine kinases by stimulating shedding of preformed EGF receptor ligands by matrix metalloproteinases (47-49) and activate the ERK1/2 and p38 MAP kinase cascades (50, 51). Regulation of the prolific ERK1/2 cascade occurs via both G protein-dependent mechanisms, and through scaffolding by β -arrestins, which recruit ERK1/2 into AT1R-based 'signalsomes' and constrain its signaling (52, 53). AT1R activation also leads to generation of reactive oxygen species via activation of the NADH-NADPH oxidase pathways (54, 55).

AT2Rs, on the other hand, stimulate responses opposite to those of AT1Rs, i.e. inhibition of contractility, apoptosis, etc. (34, 56). Structural differences are apparent as well with AT2Rs demonstrating approximately 40% AA homology with AT1Rs. Expression of these two isoforms also differs, with the AT2 expression seen mostly in fetal tissue and limited to brain, ovary, kidney and mesenteric arteries in adults (57, 58).

Adding to the potential diversity in AngII signaling are reports of cytoplasmic and nuclear Ang II binding sites in vascular smooth muscle (VSM), both in primary cultures and cloned cells lines, and in liver extracts (59-65). Microinjection of Ang II into the cytoplasm of VSM cells initiates elevations in intracellular free calcium and activation of tyrosine kinases. Several GPCRs have been described as residing either in the nuclear membrane or the nucleus itself (66, 67). The nuclear sites possessed ligand recognition properties comparable to the plasma membrane AT1Rs but displayed different physical-chemical properties (62, 63). Our work as well as that of others, have identified nuclear AngII receptors which we hypothesize are directed to the nuclear membrane as a consequence of a nuclear localization sequence found within the cytoplasmic tail of the receptor (amino acid residues 307-312; KKFKKY) (68-71). Activation of these "nuclear" AT1Rs results in induction of total RNA and mRNA for renin and angiotensinogen, suggesting the existence of a positive feedback loop for Ang II formation (72-74) and expression of cyclooxygenase 2 (75, 76).

Specific regions within the AT1R have been identified as being essential for internalization of the activated receptor. Although induction of G protein-mediated signaling and removal of AT1Rs from the cell surface may occur sequentially, residues involved with G protein activation are not necessarily involved in receptor internalization. One region of the receptor demonstrated to be required for endocytosis is amino acids 331-338 (-STKMSTLS-) located within the cytoplasmic tail of the receptor (77-79). Additional residues within the receptor tail have been identified as internalization motifs including leucine(L)³¹⁶ and

tyrosine(Y)³¹⁹(79). Substitution of asparagine or alanine for Met¹³⁴, or replacement of the highly conserved residues Asp¹²⁵ and Arg¹²⁶ (DR) with alanine, both within the second intracellular loop, results in loss of internalization, and intracellular and nuclear signaling (80).

The AT1R, analogous to other GPCRs, contains an NPX₂₋₃Y sequence, which in the AT1AR is located at residues 298-302 (NPLFY). This sequence has been demonstrated in other GPCRs to be involved in receptor internalization (81). Interestingly, with the AT1AR this is not the case. Mutations in this region do not alter internalization dynamics (79, 82).

Mutations in the N-terminal region of the third cytoplasmic loop of the AT1AR suggest that this region may be involved in transduction of the mitogenic signal elicited by Ang II. When residues 234-240 of the AT1AR were replaced by an analogous region of the AT2R in transfected CHO cells, Ang II failed to initiate proliferation, despite receptor internalization (83). Overall, internalization of the AT1R involves motifs found within the cytoplasmic tail, a region rich in phosphorylation sites.

The mechanism for internalization of the AT1R appears to mimic that seen for other GPCRs and is similar to that originally described for the β_2 -adrenergic receptor (β_2 -AR). Agonist interaction with the receptor initiates a transition of the receptor from an inactive to an active state and activates G protein-dependent signaling. Activation of GPCR kinases (GRKs) ensues, producing phosphorylation of residues within the cytoplasmic tail of the receptor and subsequent binding to β -arrestins, which halts further activation of G proteins and directs the receptor into clathrin-coated pits along the surface of the membrane. The pits are subsequently pinched off from the membrane, i.e. internalized, through the actions of dynamin, a membrane bound GTPase. The internalized coated pit containing the agonist-phosphorylated GPCR-arrestin complex then is targeted to acidic endosomes (lysosomes), where the complex is either dissociated as a result of de-phosphorylation of the receptor and rapidly recycled back to the plasma membrane (Class A GPCRs) (84-88), or, retained in the endosomes, with the β -arrestin attached (Class B GPCRs) (89, 90). The AT1R belongs to the latter class. Like the β_2 -AR, it can be internalized by a pathway that utilizes dynamin and β -arrestin (91, 92) but, unlike the β_2 -AR, demonstrates persistent arrestin-binding and slower recycling. This slow recycling is thought to be the result of enhanced interaction between the receptors and β -arrestin (88).

3.3 Peritoneal AngII receptors

Peritoneal mesothelial cells (PMC) are epithelial-like cells lining both the visceral and parietal walls of the intraperitoneal cavity. These cells are routinely bathed in a lubricating fluid, approximately 50 ml a day, which allows for the opposing surfaces of peritoneal mesothelial cells to slide over each other during routine movements. As noted, PMC possess the requisite elements of the RAS including AT1R. As a consequence of peritoneal dialysis, AT1R of PMC are exposed on a daily basis to the extremely harsh, non-physiological conditions of peritoneal dialysis fluid, i.e. elevated concentrations of glucose and metabolites ($\sim 5 \times$ plasma concentrations), physical derangements of increased fluid volume ($\sim 100 \times$), hyperosmotic stress (485 mOsm vs 280 mOsm for plasma), and low pH (~ 5.5). These harsh conditions alone may modulate AT1R signaling independent of AngII

generation, by affecting PMC expression of RAS components or producing physical/chemical stressors that alter downstream AT1R signaling.

3.4 Regulation of AngII receptors by glucose

Examination of effects of elevated glucose on expression of AT1R in PMC is limited. Increased glucose concentrations elevated total cell expression of AT1R as measured by RT-PCR and immunoblotting in PMC (11, 12). In these studies, the effects of glucose on AngII surface binding were not studied. On the other hand, the effects of glucose in non-PMC tissue [e.g. kidney, heart, vascular smooth muscle cells (VSMC)] have been studied in some detail. Supraphysiological concentrations of glucose downregulated mRNA, protein and cell surface expression of AT1R (assessed by radioligand binding and immunofluorescence) in renal proximal tubules cells (93). Cellular uptake of glucose was necessary for this, whereas glucose's hyperosmotic effects were not, because the inert sugars mannitol or L-glucose could not mimic D-glucose. In cultured rat glomerular mesangial cells, exposure to elevated glucose concentrations reduced the cell surface density of AT1R, as determined by radioligand binding studies (94).

3.5 Regulation of AngII receptors by advanced glycation end products (AGE)

AGE, which are proteins or lipids that have been non-enzymatically glycosylated by aldose sugars, are considered to be mediators of fibrosis (95). In cultured renal podocytes, exposure to AGE increased expression of AT1R (mRNA and protein content) but not AT2R, however assessment of surface binding was not performed (96). Thus, the presence of AGE seen under conditions of elevated glucose (plasma of diabetics, PD fluid) may also be involved in regulating expression of AT1R.

3.6 Regulation of AngII receptors by stretch

During PD, PMC are exposed to intraperitoneal volumes as much as 60-fold greater than those found under normal conditions. The increased volume imparts significant physical forces on PMC (stretch from fluid under pressure), which might alter normal cell physiology. A classic study in humans demonstrated that increasing IP volume causes proportional increases in intra-abdominal pressure (97). Since the increase in volume is within the peritoneal space, the pressure must stretch PMC outward. In a seminal manuscript, mechanical stretch of cardiomyocytes by flexion of the bottom of the culture well initiated cellular signaling (e.g. phosphorylation of ERK and JAK2) that was blocked by AT1R antagonists in the absence of secreted AngII, thus demonstrating stretch-induced activation of AT1R (98). Other studies have demonstrated that experimental stretch of cultured cells stimulates cell actions through AT1R; production of adrenomedullin in neonatal rat cardiac myocytes (99), expression of fibronectin in vascular smooth muscle cells (100), expression of heparin-binding epidermal growth factor in bladder smooth muscle cells (101), and upregulation of matrix metalloproteinases in neonatal rat myocytes (102). Cell stretch did not activate AT2 receptors on the cell lines studied. Mechanical stretch of human PMC by either of 2 means (shear stress by culture plate rotation or flexion of the bottoms of the wells) did not increase TGF- β 1 formation (an AT1R signal); but when this was repeated in elevated glucose medium, an increase in TGF- β 1 formation was observed (103). In this study (103), no mention of AngII receptor regulation was made.

3.7 Effects of osmotic cell stress on AngII receptors

During chronic PD, exposure of PMC to elevated intraperitoneal glucose concentrations produces an osmotic load approximately twice that found in the normal peritoneum. In cultured mouse mesangial cells, elevated glucose concentrations activated AT1R signaling, including synthesis of collagen and fibronectin, without elaboration of AngII itself, consistent with the hypothesis that osmotic stress (i.e. cell shrinkage) directly activates AT1R (104). These studies investigated the role of osmotic stress rather than stretch, since there is no elevation of pressure in cell culture.

3.8 Regulation of AngII receptors by pH

Although adjusting pH to 5.5 (compared to 7.4) stabilizes PD fluid, the acidic microenvironment appears to contribute to the development of peritoneal fibrosis. Data on the effects of pH on AngII receptors are extremely limited. It is well known that acute reductions in pH cause AngII to dissociate from its receptors. This technique is used in cell culture experiments to eliminate prior ligand occupancy, and low pH in endosomes allows AngII to separate from AT1R for recycling of the receptor. Further, systemic pH less than 7.2 in critically ill patients appears to remove pressor ligands, like AngII, from their receptors on the surface of VSMC, resulting in reductions in peripheral vascular resistance and the development of shock.

On the other hand, much less is known about regulation of AngII receptors by chronic reductions in pH. Carroll et al. showed that lowering culture medium pH from 7.4 to 6.8 for 45 minutes significantly increased AngII binding and aldosterone secretion in adrenal glomerulosa cells (105). This increased binding was also seen in crude membranes made from adrenal cells, indicating that the effect of low pH was direct and not mediated through a cellular response. More recently, a study employing both *in vivo* and *in vitro* models demonstrated enhanced AT1R expression and signaling under acidic conditions (106). After mice were exposed to an acid load for 18 hours, AT1R mRNA expression and protein levels were increased in renal cortex, compared to control animals. Similarly, proximal tubule cells cultured at pH 7.0 expressed increased total AT1R protein, AT1R cell surface expression, and AngII-induced ammoniogenic responses compared to cells grown at pH 7.4. In a hypertensive animal model of PD, peritoneal thickening and loss of PMC developed from treatment with acidic PD fluid (pH 3.5) for 42 days, but damage did not occur with PD fluid at neutral pH; peritoneal histopathology from low pH was ameliorated with oral administration of an AT1R antagonist (21).

4. The AT1R as A Therapeutic Target for Peritoneal Fibrosis

Both data from animal models and limited human trials have implicated AT1R in the pathogenesis of PD-related peritoneal fibrosis. But these studies have for the most part employed systemic, not intraperitoneal, pharmacological agents targeting RAS to imply this involvement. While it is clear that systemically administered ACE inhibitors and small molecule AT1R antagonists mitigate the peritoneal fibrotic response in animal models of PD, and do so seemingly independent of their effects on systemic blood pressure, the degree to which AT1R expressed on PMCs are responsible for the fibrosis remains to be

established. Nonetheless, PMCs clearly express AT1Rs, and elements of the RAS are present within the peritoneal cavity, suggesting that AngII signaling within that space could not only occur, but may be regulated by factors apart from systemic blood pressure. Local conditions imposed during PD, e.g. high glucose, osmotic stress, cell stretch and low pH, have been shown to affect expression of AT1Rs and/or modify AngII signaling. It thus seems likely that PD promotes local inflammatory responses, at least in large part, through local effects on PMCs, and that the fibrotic response is initiated via AT1Rs. If so, targeting AT1R signaling within the peritoneal cavity, e.g. delivering RAS modifiers via the dialysate, could prove a more effective therapeutic strategy than systemic administration.

The complexity of AT1R signaling, encompassing signals from both heterotrimeric G proteins and non-G protein effectors like the β -arrestins, introduces additional questions about the mechanism(s) underlying peritoneal fibrosis and the most effective approach to treatment. For example, we have shown in cultured PMCs that specific components of PD fluid, namely high glucose and osmotic stress, can activate AT1R, possibly even in the absence of the cognate ligand AngII (13). We have also characterized a novel pathway of NF- κ B activation and subsequent synthesis of COX-2 as a consequence of arrestin-dependent AT1R internalization (107). Further, AngII is known to stimulate transcription of transforming growth factor β 1 (TGF- β 1) in several cell types (12, 108, 109). TGF- β 1 is known to promote epithelial-to-mesenchymal transition, and to contribute to the pro-fibrogenic actions of AngII (110, 111). Consequently, administration of TGF- β 1 receptor-targeted peptides significantly reduced fibrosis and improved transport function in a mouse model of PD (112).

At present, little is known about the role β -arrestins play in these processes. Data suggest that cell membrane stretch can alter the structure and function of arrestins (113), which may in turn alter AT1R signaling in ways that 'biases' it toward a fibrotic response. The role of arrestins in promoting fibrosis has been demonstrated in a murine model of bleomycin-induced pulmonary fibrosis, wherein mice null for β -arrestin2 are protected (114). Based on our own data as well as the current literature, we hypothesize that activation of AT1R can occur by physical perturbation of the plasma membrane caused by the hyperosmotic challenge of PD fluid. The physical change in membrane architecture concomitantly alters AT1R conformation, allowing for interaction with receptor internalization machinery, i.e. arrestins, producing a 'biased', non-G protein-mediated response leading to synthesis of pro-inflammatory, pro-fibrotic mediators such as COX-2. This non-canonical pathway is shown in Figure 3.

If, as we hypothesize, the unique environmental conditions to which PMCs are exposed during PD modulates AT1R signaling either allosterically or by affecting the local production of AngII, the question remains as to the best way to inhibit the deleterious signals. One could argue, based upon animal studies and limited human trials mentioned above, that use of ACE inhibitors would be logical choices for prevention of AT1R-induced peritoneal fibrosis. However, considering findings from animal studies on hypertrophic cardiac disease, addition of ARBs alone would be suitable. ARBs conceivably would 'lock' AT1R in a non-activatable conformation and prevent arrestin-dependent pro-fibrotic signaling. Further research will be needed to establish whether global blockade of AT1R

signaling is desired, or whether some AT1R signals are in fact beneficial. While orthosteric small molecule AT1R antagonists, e.g. irbesartan and valsartan, have demonstrated benefit (when administered systemically), one might suppose that endogenous AT1R on PMCs also play physiologically adaptive roles. In that case introducing a 'neutral' antagonist that blocks all aspects of AT1R signaling may not be optimal, even if stabilizing the inactive receptor conformation through binding of a small molecule within the orthosteric site does retard maladaptive signaling.

Currently, there is considerable interest in exploring the utility of functionally selective 'biased' GPCR ligands in re-calibrating dysregulated GPCR signaling (115). As it is clear that GPCRs adopt multiple conformationally discrete states that engage downstream effectors with different efficiency, it is not surprising that structurally distinct ligands can 'bias' signaling by preferentially stabilizing conformations that favor some effectors over others (88, 116, 117). *In vivo*, i.e. in the presence of the endogenous ligand, such compounds would exert mixed agonist-antagonist effects, antagonizing the actions of the native ligand while at the same time promoting activation of selected pathways (118-120). Downstream intracellular signaling patterns are thus dependent upon the structure of the agonist interacting with the receptor. For example, Santos et al. have shown differential signaling for AT1R after exposure to AngII or the biased agonists [Sar¹, Ile⁴, Ile⁸]-AngII and [Sar¹, D-Ala⁸]-AngII (TRV027) (121). Each ligand demonstrated differential activation of arrestins, intracellular kinases, and gene expression profiles. Similarly, Masuho and colleagues have shown that biased signaling extends to distinct G protein engagement patterns or 'fingerprints' of a GPCR with multiple G proteins (122). Ligand bias can favor either selective G protein or arrestin coupling. Indeed, arrestin pathway-selective agonists for the parathyroid hormone PTH1 (123, 124) and angiotensin AT1ARs (125), and G protein pathway selective agonists for the GPR109A nicotinic acid (126, 127) and μ opioid receptors (128), have demonstrated unique and potentially therapeutic efficacy in cell-based assays and preclinical animal models. The challenge in determining whether 'biased' ligands will have utility in the management of PD-induced fibrosis is to determine which aspects of AT1R signaling, e.g. G protein versus arrestin-dependent, are deleterious, and whether AT1Rs mediate other beneficial signals that might be preserved using 'biased' ligands.

5. Summary and Future Directions

The incidence of end-stage renal disease has increased greatly over the past 30 years. Hemodialysis and PD are the bridging modalities to renal transplantation. During PD, hyperosmotic glucose solutions, instilled into the peritoneal cavity in repeated cycles, comprise the osmotic force that allows for ultrafiltration. Chronic PD results in loss of filtration function caused by peritoneal membrane fibrosis, limiting its long-term utility. As we have described above, AngII has been implicated in this process but the cellular mechanisms for this response have not been clearly elucidated. Currently, there is no pharmacological regimen utilized in PD fluid to prevent or reverse the inevitable peritoneal fibrotic response. Future studies will hopefully clarify the nature of AngII pro-fibrotic signaling in PMCs and may lead to new, targeted intraperitoneal interventions that may reduce peritoneum fibrosis during chronic PD. In addition, new mechanistic information on

the role of AT1R in PD-induced peritoneal fibrosis would have far-reaching implications for fibrotic injury of other organs, including the heart, kidneys and vasculature.

Acknowledgments

The work was supported by Dialysis Clinic, Incorporated (T.A.M./M.E.U.), National Institutes of Health Grant R01 DK55524 AND GM095497 (L.M.L.) and the Research Service of the Charleston, SC Veterans Affairs Medical Center (L.M.L./M.E.U.). The contents of this article do not represent the views of the Department of Veterans Affairs or the United States Government

References

1. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, Funke R, Gage D, Harris K, Heaford A, Howland J, Kann L, Lehoczky J, LeVine R, McEwan P, McKernan K, Meldrim J, Mesirov JP, Miranda C, Morris W, Naylor J, Raymond C, Rosetti M, Santos R, Sheridan A, Sougnez C, Stange-Thomann Y, Stojanovic N, Subramanian A, Wyman D, Rogers J, Sulston J, Ainscough R, Beck S, Bentley D, Burton J, Clee C, Carter N, Coulson A, Deadman R, Deloukas P, Dunham A, Dunham I, Durbin R, French L, Grafham D, Gregory S, Hubbard T, Humphray S, Hunt A, Jones M, Lloyd C, McMurray A, Matthews L, Mercer S, Milne S, Mullikin JC, Mungall A, Plumb R, Ross M, Shownkeen R, Sims S, Waterston RH, Wilson RK, Hillier LW, McPherson JD, Marra MA, Mardis ER, Fulton LA, Chinwalla AT, Pepin KH, Gish WR, Chissole SL, Wendl MC, Delehaunty KD, Miner TL, Delehaunty A, Kramer JB, Cook LL, Fulton RS, Johnson DL, Minx PJ, Clifton SW, Hawkins T, Branscomb E, Predki P, Richardson P, Wenning S, Slezak T, Doggett N, Cheng JF, Olsen A, Lucas S, Elkin C, Uberbacher E, Frazier M, Gibbs RA, Muzny DM, Scherer SE, Bouck JB, Sodergren EJ, Worley KC, Rives CM, Gorrell JH, Metzker ML, Naylor SL, Kucherlapati RS, Nelson DL, Weinstock GM, Sakaki Y, Fujiyama A, Hattori M, Yada T, Toyoda A, Itoh T, Kawagoe C, Watanabe H, Totoki Y, Taylor T, Weissbach J, Heilig R, Saurin W, Artiguenave F, Brottier P, Bruls T, Pelletier E, Robert C, Wincker P, Smith DR, Doucette-Stamm L, Rubinfeld M, Weinstock K, Lee HM, Dubois J, Rosenthal A, Platzer M, Nyakatura G, Taudien S, Rump A, Yang H, Yu J, Wang J, Huang G, Gu J, Hood L, Rowen L, Madan A, Qin S, Davis RW, Federspiel NA, Abola AP, Proctor MJ, Myers RM, Schmutz J, Dickson M, Grimwood J, Cox DR, Olson MV, Kaul R, Raymond C, Shimizu N, Kawasaki K, Minoshima S, Evans GA, Athanasiou M, Schultz R, Roe BA, Chen F, Pan H, Ramser J, Lehrach H, Reinhardt R, McCombie WR, de la Bastide M, Dedhia N, Blocker H, Hornischer K, Nordsiek G, Agarwala R, Aravind L, Bailey JA, Bateman A, Batzoglu S, Birney E, Bork P, Brown DG, Burge CB, Cerutti L, Chen HC, Church D, Clamp M, Copley RR, Doerks T, Eddy SR, Eichler EE, Furey TS, Galagan J, Gilbert JG, Harmon C, Hayashizaki Y, Haussler D, Hermjakob H, Hokamp K, Jang W, Johnson LS, Jones TA, Kasif S, Kasprzyk A, Kennedy S, Kent WJ, Kitts P, Koonin EV, Korf I, Kulp D, Lancet D, Lowe TM, McLysaght A, Mikkelsen T, Moran JV, Mulder N, Pollara VJ, Ponting CP, Schuler G, Schultz J, Slater G, Smit AF, Stupka E, Szustakowski J, Thierry-Mieg D, Thierry-Mieg J, Wagner L, Wallis J, Wheeler R, Williams A, Wolf YI, Wolfe KH, Yang SP, Yeh RF, Collins F, Guyer MS, Peterson J, Felsenfeld A, Wetterstrand KA, Patrinos A, Morgan MJ, de Jong P, Catanese JJ, Osoegawa K, Shizuya H, Choi S, Chen YJ, Szustakowski J. International Human Genome Sequencing C. Initial sequencing and analysis of the human genome. *Nature*. 2001; 409(6822):860–921. DOI: 10.1038/35057062 [PubMed: 11237011]
2. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, Gocayne JD, Amanatides P, Ballew RM, Huson DH, Wortman JR, Zhang Q, Kodira CD, Zheng XH, Chen L, Skupski M, Subramanian G, Thomas PD, Zhang J, Gabor Miklos GL, Nelson C, Broder S, Clark AG, Nadeau J, McKusick VA, Zinder N, Levine AJ, Roberts RJ, Simon M, Slayman C, Hunkapiller M, Bolanos R, Delcher A, Dew I, Fasulo D, Flanigan M, Florea L, Halpern A, Hannenhalli S, Kravitz S, Levy S, Mobarry C, Reinert K, Remington K, Abu-Threideh J, Beasley E, Biddick K, Bonazzi V, Brandon R, Cargill M, Chandramouliswaran I, Charlab R, Chaturvedi K, Deng Z, Di Francesco V, Dunn P, Eilbeck K, Evangelista C, Gabrielian AE, Gan W, Ge W, Gong F, Gu Z, Guan P, Heiman TJ, Higgins ME, Ji RR, Ke Z, Ketchum KA, Lai Z, Lei Y, Li Z, Li J, Liang Y, Lin X, Lu F, Merkulov GV, Milshina N, Moore HM, Naik AK, Narayan VA, Neelam B, Nusskern D, Rusch DB, Salzberg S, Shao W, Shue B, Sun J, Wang Z, Wang A, Wang X, Wang J, Wei M,

Wides R, Xiao C, Yan C, Yao A, Ye J, Zhan M, Zhang W, Zhang H, Zhao Q, Zheng L, Zhong F, Zhong W, Zhu S, Zhao S, Gilbert D, Baumhueter S, Spier G, Carter C, Cravchik A, Woodage T, Ali F, An H, Awe A, Baldwin D, Baden H, Barnstead M, Barrow I, Beeson K, Busam D, Carver A, Center A, Cheng ML, Curry L, Danaher S, Davenport L, Desilets R, Dietz S, Dodson K, Doup L, Ferriera S, Garg N, Gluecksmann A, Hart B, Haynes J, Haynes C, Heiner C, Hladun S, Hostin D, Houck J, Howland T, Ibegwam C, Johnson J, Kalush F, Kline L, Koduru S, Love A, Mann F, May D, McCawley S, McIntosh T, McMullen I, Moy M, Moy L, Murphy B, Nelson K, Pfannkoch C, Pratts E, Puri V, Qureshi H, Reardon M, Rodriguez R, Rogers YH, Romblad D, Ruhfel B, Scott R, Sitter C, Smallwood M, Stewart E, Strong R, Suh E, Thomas R, Tint NN, Tse S, Vech C, Wang G, Wetter J, Williams S, Williams M, Windsor S, Winn-Deen E, Wolfe K, Zaveri J, Zaveri K, Abril JF, Guigo R, Campbell MJ, Sjolander KV, Karlak B, Kejariwal A, Mi H, Lazareva B, Hatton T, Narechania A, Diemer K, Muruganujan A, Guo N, Sato S, Bafna V, Istrail S, Lippert R, Schwartz R, Walenz B, Yooseph S, Allen D, Basu A, Baxendale J, Blick L, Caminha M, Carnes-Stine J, Caulk P, Chiang YH, Coyne M, Dahlke C, Mays A, Dombroski M, Donnelly M, Ely D, Esparham S, Fosler C, Gire H, Glanowski S, Glasser K, Glodek A, Gorokhov M, Graham K, Gropman B, Harris M, Heil J, Henderson S, Hoover J, Jennings D, Jordan C, Jordan J, Kasha J, Kagan L, Kraft C, Levitsky A, Lewis M, Liu X, Lopez J, Ma D, Majoros W, McDaniel J, Murphy S, Newman M, Nguyen T, Nguyen N, Nodell M, Pan S, Peck J, Peterson M, Rowe W, Sanders R, Scott J, Simpson M, Smith T, Sprague A, Stockwell T, Turner R, Venter E, Wang M, Wen M, Wu D, Wu M, Xia A, Zandieh A, Zhu X. The sequence of the human genome. *Science*. 2001; 291(5507):1304–51. DOI: 10.1126/science.1058040 [PubMed: 11181995]

3. Flower DR. Modelling G-protein-coupled receptors for drug design. *Biochim Biophys Acta*. 1999; 1422(3):207–34. [PubMed: 10548717]
4. Luttrell LM. Transmembrane signaling by G protein-coupled receptors. *Methods Mol Biol*. 2006; 332:3–49. DOI: 10.1385/1-59745-048-0:1 [PubMed: 16878684]
5. Luttrell LM, Gesty-Palmer D. Beyond desensitization: physiological relevance of arrestin-dependent signaling. *Pharmacol Rev*. 2010; 62(2):305–30. DOI: 10.1124/pr.109.002436 [PubMed: 20427692]
6. Luttrell LM. Arrestin pathways as drug targets. *Prog Mol Biol Transl Sci*. 2013; 118:469–97. DOI: 10.1016/B978-0-12-394440-5.00018-8 [PubMed: 23764065]
7. Gesty-Palmer D, Luttrell LM. Refining efficacy: exploiting functional selectivity for drug discovery. *Adv Pharmacol*. 2011; 62:79–107. DOI: 10.1016/B978-0-12-385952-5.00009-9 [PubMed: 21907907]
8. Maudsley S, Martin B, Luttrell LM. The origins of diversity and specificity in g protein-coupled receptor signaling. *J Pharmacol Exp Ther*. 2005; 314(2):485–94. DOI: 10.1124/jpet.105.083121 [PubMed: 15805429]
9. van der Westhuizen ET, Valant C, Sexton PM, Christopoulos A. Endogenous allosteric modulators of G protein-coupled receptors. *J Pharmacol Exp Ther*. 2015; 353(2):246–60. DOI: 10.1124/jpet.114.221606 [PubMed: 25650376]
10. Kiribayashi K, Masaki T, Naito T, Ogawa T, Ito T, Yorioka N, Kohno N. Angiotensin II induces fibronectin expression in human peritoneal mesothelial cells via ERK1/2 and p38 MAPK. *Kidney Int*. 2005; 67(3):1126–35. Epub 2005/02/09. DOI: 10.1111/j.1523-1755.2005.00179.x [PubMed: 15698454]
11. Kyuden Y, Ito T, Masaki T, Yorioka N, Kohno N. Tgf-beta1 induced by high glucose is controlled by angiotensin-converting enzyme inhibitor and angiotensin II receptor blocker on cultured human peritoneal mesothelial cells. *Perit Dial Int*. 2005; 25(5):483–91. Epub 2005/09/24. [PubMed: 16178483]
12. Noh H, Ha H, Yu MR, Kim YO, Kim JH, Lee HB. Angiotensin II mediates high glucose- induced TGF-beta1 and fibronectin upregulation in HPMC through reactive oxygen species. *Perit Dial Int*. 2005; 25(1):38–47. Epub 2005/03/18. [PubMed: 15770925]
13. Ullian ME, Luttrell LM, Lee MH, Morinelli TA. Stimulation of cyclooxygenase 2 expression in rat peritoneal mesothelial cells. *Nephron, Experimental Nephrology*. 2014; 128(3- 4):89–97. Epub December 17, 2014. DOI: 10.1159/000368673
14. Sawada T, Ishii Y, Tojimbara T, Nakajima I, Fuchinoue S, Teraoka S. The ACE inhibitor, quinapril, ameliorates peritoneal fibrosis in an encapsulating peritoneal sclerosis model in mice. *Pharmacol Res*. 2002; 46(6):505–10. Epub 2002/11/30. [PubMed: 12457623]

15. Bozkurt D, Cetin P, Sipahi S, Hur E, Nar H, Ertlav M, Sezak M, Duman S. The effects of renin-angiotensin system inhibition on regression of encapsulating peritoneal sclerosis. *Perit Dial Int.* 2008; 28(Suppl 5):S38–42. [PubMed: 19008539]
16. Ersoy R, Celik A, Yilmaz O, Sarioglu S, Sis B, Akan P, Yenicerioglu Y, Ormen M, Camsari T. The effects of irbesartan and spironolactone in prevention of peritoneal fibrosis in rats. *Perit Dial Int.* 2007; 27(4):424–31. Epub 2007/07/03. [PubMed: 17602151]
17. Stojimirovic B, Jovanovic N, Trbojevic-Stankovic J, Nestic DM, Brasanac T, Zunic-Bozinovski S. Levels of transforming growth factor beta1 during first six months of peritoneal dialysis. *Ren Fail.* 2015; 37(4):640–5. DOI: 10.3109/0886022X.2015.1010417 [PubMed: 25682975]
18. Duman S, Gunal AI, Sen S, Asci G, Ozkahya M, Terzioglu E, Akcicek F, Atabay G. Does enalapril prevent peritoneal fibrosis induced by hypertonic (3.86%) peritoneal dialysis solution? *Perit Dial Int.* 2001; 21(2):219–24. Epub 2001/05/02. [PubMed: 11330572]
19. Duman S, Sen S, Duman C, Oreopoulos DG. Effect of valsartan versus lisinopril on peritoneal sclerosis in rats. *Int J Artif Organs.* 2005; 28(2):156–63. [PubMed: 15770604]
20. Schuinski AF, Baroni G, Pecoits Filho RF, Meyer F, Cerqueira ML, da Silva MA, de Carvalho V. Evaluation of the use of captopril on peritoneal fibrosis induced in rats by the use of glucose solution 4.25%. *J Bras Nefrol.* 2013; 35(4):273–8. DOI: 10.5935/0101-2800.20130046 [PubMed: 24402107]
21. Nakamoto H, Imai H, Fukushima R, Ishida Y, Yamanouchi Y, Suzuki H. Role of the renin-angiotensin system in the pathogenesis of peritoneal fibrosis. *Perit Dial Int.* 2008; 28(Suppl 3):S83–7. Epub 2008/09/20. [PubMed: 18552271]
22. Jing S, Kezhou Y, Hong Z, Qun W, Rong W. Effect of renin-angiotensin system inhibitors on prevention of peritoneal fibrosis in peritoneal dialysis patients. *Nephrology.* 2010; 15(1):27–32. Epub 2010/04/10. DOI: 10.1111/j.1440-1797.2009.01162.x [PubMed: 20377768]
23. Kolesnyk I, Dekker FW, Noordzij M, le Cessie S, Struijk DG, Krediet RT. Impact of ACE inhibitors and AII receptor blockers on peritoneal membrane transport characteristics in long-term peritoneal dialysis patients. *Perit Dial Int.* 2007; 27(4):446–53. Epub 2007/07/03. [PubMed: 17602154]
24. Kolesnyk I, Noordzij M, Dekker FW, Boeschoten EW, Krediet RT. A positive effect of AII inhibitors on peritoneal membrane function in long-term PD patients. *Nephrol Dial Transplant.* 2009; 24(1):272–7. Epub 2008/08/05. DOI: 10.1093/ndt/gfn421 [PubMed: 18676349]
25. Coronel F, Berni A, Cigarran S, Calvo N, Herrero JA. Effects of angiotensin II receptor blocker (irbesartan) on peritoneal membrane functions. *Adv Perit Dial.* 2004; 20:27–30. Epub 2004/09/24. [PubMed: 15384790]
26. de Gasparo M, Catt KJ, Inagami T, Wright JW, Unger T. International Union of Pharmacology. XXIII. The Angiotensin II Receptors. *Pharmacol Rev.* 2000; 52(3):415–72. [PubMed: 10977869]
27. Humphrey P, Spedding M, Vanhoutte P. Receptor classification and nomenclature: the revolution and the resolution. *Trends Pharmacol Sci.* 1994; 15(7):203–4. [PubMed: 7940979]
28. Velez JC, Janech MG, Hicks MP, Morinelli TA, Rodgers J, Self SE, Arthur JM, Fitzgibbon WR. Lack of renoprotective effect of chronic intravenous Angiotensin-(1-7) or Angiotensin-(2-10) in a rat model of focal segmental glomerulosclerosis. *PLoS One.* 2014; 9(10):e110083. Epub 2014/10/23. doi: 10.1371/journal.pone.0110083 [PubMed: 25337950]
29. S RA, SeS AC, C Maric, D S, M RP, dB I, H-W S, P SV, L MT, B M, M EP, L VS, C-S MJ, S HP, S R, W T. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proc Natl Acad Sci U S A.* 2003; 100(14):8258–63. [PubMed: 12829792]
30. Blair-West JR, Coghlan JP, Denton DA, Funder JW, Scoggins BA, Wright RD. The effect of the heptapeptide (2-8) and hexapeptide (3-8) fragments of angiotensin II on aldosterone secretion. *J Clin Endocrinol Metab.* 1971; 32(4):575–8. DOI: 10.1210/jcem-32-4-575 [PubMed: 4323125]
31. Sim MK. Des-aspartate-angiotensin I, a novel angiotensin AT(1) receptor drug. *Eur J Pharmacol.* 2015; 760:36–41. DOI: 10.1016/j.ejphar.2015.04.004 [PubMed: 25891368]
32. Ito M, Oliverio MI, Mannon PJ, Best CF, Maeda N, Smithies O, Coffman TM. Regulation of Blood Pressure by the Type 1A Angiotensin II Receptor Gene. *Proceedings of the National Academy of Science.* 1995; 92:3521–5.

33. Sugaya T, Nishimatsu Si, Tanimoto K, Takimoto E, Yamagishi T, Imamura K, Goto S, Imaizumi K, Hisada Y, Otsuka A, Uchida H, Sugiura M, Fukuta K, Fukamizu A, Murakami K. Angiotensin II Type 1a Receptor-deficient Mice with Hypotension and Hyperreninemia. *Journal of Biological Chemistry*. 1995; 270:18719–22. [PubMed: 7642517]
34. Oliverio MI, Kim HS, Ito M, Le T, Audoly L, Best CF, Hiller S, Kluckman K, Maeda N, Smithies O, Coffman TM. Reduced growth, abnormal kidney structure, and type 2 (AT₂) angiotensin receptor-mediated blood pressure regulation in mice lacking both AT_{1A} and AT_{1B} receptors for angiotensin II. *Proceedings of the National Academy of Science*. 1998; 95:15496–501.
35. Bonnardeaux A, Davies E, Jeunemaitre X, Fery I, Charru A, Clauser E, Tiret L, Cambien F, Corval P, Soubrier F. Angiotensin II type 1 receptor gene polymorphisms in human essential hypertension. *Hypertension*. 1994; 24:63–9. [PubMed: 8021009]
36. Geel, PPv; Pinto, YM.; Buikema, H.; Gilst, WHv. Is the A1166C polymorphism of the angiotensin II type 1 receptor involved in cardiovascular disease? *European Heart Journal*. 1998; 19(Supplement):G13–7. [PubMed: 9717050]
37. Paradis P, Dali-Youcef N, Paradis FW, Thibault G, Nemer M. Overexpression of angiotensin II type I receptor in cardiomyocytes induces cardiac hypertrophy and remodeling. *Proceedings of the National Academy of Sciences*. 2000; 97(2):931–6.
38. Hein L. Genetic deletion and overexpression of angiotensin II receptors. *Journal of molecular medicine*. 1998; 76(11):756–63. [PubMed: 9826120]
39. Hein L, Stevens ME, Barsh GS, Pratt RE, Kobilka BK, Dzau VJ. Overexpression of angiotensin AT1 receptor transgene in the mouse myocardium produces a lethal phenotype associated with myocyte hyperplasia and heart block. *Proceedings of the National Academy of Sciences*. 1997; 94:6391–6.
40. Gasparo, Md; Catt, KJ.; Inagami, T.; Wright, JW.; Unger, T. International Union of Pharmacology. XXIII. The Agiotensin II Receptors. *Pharmacol Rev*. 2000; 52(No. 3):415–72. [PubMed: 10977869]
41. Marrero MB, Schieffer B, Paxton WG, Heerdt L, Berk BC, Delafontaine P, Bernstein KE. Direct stimulation of Jak/STAT pathway by the angiotensin II AT1 receptor. *Nature*. 1995; 375(6528): 247–50. DOI: 10.1038/375247a0 [PubMed: 7746328]
42. Sayeski PP, Ali MS, Hawks K, Frank SJ, Bernstein KE. The angiotensin II-dependent association of Jak2 and c-Src requires the N-terminus of Jak2 and the SH2 domain of c-Src. *Circ Res*. 1999; 84(11):1332–8. [PubMed: 10364571]
43. Marrero MB, Schieffer B, Paxton WG, Schieffer E, Bernstein KE. Electroporation of pp60c-src antibodies inhibits the angiotensin II activation of phospholipase C-gamma 1 in rat aortic smooth muscle cells. *J Biol Chem*. 1995; 270(26):15734–8. [PubMed: 7541047]
44. Natarajan K, Yin G, Berk BC. Scaffolds direct Src-specific signaling in response to angiotensin II: new roles for Cas and GIT1. *Mol Pharmacol*. 2004; 65(4):822–5. DOI: 10.1124/mol.65.4.822 [PubMed: 15044610]
45. Pang J, Yan C, Natarajan K, Cavet ME, Massett MP, Yin G, Berk BC. GIT1 mediates HDAC5 activation by angiotensin II in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol*. 2008; 28(5):892–8. Epub 2008/02/23. doi:ATVBAHA.107.161349[pii]10.1161/ATVBAHA.107.161349. [PubMed: 18292392]
46. McAllister-Lucas LM, Ruland J, Siu K, Jin X, Gu S, Kim DS, Kuffa P, Kohrt D, Mak TW, Nunez G, Lucas PC. CARMA3/Bcl10/MALT1-dependent NF-kappaB activation mediates angiotensin II-responsive inflammatory signaling in nonimmune cells. *Proc Natl Acad Sci U S A*. 2007; 104(1): 139–44. Epub 2006/11/15. doi:0601947103[pii]10.1073/pnas.0601947103. [PubMed: 17101977]
47. Chen J, Chen JK, Harris RC. Angiotensin II induces epithelial-to-mesenchymal transition in renal epithelial cells through reactive oxygen species/Src/caveolin-mediated activation of an epidermal growth factor receptor-extracellular signal-regulated kinase signaling pathway. *Mol Cell Biol*. 2012; 32(5):981–91. Epub 2012/01/05. DOI: 10.1128/MCB.06410-11 [PubMed: 22215616]
48. Takayanagi T, Crawford KJ, Kobayashi T, Obama T, Tsuji T, Elliott KJ, Hashimoto T, Rizzo V, Eguchi S. Caveolin 1 is critical for abdominal aortic aneurysm formation induced by angiotensin II and inhibition of lysyl oxidase. *Clin Sci (Lond)*. 2014; 126(11):785–94. Epub 2013/12/18. DOI: 10.1042/CS20130660 [PubMed: 24329494]

49. Ishizaka N, Griendling KK, Lassegue B, Alexander RW. Angiotensin II type 1 receptor: relationship with caveolae and caveolin after initial agonist stimulation. *Hypertension*. 1998; 32(3):459–66. Epub 1998/09/18. [PubMed: 9740611]
50. Ahn S, Shenoy SK, Wei H, Lefkowitz RJ. Differential Kinetic and Spatial Patterns of {beta}-Arrestin and G Protein-mediated ERK Activation by the Angiotensin II Receptor. *J Biol Chem*. 2004; 279(34):35518–25. [PubMed: 15205453]
51. Touyz RM, He G, El Mabrouk M, Schiffrin EL. p38 Map kinase regulates vascular smooth muscle cell collagen synthesis by angiotensin II in SHR but not in WKY. *Hypertension*. 2001; 37(2 Pt 2): 574–80. [PubMed: 11230337]
52. Luttrell LM, Roudabush FL, Choy EW, Miller WE, Field ME, Pierce KL, Lefkowitz RJ. Activation and targeting of extracellular signal-regulated kinases by b-arrestin scaffolds. *Proceedings of the National Academy of Sciences*. 2001; 98(No. 5):2449–54.
53. Tohgo A, Choy EW, Gesty-Palmer D, Pierce KL, Laporte S, Oakley RH, Caron MG, Lefkowitz RJ, Luttrell LM. The stability of the G protein-coupled receptor-beta-arrestin interaction determines the mechanism and functional consequence of ERK activation. *J Biol Chem*. 2003; 278(8):6258–67. [PubMed: 12473660]
54. Griendling KK, Ushio-Fukai M. Reactive oxygen species as mediators of angiotensin II signaling. *Regul Pept*. 2000; 91:21–7. [PubMed: 10967199]
55. Yan C, Kim D, Aizawa T, Berk BC. Functional interplay between angiotensin II and nitric oxide: cyclic GMP as a key mediator. *Arterioscler Thromb Vasc Biol*. 2003; 23(1):26–36. [PubMed: 12524221]
56. Siragy HM. The role of the AT2 receptor in hypertension. *Am J Hypertens*. 2000; 13:S62–S7.
57. de Gasparo M, Siragy HM. The AT2 receptor: fact, fancy and fantasy. *Regul Pept*. 1999; 81(1-3): 11–24. [PubMed: 10395404]
58. Siragy HM, Carey RM. Angiotensin type 2 receptors: potential importance in the regulation of blood pressure. *Curr Opin Nephrol Hypertens*. 10(1):99–103. [PubMed: 11195059]
59. Lu D, Yang H, Shaw G, Raizada MK. Angiotensin II-induced nuclear targeting of the angiotensin type 1 (AT1) receptor in brain neurons. *Endocrinology*. 1998; 139(1):365–75. [PubMed: 9421435]
60. Lee DK, Lanca AJ, Cheng R, Nguyen T, Ji XD, Gobeil F Jr, Chemtob S, George SR, O'Dowd BF. Agonist-independent Nuclear Localization of the Apelin, Angiotensin AT1, and Bradykinin B2 Receptors. *J Biol Chem*. 2004; 279(9):7901–8. [PubMed: 14645236]
61. Haller H, Lindschau C, Quass P, Luft FC. Intracellular Actions of Angiotensin II in Vascular Smooth Muscle Cells. *Journal of the American Society of Nephrology*. 1999; 10(Suppl 11):S75–S83. [PubMed: 9892144]
62. Tang SS, Rogg H, Schumacher R, Dzau VJ. Characterization of nuclear angiotensin-II-binding sites in rat liver and comparison with plasma membrane receptors. *Endocrinology*. 1992; 131(1): 374–80. [PubMed: 1612017]
63. Booz GW, Conrad KM, Hess AL, Singer HA, Baker KM. Angiotensin-II-binding sites on hepatocyte nuclei. *Endocrinology*. 1992; 130(6):3641–9. [PubMed: 1597161]
64. Filipeanu CM, Brailoiu E, Kok JW, Henning RH, De Zeeuw D, Nelemans SA. Intracellular angiotensin II elicits Ca²⁺ increases in A7r5 vascular smooth muscle cells. *Eur J Pharmacol*. 2001; 420(1):9–18. [PubMed: 11412834]
65. Re RN, LaBiche RA, Bryan SE. Nuclear hormone mediated changes in chromatin solubility. *Biochem Biophys Res Commun*. 1983; 110(No. 1):61–8. [PubMed: 6838524]
66. Watson PH, Pickard BW. Nuclear trafficking of the G-protein-coupled parathyroid hormone receptor. *Crit Rev Eukaryot Gene Expr*. 2008; 18(2):151–61. [PubMed: 18304029]
67. Tadevosyan A, Vaniotis G, Allen BG, Hebert TE, Nattel S. G protein-coupled receptor signalling in the cardiac nuclear membrane: evidence and possible roles in physiological and pathophysiological function. *J Physiol*. 2012; 590(Pt 6):1313–30. DOI: 10.1113/jphysiol.2011.222794 [PubMed: 22183719]
68. Chen R, Mukhin YV, Garnovskaya MN, Thielin TE, Iijima Y, Huang C, Raymond JR, Ullian ME, Paul RV. A functional angiotensin II receptor-GFP fusion protein: evidence for agonist-dependent nuclear translocation. *American Journal of Physiology*. 2000; 279(Renal Fluid & Electrolyte Physiology):F440–F8. [PubMed: 10966923]

69. Morinelli TA, Raymond JR, Baldys A, Yang Q, Lee MH, Luttrell L, Ullian ME. Identification of a putative nuclear localization sequence within ANG II AT₁(1A) receptor associated with nuclear activation. *Am J Physiol Cell Physiol.* 2007; 292(4):C1398–408. Epub 2006/12/15. DOI: 10.1152/ajpcell.00337.2006 [PubMed: 17166941]
70. Tadevosyan A, Maguy A, Villeneuve LR, Babin J, Bonnefoy A, Allen BG, Nattel S. Nuclear-delimited angiotensin receptor-mediated signaling regulates cardiomyocyte gene expression. *J Biol Chem.* 2010; 285:22338–49. Epub 2010/05/14. doi:M110.121749[pil]10.1074/jbc.M110.121749. [PubMed: 20463030]
71. Cook JL, Mills SJ, Naquin RT, Alam J, Re RN. Cleavage of the Angiotensin AT₁ Receptor and Nuclear Accumulation of the Cytoplasmic Carboxy-Terminal Fragment. *Am J Physiol Cell Physiol.* 2007; 292(Apr):C1313–C22. DOI: 10.1152/ajpcell.00454.2006 [PubMed: 17122414]
72. Re R, Parab M. Effect of angiotensin II on RNA synthesis by isolated nuclei. *Life Sci.* 1984; 34:647–51. [PubMed: 6199638]
73. Eggena P, Zhu JH, Sereevinyayut S, Giordani M, Clegg K, Andersen PC, Hyun P, Barrett JD. Hepatic angiotensin II nuclear receptors and transcription of growth-related factors. *J Hypertens.* 1996; 14(8):961–8. [PubMed: 8884550]
74. Eggena P, Zhu JH, Clegg K, Barrett JD. Nuclear angiotensin receptors induce transcription of renin and angiotensinogen mRNA. *Hypertension.* 1993; 22(4):496–501. [PubMed: 8406654]
75. Morinelli TA, Raymond JR, Baldys A, Yang Q, Lee Mh, Luttrell L, Ullian ME. Identification Of A Putative Nuclear Localization Sequence Within The Angiotensin II AT₁A Receptor Associated with Nuclear Activation. *Am J Physiol Cell Physiol.* 2007; 292(4):C1398–408. [PubMed: 17166941]
76. Morinelli TA, Walker LP, Ullian ME. COX-2 expression stimulated by Angiotensin II depends upon AT₁ receptor internalization in vascular smooth muscle cells. *Biochim Biophys Acta.* 2008; 1783(6):1048–54. Epub 2008/02/13. DOI: 10.1016/j.bbamcr.2008.01.012 [PubMed: 18267125]
77. Smith RD, Hunyady L, Olivares-Reyes JA, Mihalik B, Jayadev S, Catt KJ. Agonist-Induced Phosphorylation of the Angiotensin AT₁a Receptor Is Localized to a Serine/Threonine-Rich Region of Its Cytoplasmic Tail. *Mol Pharmacol.* 1998; 54(6):935–41. [PubMed: 9855619]
78. Hunyady L, Bor M, Balla T, Catt KJ. Identification of a cytoplasmic Ser-Thr-Leu motif that determines agonist-induced internalization of the AT₁ angiotensin receptor. *Journal of Biological Chemistry.* 1994; 269(No. 50):31378–82. [PubMed: 7989302]
79. Thomas WG, Baker KM, Motel TJ, Thekkumkara TJ. Angiotensin II Receptor Endocytosis Involves Two Distinct Regions of the Cytoplasmic Tail A Role for Residues on The Hydrophobic Face of a Putative Amphipathic Helix. *Journal of Biological Chemistry.* 1995; 270(38):22153–9. [PubMed: 7673193]
80. Gaborik Z, Jagadeesh G, Zhang M, Spat A, Catt KJ, Hunyady L. The Role of a Conserved Region of the Second Intracellular Loop in AT₁ Angiotensin Receptor Activation and Signaling. *Endocrinology.* 2003; 144(6):2220–8. [PubMed: 12746278]
81. Barak LS, Tiberi M, Freedman NJ, Kwatra MM, Lefkowitz RJ, Caron MG. A highly conserved tryosine residue in G protien-coupled receptors is required for agonist-mediated b2-adrenergic receptor sequestration. *Journal of Biological Chemistry.* 1994; 269(No. 4, January 28):2790–5. [PubMed: 7507928]
82. Hunyady L, Bor M, Baukal AJ, Balla T, Catt KJ. A Conserved NPLFY Sequence Contributes to Agonist Binding and Signal Transduction but Is Not an Internalization Signal for the Type 1 Angiotensin II Receptor. *Journal of Biolgical Chemistry.* 1995; 270(28):16602–9.
83. Conchon S, Barrault MB, Miserey S, Corvol P, Clauser E. The C-terminal third intracellular loop of the rat AT₁a angiotensin receptor plays a key role in G protein coupling specificity and transduction of the mitogenic signal. *Journal of Biological Chemistry.* 1997; 272(October 10): 2556–25572.
84. Thomas WG, Thekkumkara TJ, Motel TJ, Thekkumkara TJ. Cardiac effects of AII. AT₁A receptor signaling, desensitization and internalization. *Adv Exp Med Biol.* 1996; 396:59–69. [PubMed: 8726686]

85. Hunyady L, Merelli F, Baukal A, Balla T, Catt K. Agonist-induced endocytosis and signal generation in adrenal glomerulosa cells. A potential mechanism for receptor-operate calcium entry. *Journal of Biological Chemistry*. 1991; 266(5):2783–8. [PubMed: 1993657]
86. Nickenig G, Murphy T. Down-regulation by growth factors of vascular smooth muscle angiotensin receptor gene expression. *Mol Pharmacol*. 1994; 46:653–9. [PubMed: 7969043]
87. Ullian ME, Linas SL. Role of receptor cycling in the regulation of angiotensin II surface receptor number and angiotensin II uptake in rat vascular smooth muscle cells. *Journal of Clinical Investigation*. 1989; 84(3):840–6. [PubMed: 2760215]
88. Luttrell LM, Lefkowitz RJ. The role of {beta}-arrestins in the termination and transduction of G-protein-coupled receptor signals. *J Cell Sci*. 2002; 115(3):455–65. [PubMed: 11861753]
89. Oakley RH, Laporte SA, Holt JA, Barak LS, Caron MG. Molecular Determinants Underlying the Formation of Stable Intracellular G Protein-coupled Receptor-beta-Arrestin Complexes after Receptor Endocytosis*. *J Biol Chem*. 2001; 276(22):19452–60. [PubMed: 11279203]
90. Reilly CF. Rat vascular smooth muscle cells immortalized with SV40 Large T antigen possess defined smooth muscle cell characteristics including growth inhibition by heparin. *Journal of Cellular Physiology*. 1990; 142:342–51. [PubMed: 2154504]
91. Gaborik Z, Szaszak M, Szidonya L, Balla B, Paku S, Catt KJ, Clark AJL, Hunyady L. b-Arrestin- and dynamin-dependent endocytosis of the AT1 angiotensin receptor. *Mol Pharmacol*. 2001; 59:239–47. [PubMed: 11160859]
92. Kohout TA, Lin FT, Perry SJ, Conner DA, Lefkowitz RJ. b-Arrestin 1 and 2 differentially regulate heptahelical receptor signaling and trafficking. *Proceedings of the National Academy of Sciences*. 2001; 98(4):1601–6.
93. Yesudas R, Snyder R, Abbruscato T, Thekkumkara T. Functional Role of Sodium Glucose Transporter in High Glucose Mediated Angiotensin Type 1 Receptor Down-Regulation in Human Proximal Tubule Cells. *Am J Physiol Renal Physiol*. 2012; Epub 2012/06/01. doi: 10.1152/ajprenal.00651.2011
94. Amiri F, Garcia R. Regulation of angiotensin II receptors and PKC isoforms by glucose in rat mesangial cells. *Am J Physiol*. 1999; 276(5 Pt 2):F691–9. Epub 1999/05/18. [PubMed: 10330051]
95. Ramasamy R, Yan SF, Schmidt AM. Advanced glycation endproducts: from precursors to RAGE: round and round we go. *Amino Acids*. 2012; 42(4):1151–61. Epub 2010/10/20. DOI: 10.1007/s00726-010-0773-2 [PubMed: 20957395]
96. Cheng CL, Tang Y, Zheng Z, Liu X, Ye ZC, Wang C, Lou TQ. Advanced glycation end-products activate the renin-angiotensin system through the RAGE/PI3-K signaling pathway in podocytes. *Clin Invest Med*. 2012; 35(5):E282. Epub 2012/10/10. [PubMed: 23043709]
97. Nevado J, Peiro C, Vallejo S, El-Assar M, Lafuente N, Matesanz N, Azcutia V, Cercas E, Sanchez-Ferrer CF, Rodriguez-Manas L. Amadori adducts activate nuclear factor-kappaB-related proinflammatory genes in cultured human peritoneal mesothelial cells. *British journal of pharmacology*. 2005; 146(2):268–79. Epub 2005/07/06. DOI: 10.1038/sj.bjp.0706309
98. Zou Y, Akazawa H, Qin Y, Sano M, Takano H, Minamino T, Makita N, Iwanaga K, Zhu W, Kudoh S, Toko H, Tamura K, Kihara M, Nagai I, Fukamizu A, Umemura S, Iiri T, Fujita T, Komuro I. Mechanical stress activates angiotensin II type 1 receptor without the involvement of angiotensin II. *Nat Cell Biol*. 2004; 6(6):499–506. Epub 2004/05/18. doi:10.1038/ncb1137 ncb1137[pii]. [PubMed: 15146194]
99. Forbes JM, Soulis T, Thallas V, Panagiotopoulos S, Long DM, Vasan S, Wagle D, Jerums G, Cooper ME. Renoprotective effects of a novel inhibitor of advanced glycation. *Diabetologia*. 2001; 44(1):108–14. Epub 2001/02/24. DOI: 10.1007/s001250051587 [PubMed: 11206401]
100. Tamura K, Umemura S, Nyui N, Hibi K, Ishigami T, Kihara M, Toya Y, Ishii M. Activation of angiotensinogen gene in cardiac myocytes by angiotensin II and mechanical stretch. *Am J Physiol*. 1998; 275(1 Pt 2):R1–9. Epub 1998/08/05. [PubMed: 9688953]
101. Nguyen HT, Adam RM, Bride SH, Park JM, Peters CA, Freeman MR. Cyclic stretch activates p38 SAPK2-, ErbB2-, and AT1-dependent signaling in bladder smooth muscle cells. *Am J Physiol Cell Physiol*. 2000; 279(4):C1155–67. Epub 2000/09/26. [PubMed: 11003596]
102. Saygili E, Rana OR, Meyer C, Gemein C, Andrzejewski MG, Ludwig A, Weber C, Schotten U, Kruttgen A, Weis J, Schwinger RH, Mischke K, Rassaf T, Kelm M, Schauer P. The

angiotensin-calcineurin-NFAT pathway mediates stretch-induced up-regulation of matrix metalloproteinases-2/-9 in atrial myocytes. *Basic Res Cardiol*. 2009; 104(4):435–48. Epub 2009/01/17. DOI: 10.1007/s00395-008-0772-6 [PubMed: 19148693]

103. Morgera S, Schlenstedt J, Giessing M, Deger S, Hoher B, Neumayer HH. Glucose-mediated transforming growth factor-beta1 release in human mesothelial cells is endothelin independent. *J Cardiovasc Pharmacol*. 2004; 44(Suppl 1):S216–8. Epub 2005/04/20. [PubMed: 15838283]
104. Yano N, Suzuki D, Endoh M, Cao TN, Dahdah JR, Tseng A, Stabila JP, McGonnigal BG, Padbury JF, Tseng YT. High ambient glucose induces angiotensin-independent AT-1 receptor activation, leading to increases in proliferation and extracellular matrix accumulation in MES-13 mesangial cells. *Biochem J*. 2009; 423(1):129–43. Epub 2009/07/17. DOI: 10.1042/BJ20082277 [PubMed: 19604148]
105. Carroll JE, Landry AS, Elliot ME, Goodfriend TL. Effects of pH on adrenal angiotensin receptors and responses. *J Lab Clin Med*. 1986; 108(1):23–9. Epub 1986/07/01. [PubMed: 3011931]
106. Nagami GT, Chang JA, Plato ME, Santamaria R. Acid loading in vivo and low pH in culture increase angiotensin receptor expression: enhanced ammoniagenic response to angiotensin II. *Am J Physiol Renal Physiol*. 2008; 295(6):F1864–70. Epub 2008/10/24. DOI: 10.1152/ajprenal.90410.2008 [PubMed: 18945827]
107. Morinelli TA, Lee MH, Kendall RT, Luttrell LM, Walker LP, Ullian ME. Angiotensin II activates NF-kappaB through AT1A receptor recruitment of beta-arrestin in cultured rat vascular smooth muscle cells. *American journal of physiology Cell physiology*. 2013; 304(12):C1176–86. Epub 2013/04/12. DOI: 10.1152/ajpcell.00235.2012 [PubMed: 23576578]
108. Weigert C, Brodbeck K, Klopfer K, Haring HU, Schleicher ED. Angiotensin II induces human TGF-beta 1 promoter activation: similarity to hyperglycaemia. *Diabetologia*. 2002; 45(6):890–8. Epub 2002/07/11. DOI: 10.1007/s00125-002-0843-4 [PubMed: 12107734]
109. Fukami K, Ueda S, Yamagishi S, Kato S, Inagaki Y, Takeuchi M, Motomiya Y, Bucala R, Iida S, Tamaki K, Imaizumi T, Cooper ME, Okuda S. AGEs activate mesangial TGF-beta-Smad signaling via an angiotensin II type I receptor interaction. *Kidney Int*. 2004; 66(6):2137–47. Epub 2004/12/01. DOI: 10.1111/j.1523-1755.2004.66004.x [PubMed: 15569303]
110. Chen K, Mehta JL, Li D, Joseph L, Joseph J. Transforming growth factor beta receptor endoglin is expressed in cardiac fibroblasts and modulates profibrogenic actions of angiotensin II. *Circ Res*. 2004; 95(12):1167–73. Epub 2004/11/13. DOI: 10.1161/01.RES.0000150369.68826.2f [PubMed: 15539634]
111. Border WA, Noble NA. Transforming growth factor beta in tissue fibrosis. *The New England journal of medicine*. 1994; 331(19):1286–92. DOI: 10.1056/NEJM199411103311907 [PubMed: 7935686]
112. Loureiro J, Aguilera A, Selgas R, Sandoval P, Albar-Vizcaino P, Perez-Lozano ML, Ruiz-Carpio V, Majano PL, Lamas S, Rodriguez-Pascual F, Borrás-Cuesta F, Dotor J, Lopez-Cabrera M. Blocking TGF-beta1 protects the peritoneal membrane from dialysate-induced damage. *J Am Soc Nephrol*. 2011; 22(9):1682–95. Epub 2011/07/12. DOI: 10.1681/ASN.2010111197 [PubMed: 21742730]
113. Tang W, Strachan RT, Lefkowitz RJ, Rockman HA. Allosteric Modulation of beta-Arrestin-Biased AT1R Signaling by Membrane Stretch. *J Biol Chem*. 2014; Epub 2014/08/30. doi: 10.1074/jbc.M114.585067
114. Lovgren AK, Kovacs JJ, Xie T, Potts EN, Li Y, Foster WM, Liang J, Meltzer EB, Jiang D, Lefkowitz RJ, Noble PW. beta-arrestin deficiency protects against pulmonary fibrosis in mice and prevents fibroblast invasion of extracellular matrix. *Sci Transl Med*. 2011; 3(74):74ra23. Epub 2011/03/18. doi: 10.1126/scitranslmed.3001564
115. Maudsley S, Patel SA, Park SS, Luttrell LM, Martin B. Functional signaling biases in G protein-coupled receptors: Game Theory and receptor dynamics. *Mini Rev Med Chem*. 2012; 12(9):831–40. [PubMed: 22681251]
116. Smith NJ, Luttrell LM. Signal switching, crosstalk, and arrestin scaffolds: novel G protein-coupled receptor signaling in cardiovascular disease. *Hypertension*. 2006; 48(2):173–9. [PubMed: 16818801]
117. Kendall RT, Strungs EG, Rachidi SM, Lee MH, El-Shewy HM, Luttrell DK, Janech MG, Luttrell LM. The beta-arrestin pathway-selective type 1A angiotensin receptor (AT1A) agonist

- [Sar1,Ile4,Ile8]angiotensin II regulates a robust G protein-independent signaling network. *J Biol Chem.* 2011; 286(22):19880–91. Epub 2011/04/20. DOI: 10.1074/jbc.M111.233080 [PubMed: 21502318]
118. Wei H, Ahn S, Shenoy SK, Karnik SS, Hunyady L, Luttrell LM, Lefkowitz RJ. Independent {beta}-arrestin 2 and G protein-mediated pathways for angiotensin II activation of extracellular signal-regulated kinases 1 and 2. *PNAS.* 2003; 100(19):10782–7. [PubMed: 12949261]
 119. Gesty-Palmer D, El Shewy H, Kohout TA, Luttrell LM. beta-Arrestin 2 expression determines the transcriptional response to lysophosphatidic acid stimulation in murine embryo fibroblasts. *J Biol Chem.* 2005; 280(37):32157–67. Epub 2005/07/20. DOI: 10.1074/jbc.M507460200 [PubMed: 16027114]
 120. Azzi M, Charest PG, Angers S, Rousseau G, Kohout T, Bouvier M, Pineyro G. Beta-arrestin-mediated activation of MAPK by inverse agonists reveals distinct active conformations for G protein-coupled receptors. *Proc Natl Acad Sci U S A.* 2003; 100(20):11406–11. Epub 2003/09/19. DOI: 10.1073/pnas.1936664100 [PubMed: 13679574]
 121. Santos GA, Duarte DA, Parreiras ESLT, Teixeira FR, Silva-Rocha R, Oliveira EB, Bouvier M, Costa-Neto CM. Comparative analyses of downstream signal transduction targets modulated after activation of the AT1 receptor by two beta-arrestin-biased agonists. *Frontiers in pharmacology.* 2015; 6:131.doi: 10.3389/fphar.2015.00131 [PubMed: 26191004]
 122. Masuho I, Ostrovskaya O, Kramer GM, Jones CD, Xie K, Martemyanov KA. Distinct profiles of functional discrimination among G proteins determine the actions of G protein-coupled receptors. *Sci Signal.* 2015; 8(405):ra123.doi: 10.1126/scisignal.aab4068 [PubMed: 26628681]
 123. Gesty-Palmer D, Flannery P, Yuan L, Corsino L, Spurney R, Lefkowitz RJ, Luttrell LM. A beta-arrestin-biased agonist of the parathyroid hormone receptor (PTH1R) promotes bone formation independent of G protein activation. *Sci Transl Med.* 2009; 1(1):1ra. Epub 2010/04/07. doi: 10.1126/scitranslmed.3000071
 124. Gesty-Palmer D, Yuan L, Martin B, Wood WH 3rd, Lee MH, Janech MG, Tsoi LC, Zheng WJ, Luttrell LM, Maudsley S. beta-arrestin-selective G protein-coupled receptor agonists engender unique biological efficacy in vivo. *Molecular endocrinology.* 2013; 27(2):296–314. Epub 2013/01/15. DOI: 10.1210/me.2012-1091 [PubMed: 23315939]
 125. Violin JD, DeWire SM, Yamashita D, Rominger DH, Nguyen L, Schiller K, Whalen EJ, Gowen M, Lark MW. Selectively engaging beta-arrestins at the angiotensin II type 1 receptor reduces blood pressure and increases cardiac performance. *J Pharmacol Exp Ther.* 2010; 335(3):572–9. Epub 2010/08/31. DOI: 10.1124/jpet.110.173005 [PubMed: 20801892]
 126. Walters RW, Shukla AK, Kovacs JJ, Violin JD, DeWire SM, Lam CM, Chen JR, Muehlbauer MJ, Whalen EJ, Lefkowitz RJ. beta-Arrestin1 mediates nicotinic acid-induced flushing, but not its antilipolytic effect, in mice. *J Clin Invest.* 2009; 119(5):1312–21. Epub 2009/04/08. DOI: 10.1172/JCI36806 [PubMed: 19349687]
 127. Semple G, Skinner PJ, Gharbaoui T, Shin YJ, Jung JK, Cherrier MC, Webb PJ, Tamura SY, Boatman PD, Sage CR, Schrader TO, Chen R, Colletti SL, Tata JR, Waters MG, Cheng K, Taggart AK, Cai TQ, Carballo-Jane E, Behan DP, Connolly DT, Richman JG. 3-(1H-tetrazol-5-yl)-1,4,5,6-tetrahydro-cyclopentapyrazole (MK-0354): a partial agonist of the nicotinic acid receptor, G-protein coupled receptor 109a, with antilipolytic but no vasodilatory activity in mice. *J Med Chem.* 2008; 51(16):5101–8. Epub 2008/07/31. DOI: 10.1021/jm800258p [PubMed: 18665582]
 128. DeWire SM, Yamashita DS, Rominger DH, Liu G, Cowan CL, Graczyk TM, Chen XT, Pitis PM, Gotchev D, Yuan C, Koblish M, Lark MW, Violin JD. A G protein-biased ligand at the mu-opioid receptor is potently analgesic with reduced gastrointestinal and respiratory dysfunction compared with morphine. *J Pharmacol Exp Ther.* 2013; 344(3):708–17. Epub 2013/01/10. DOI: 10.1124/jpet.112.201616 [PubMed: 23300227]

Abbreviations

AT1R	Angiotensin II receptor
®-Arr	Beta-arrestin

NF-κB	Nuclear Factor κ B
TGF-β1	Transforming Growth Factor β 1
GPCR	G protein-coupled receptor
ESRD	end-stage renal disease
PD	peritoneal dialysis
ARBs	angiotensin II receptor blockers

Figure 1.

Effects of chronic PD on sub-mesothelial morphology of the peritoneal cavity. Under basal conditions, peritoneal mesothelial cells (PMC) form a continuous monolayer of cells forming a permeability barrier between the fluid of the peritoneal cavity and the submesothelial capillary bed. Under basal conditions, AT1Rs of PMC reside in the cellular plasma membrane (see inset). The submesothelial matrix composed mostly of collagen 1, includes few fibroblasts, mast cells and capillaries. Chronic PD results in disruption of the PMC monolayer, a breakdown in the permeability barrier, influx of mast cells and fibroblasts and an increase in extracellular matrix accumulation and increase in capillary number. Under these conditions, we hypothesize that the hyperosmotic PD fluid promotes the internalization of AT1Rs into cytosolic endosomes (see inset) promoting activation and nuclear localization of NF- κ B (see Figure 3).

Figure 2.

AngII AT1R intracellular signaling pathways. IP3, Inositol 1,4,5-trisphosphate; ROS, reactive oxygen species; GRK, G protein receptor kinase; PKC, protein kinase C; ERK, extracellular-regulated kinase; JNK, Jun amino-terminal kinase; NF- κ B, nuclear factor – kappa B; EGFR, Epidermal Growth Factor Receptor.

Figure 3.

Proposed pathway for PD fluid-induced activation of AT1R in PMCs. Exposure of PMCs to hyperosmotic PD fluid produces structural alterations in cell membrane morphology producing re-arrangement and activation of the AT1R. (1). AT1R re-arrangement (activation) results in recruitment of β -arrestin 1 and 2 (AR) to the physically-activated receptor (2). Internalization of the complex into endosomal vesicles follows (3). Along with the recruitment of arrestins, activation of attached NF- κ B ensues, ie phosphorylation and release of I κ B α (4). The activated p50/p65 NF- κ B along with the attached arrestins/AT1R scaffold, traffics to the nuclear membrane (5) at which point the p50/p65 complex is released into the nucleus, binds with specific NF- κ B consensus sequences (6) and initiates transcription of targeted pro-inflammatory/pro-fibrotic mediators such as COX-2 (7).

Table 1

AT1 and AT2 receptor ligands. Metabolites of AngII and synthetic analogues known to interact with AT1R or AT2R. (+) interacts; (-) does not interact; (?) may or may not interact.

LIGAND	AGONIST	ANTAGONIST	AT1R	AT2R
[des-Asp1]Angiotensin I	+		+?	-
Angiotensin II	+		+	+
Angiotensin(2-10)	?	?	-+	+
Angiotensin(1-7)	+(MAS RECEPTOR)		--+	+
Losartan (Valsartan, etc)		+	+	-
PD123177		+	-	+
CGP42112	+		-	+