Hypertension

REVIEW

Angiotensin II Type 2 Receptor

A Target for Protection Against Hypertension, Metabolic Dysfunction, and Organ Remodeling

Naureen Fatima, Sanket N. Patel, Tahir Hussain

ABSTRACT: The renin-angiotensin system is of vital significance not only in the maintenance of blood pressure but also because of its role in the pathophysiology of different organ systems in the body. Of the 2 Ang II (angiotensin II) receptors, the AT_1R (Ang II type 1 receptor) has been extensively studied for its role in mediating the classical functions of Ang II, including vasoconstriction, stimulation of renal tubular sodium reabsorption, hormonal secretion, cell proliferation, inflammation, and oxidative stress. The other receptor, AT_2R (Ang II type 2 receptor), is abundantly expressed in both immune and nonimmune cells in fetal tissue. However, its expression is increased under pathological conditions in adult tissues. The role of AT_2R in counteracting AT_1R function has been discussed in the past 2 decades. However, with the discovery of the nonpeptide agonist C21, the significance of AT_2R in various pathologies such as obesity, hypertension, and kidney diseases have been examined. This review focuses on the most recent findings on the beneficial effects of AT_2R by summarizing both gene knockout studies as well as pharmacological studies, specifically highlighting its importance in blood pressure regulation, obesity/metabolism, organ protection, and relevance in the treatment of coronavirus disease 2019 (COVID-19).

Key Words: alamandine ■ blood pressure ■ hypertension ■ inflammation ■ obesity

AT_2R (Ang II [angiotensin II] type-2 receptor) is a lesser-known component of the renin-angiotensin system (RAS). Although AT_2R was discovered and cloned in the early 1990s,[1] its role in pathophysiology continues to be unraveled, and the significance associated with these functions has been ever-evolving. AT_2R is activated by the peptides Ang II/III/-(1–7) and potentially by alamandine that acts as the endogenous agonist.[2–7] Typically, AT_2R is considered as a functional antagonist of the well-known and predominantly (relative to AT_2R) expressed AT_1R (Ang II type-1 receptor),[8] which is involved in renal and cardiovascular diseases, hypertension, and tissue injury.[9] With the availability of preferential agonists and antagonists, particularly in the recent past, researchers have been able to make strides in understanding the underlying AT_2R biology in health and diseases at the molecular, cellular, and whole-organism levels. The results of the pharmacological studies are convincing and suggest that selective activation of AT_2R may mediate pronatriuretic[10] and anti-inflammatory[11-13] effects and improves insulin resistance and metabolism,[14,15] which is associated with antiadiposity/obesity, antihypertensive, and renoprotective and cardioprotective outcomes. Moreover, AT_2R has been recently crystallized,[16] which extends our insight into receptor chemistry. This is a critical step in developing more selective and potent drugs to target AT_2R to exert desired effects. The purpose of this review is to summarize the recent discoveries covering various aspects of AT_2R structure, signaling, and biology, from molecules to whole animals. Since AT_2R is a part of the protective arm of the RAS, namely Ang (1–7)/receptor Mas (MasR) and functional similarities and interdependence between AT_2R and MasR exist,[17] the coronavirus disease 2019 (COVID-19) relevance of the pharmacological activation of AT_2R is also briefly discussed.

AT_2R CHEMISTRY AND SIGNALING

AT_2R is a member of the GPCR (G protein-coupled receptor) family. However, its unique chemistry and cell

Correspondence to: Tahir Hussain, Professor and Associate Dean of Research and Graduate Programs, College of Pharmacy, Health Bldg-2, 4849 Calhoun, Room 3044, University of Houston, Houston, TX 77204. Email thussain@central.uh.edu

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Nonstandard Abbreviations and Acronyms

Ang II	angiotensin II
AT1R	Ang II type-1 receptor
AT2R	Ang II type-2 receptor
BP	blood pressure
cGMP	cyclic guanosine monophosphate
COVID-19	coronavirus disease 2019
EnaC	epithelial sodium channel
GPCR	G protein-coupled receptor
HSD	high-salt diet
IL-2	interleukin 2
MI	myocardial infarction
MKP-1	mitogen-activated protein kinase phosphatase 1
NCC	Na-Cl co-transporter
NO	nitric oxide
NOS	NO synthase
OZR	obese Zucker rat
PP2A	protein phosphatase 2A
RAS	renin-angiotensin system
SHP-1	SH2 domain-containing phosphatase 1
WT	wild type
α-SMA	α-skeletal muscle actin

signaling are puzzling and yet to be fully understood. AT_2R is coupled to stimulatory ($G_{\alpha s}$) or inhibitory ($G_{\alpha i/o}$) proteins or G protein-independent pathways that include AT_2R binding protein.[18-20] The activation of G protein is also associated with the activation of Tyr phosphatase SHP-1 (SH2 domain-containing phosphatase 1),[21] Ser/Thr phosphatases MKP-1 (mitogen-activated protein kinase phosphatase 1),[22] and PP2A (protein phosphatase 2A).[23] Most of the cellular and biological effects such as vasodilatation and natriuresis associated with AT_2R activation are mediated via nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) activation by phosphatase-mediated activation of NOS (NO synthase). A recent study shows that these Ser/Thr and Tyr phosphatases serve as the regulating enzymes for AT_2R to dephosphorylate (Tyr^{657}, Thr^{495}) and phosphorylate (Ser^{1177}) endothelial NOS, thereby stimulating its activity in human aortic endothelial cells.[23] The AT_2R-mediated activation of endothelial NOS also involves activation of the PI3K/Akt pathway.[23]

We have observed opposing effects in response to AT_2R activation.[24] For instance, the AT_2R agonist produced a natriuretic and anti-inflammatory response in obese Zucker rats (OZR) but no natriuresis or proinflammatory response in lean animals.[24] A difference was observed in the signaling mechanism. AT_2R activation inhibited cAMP accumulation in the proximal tubules of both lean and OZR, whereas NO/cGMP stimulation was observed in the proximal tubules of OZR.[25] The reasons for such a discrepancy in signaling and function are unknown. However, according to a recently reported crystal structure of AT_2R, the AT_2R's helix VIII may be responsible for such opposing effects. The helix VIII renders AT_2R in an active state and blocks the recruitment of the G proteins and β-arrestin.[16] This blocking of the G proteins and β-arrestin and other features such as fewer serine residues on the third intracellular loop of the receptor may additionally explain why AT_2R, unlike typical GPCRs such as β-adrenoceptor, is not desensitized, internalized, and degraded in response to chronic agonist exposure.[26,27] Such chemical features that render AT_2Rs resistant to desensitization/internalization make AT_2R a potential target of drug discovery, particularly in terms of pharmacological activation.

BLOOD PRESSURE REGULATION

AT_2R has been studied as a target to examine its role in renovascular function, particularly in BP regulation. AT_2R is widely expressed in the vasculature (particularly endothelial cells),[28,29] heart (myocytes),[30] various kidney regions,[31-34] brain,[35,36] and immune/blood cells.[37] Moreover, AT_2R expression and functions are increased under pathological conditions in the vasculature,[38] diabetes (in both humans and animals),[39,40] heart failure,[41,42] and diabetic/obese kidney (vide infra).[43,44] Early gene knockout studies suggest that AT_2R may (a modest) or may not decrease BP in rodents.[45,46] Pharmacological studies suggest that AT_2R activation alone may not be sufficient to lower BP. However, it enhanced the BP-lowering effects of AT_1R antagonist in the spontaneously hypertensive rat model.[47] Conversely, blockade of the AT_2R reversed the blood pressure (BP)-lowering effects of the AT_1R antagonist in normotensive animals.[48] However, these pharmacological studies were acute and short-term. A recent article[49] reviewed various mechanisms of AT_2R-mediated BP regulation, including peripheral and central mechanisms. In this article, we briefly discuss the peripheral mechanisms of BP regulation, particularly long-term BP control. Orally active AT_2R preferential agonist compound-21 (C21) helped examine the role of AT_2R in long-term BP regulation. The C21 treatment administered via subcutaneously implanted osmotic pumps over a period of 2-weeks completely prevented salt-induced hypertension in OZR[50] and Ang II–induced hypertension in Sprague-Dawley rats.[51] In both the studies, prevention of the increase in BP by C21 was associated with an increase in the urinary sodium excretion, preventing salt and water retention and the buildup of body fluid. The Ang II infusion hypertension model provides more direct evidence of the role of kidney AT_2R in BP regulation as C21 was specifically delivered to the kidney. In our studies,[52] the 2 RAS axes, namely the classical Ang II/AT_1R and the protective ACE2/Ang (1–7)/MasR, were evaluated in the kidneys of OZR who were administered a high-salt diet (HSD) and AT_2R agonist

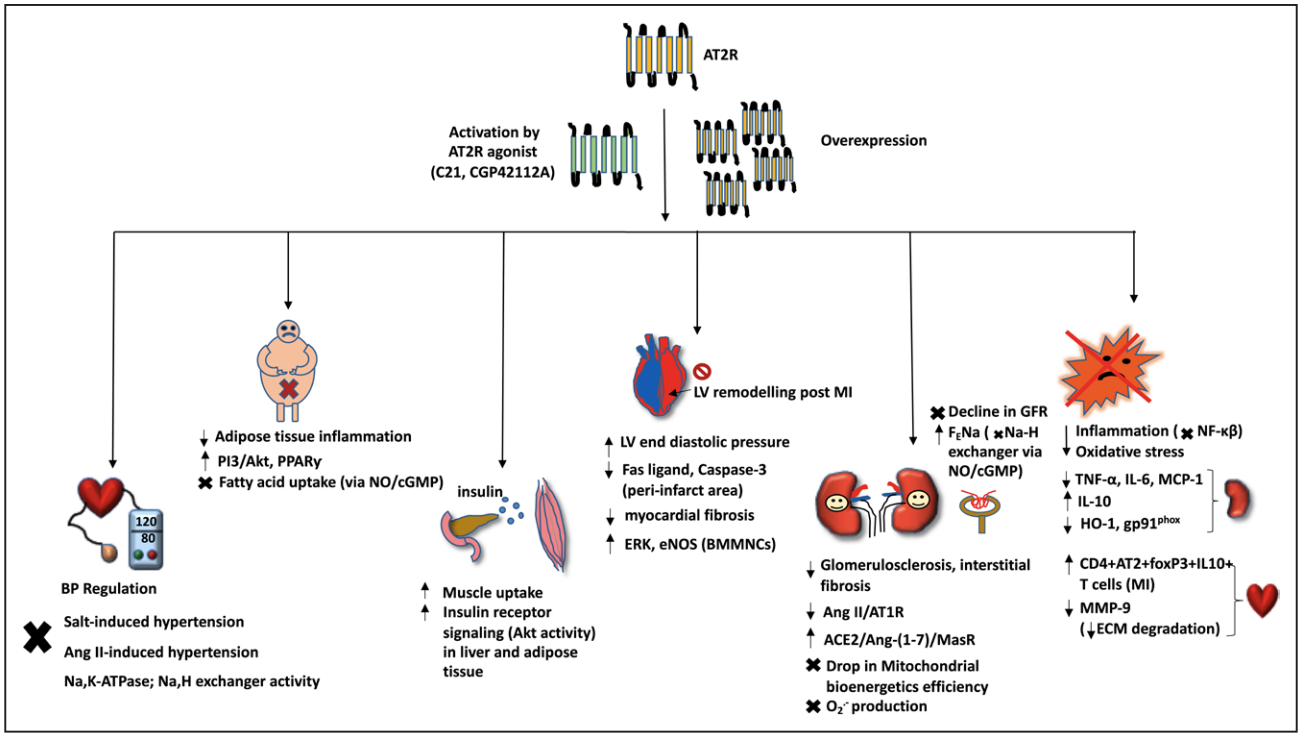

Figure. AT_2R (Ang II [angiotensin II] type 2 receptor) is advantageous in metabolism, organ protection, and inflammation. This figure highlights the main signaling molecules involved in AT2R-mediated beneficial effects in blood pressure (BP) regulation, obesity and metabolism, cardiovascular protection, renoprotection, and inflammation accompanying organ remodeling. The arrows pointing upwards (↑) indicate an increase or upregulation, the arrows pointing downward (↓) indicate decrease or downregulation, and the cross (X) symbol indicates inhibition. ACE2 indicates angiotensin-converting enzyme-2; Ang II, angiotensin II; AT_1R, Ang II type 1 receptor; cGMP, cyclic guanosine monophosphate; ECM, extracellular matrix; eNOS, endothelial NOS; ERK, extracellular signal-regulated kinase; HO-1, heme oxygenase-1; LV, left ventricular; MCP-1, monocyte chemoattractant protein-1; MI, myocardial infarction; MMP-9, matrix mettaloproteinase-9; NFκB, nuclear factor kappa B; NO, nitric oxide; PPAR, peroxisome proliferator-activated receptors; and TNF-α, tumor necrosis factor-α.

C21. The BP increase in HSD-fed OZR was associated with a remarkable increase in the Ang II levels (pro hypertensive) and a decrease in the ACE2/Ang (1–7) levels (antihypertensive) in the kidney.[52] C21 treatment reversed this trend or normalized these changes, inducing a further reduction in AT_1R expression, thus leaving open questions whether the BP decrease was a direct consequence of C21 treatment or secondary to the changes in the Ang II/AT_1R versus ACE2/Ang (1–7) balance or a combination of both. Nonetheless, studies are required to further evaluate the antihypertensive mechanisms of AT_2R.

Natriuresis

Renal function in maintaining sodium and fluid homeostasis plays a critical role in long-term BP regulation. Numerous studies have demonstrated that acute as well as chronic AT_2R activation by the AT_2R agonists C21 and CGP42112A promotes natriuresis under normal and pathological conditions.[10,51,53-56] AT_2R-mediated natriuresis seems to originate at the tubular level as the renal hemodynamics involving the GFR and renal blood flow are not affected by AT_2R agonists.[53,56] The highest density of AT_2R is present in the proximal tubule,[57] a site of major sodium and water reabsorption. However, the receptor is also expressed in other parts of the nephron, including the distal tubule, a site of fine-tuning of sodium reabsorption. An increase in the fractional sodium and lithium excretion in the presence and absence of distal tubule sodium transport (NCC [Na-Cl co-transporter] and EnaC [epithelial sodium channel]) inhibitors indicated that proximal tubule may be a primary site of AT_2R action, leading to natriuresis.[53] At the cellular and molecular level, proximal tubule AT_2R activation inhibited the activities of 2 major sodium transporters, namely NaK-ATPase (sodium pump) and NaH-exchanger via NO/cGMP pathway,[25,54,58] suggesting a potential mechanism of inhibition of tubular sodium transport, resulting in natriuresis. A study in the distal tubules isolated from K-channel knockout and wild-type (WT) mice implicated AT_2R in the inhibition of NCC and K-channel (Kir4.1/5.1), leading to an increase in Na and potassium excretion.[59] All these studies highlight the significant role of AT_2Rs and the potential mechanism involved in maintaining sodium hemostasis and long-term BP regulation. Recent discoveries of selective peptidic AT_2R ligands β-Pro^7-Ang III, β-Tyr^4-Ang II, and β-Ile^5-Ang II showed their in vitro vasorelaxant and in vivo depressor effects in conscious spontaneously hypertensive rat model. These observations have further strengthened the antihypertensive

role of the AT_2R; however, the substituted Ang II peptides required the presence of an AT_1R blocker to obtain a decrease in mean arterial pressure.[60-62] The Ang II-derived cyclic ligands of AT_2R (LP2)[63,64] and MasR (cAng [1–7])[64-66] are more stable and resistant to peptidases due to the presence of thioester linkage and are studied for their vasorelaxant and organ protective effects.[67]

Sex-Specific Differences and Pregnancy

Sex-specific differences in various RAS components in terms of their expression and function have been documented. Numerous studies suggest a positive feedback regulation between levels of estrogen, its receptors, and AT_2R expression.[68-72] Female rodent kidneys expressed higher AT_2R,[73] which was decreased in ovariectomized mice. Estrogen supplementation restored the AT_2R expression to normal,[68,69] likely via the activation of ERα, as demonstrated by the ERα-knockout mice model and AT_2R antagonist treatment.[74,75] In contrast, ERβ (not ERα) is upregulated in pregnancy and binds with and transactivates ER-responsive elements in the AT_2R promoter in the uterine arteries of pregnant mice; this effect was mediated by ERα in nonpregnant mice.[72] This finding suggests a shift towards ERβ/AT_2R-mediated BP regulation in pregnancy. In contrast, AT_2R activation increased 17-β-estradiol levels, as shown in ovary cells in vitro[71] and HFD-fed mice treated with AT_2R agonist C21.[70] The enhanced AT_2R expression may be responsible for higher BP-lowering effects under the conditions of preeclampsia.[76] The Denton group has particularly reported sex-specific differences in the role of AT_2R in BP-regulating mechanisms such as pressure-natriuresis and tubuloglomerular feedback.[77-81] In Ang II–induced hypertension developed in WT and AT_2R-knockout mice, AT_2R-mediated protection against BP increase was only found in females and not in males.[77] The study showed that WT (28 mm Hg) and AT_2R-knockout (26 mm Hg) males showed a similar increase in mean arterial pressure in response to Ang II infusion. However, WT females demonstrated only a 12 mm Hg rise in BP compared with 26 mm Hg in AT_2R-knockout females. In another study,[79] rise in mean arterial pressure was lower (9 mm Hg) in females than that in males. However, this difference was lost in AT_2R-knockout mice, demonstrating more potent effects of AT_2R in protection against BP increase in females. Moreover, chronic pressure-natriuresis in female WT mice was shifted leftward compared with AT_2R-knockout mice. However, this shift was lost with age; female aging mice demonstrated lower renal AT_2R expression, indicating an association between reduced AT_2R expression and loss in pressure-natriuresis shift.[79] Ovariectomy of aged mice did not result in a further difference in AT_2R expression or pressure-natriuresis in females. This study highlights the fact that AT_2R expression and function are dependent on changes in estrogen levels, which are reduced with age. However, it would be interesting to treat aged mice with an AT_2R agonist or supplement with estrogen to examine whether positive feedback comes into play between AT_2R and estrogen and improves pressure-natriuresis in these animals.

Augmented AT_2R expression during pregnancy is responsible for an increase in the uterine blood flow induced by elevated Ang II levels. Estradiol exposure leads to the upregulation of AT_2R expression, which is inhibited by the ERα antagonist.[74] This study suggests that AT_2R expression and function are increased during pregnancy and may play a role in attenuating BP-related complications. A similar conclusion was revealed by another study in an AT_1a knockout mouse model.[82] This study showed mid-gestation systolic BP decline, which was abolished by treatment with the AT_2R antagonist PD123319, suggesting that mid-gestation decline in BP is an outcome of AT_2R activation mediated by Ang II.

OBESITY, METABOLISM, AND INSULIN SENSITIVITY

Obesity is a major cause of metabolic dysfunction, leading to hyperinsulinemia, hyperglycemia (diabetes), and dyslipidemia, which show inflammation and cell and organ injury, thereby affecting organ function. Other studies have revealed that AT_2R expression in adipose and nonadipose tissues such as kidney and blood vessels is increased in obesity and diabetes[10,70,83-85] indicating an enhanced role in these pathologies. Hyperglycemia is one of the factors that lead to an increase in AT_2R expression.[86] Therefore, understanding the role of AT_2R in obesity, metabolism, insulin sensitivity, and renal-cardiovascular function associated with these pathologies is crucial and currently being studied.

Insulin Resistance

Several studies have implicated AT_2R activation in reducing adiposity/obesity and improving metabolism, resulting in improvement in insulin sensitivity, glucose disposal, and blood lipid profile.[14,79,87-90] Insulin sensitivity/resistance and pancreatic function in relation to the role of AT_2R have been a major focus in both normal and diabetic conditions. Chronic and acute blockade of AT_2R with the antagonist PD123319 reduced glucose uptake in the muscles[91] and insulin receptor signaling in terms of PI3K/Akt activation in the liver and adipose tissue,[92] suggesting a physiological role of AT_2R. The physiological role of AT_2R was also confirmed by a study in AT_2R-knockout mice, which showed that streptozotocin-induced glycemia was higher in AT_2R-knockout mice coupled with lower pancreatic insulin levels.[87]

Studies examining the pharmacological activation of AT_2R with C21 have been particularly helpful to test the therapeutic potential of AT_2R in improving insulin

resistance and metabolism. A recent study in normal mice suggested that C21 treatment for 12 weeks reduced adipocyte size and enhanced insulin receptor signaling in terms of Akt activity in the liver and adipose tissues.[88] Several studies performed in animal models of diabetes and insulin resistance indicated the relevance of AT_2R in these pathologies. For instance, C21 administration promoted adipocyte differentiation and improved adipose insulin sensitivity in high-fat/high-fructose diet-induced insulin resistance in rats.[15] These changes were associated with PI3K/Akt-mediated increase in peroxisome proliferator-activated receptor-γ expression,[15] a transcription factor known to improve insulin sensitivity by altering the expression of various genes associated with insulin receptor signaling. Another study performed in type 2 diabetic mice shows that AT_2R activation with C21 enhanced PPARγ activation and ameliorated insulin resistance, partly due to adipocyte differentiation and protection of pancreatic β-cells.[93] Previously, the role of AT_2R in PPARγ activation has been shown under in vitro conditions in PC12W cells.[94] Antioxidative stress, antiapoptosis, improved microvascular perfusion, and efficient delivery of insulin to tissues, such as muscles, and increase in adiponectin demonstrated by various studies may also be linked to AT_2R-mediated improvement in insulin resistance, glycemia, and pancreatic function.[88,89,95–97] AT_2R activation also reduced the levels of inflammatory cytokines, such as TNF-α, in these aforementioned studies. Since inflammation is a causative factor for insulin resistance,[98,99] anti-inflammatory activity of AT_2R is likely another mechanism contributing to an improvement in insulin sensitivity and metabolism.

Obesity

As insulin resistance is associated with obesity, the role of AT_2R in obesity and adiposity has been documented in animals treated with AT_2R agonist and AT_2R-knockout mice.[100-102] Overall AT_2R-knockout models have shown inconclusive results. For example, deletion of AT_2R-knockout protected against HFD-induced obesity and insulin resistance, which involves altered lipogenic and lipolytic enzymes, increased total energy expenditure, whole-body lipid oxidation, and decreased food intake.[102-104] In contrast, AT_2R deletion in ApoE-null mice did not impact weight gain, per se, but increased adipose tissue mass, decreased the number of adipocytes and insulin receptor substrate-1 levels, increased plasma cholesterol levels and free fatty acids upon intake of high cholesterol diet and worsened oxidative stress and atherosclerotic lesions.[100] Our study in male and female AT_2R-knockout mice showed that males had higher calorie intake but less weight gain and gonadal adiposity as compared with females, suggesting decreased metabolic capacity in females.[101] Nonetheless, these genetic studies show the involvement of AT_2R in both preadipocytes differentiation and mature adipocyte metabolism and glucose utilization.[102] However, recent pharmacological studies have explicitly suggested that AT_2R activation reduces adiposity and obesity. For example, we have reported that C21 administration in male mice attenuated adiposity and improved lipid metabolism, likely by decreasing lipid synthesis and enhancing lipid degradation.[14] Moreover, in vitro studies in freshly isolated adipocytes from mice suggested that AT_2R activation inhibits fatty acid uptake via the NO/cGMP pathway.[14] However, as we know that AT_2R agonist treatment lowers free fatty acids and triglyceride levels in the plasma,[14] the fate of free fatty acids, which are not internalized by the adipose tissue and are not circulating needs to be investigated.

Sex-Specific Differences in Obesity

We have reported that AT_2 null mice were protected against HFD-induced weight gain for 16 weeks. However, this observation was only recorded in males; females, in contrast, gained more weight than WT, suggesting a sex-specific difference.[101] The antiobesity role of AT_2R was also confirmed in our pharmacological studies.[70] We have reported that HFD-induced (16-weeks) weight gain and adiposity in female mice were attenuated by treatment with systemic AT_2R agonist C21, which was associated with reduced adipocyte size, plasma insulin, and plasma fatty acids, and improved glucose tolerance.[70] Similar improvements in metabolic changes were observed after treatment of HFD-fed ovary-intact as well as ovariectomized mice with C21 for 2 weeks,[70] suggesting an estrogen-independent role of AT_2R. However, estrogen supplementation of ovariectomized mice treated with the AT_2R agonist C21 caused a remarkable improvement in the metabolic indices. Since the estrogen dose administered to these mice was high, a definite conclusion regarding the synergism between AT_2R activation and estrogen could not be established in this study. Consistent with knockout and pharmacological studies in females, female diabetic *db/db* mice treated with C21 for 1 month demonstrated improvements in the glucose/pyruvate tolerance and fatty liver via the NO pathway.[97]

Overall, we conclude that while AT_2R-knockout studies are inconclusive in males but show a sex-specific difference. Pharmacological activation of AT_2R improves metabolic dysfunction in various animal models irrespective of sex. However, the cellular signaling pathway responsible for the various beneficial effects of AT_2R activation remains to be elucidated.

ORGAN PROTECTION

Cardiovascular Protection

Hypertension, myocardial ischemia, and metabolic dysfunction, including obesity and diabetes, are known risk factors for cardiac hypertrophy, leading to cardiac remodeling and heart disease. Numerous studies have explored

the protective role of AT_2R in cardiac hypertrophy/remodeling using pharmacological and genetic approaches in various animal models of myocardial infarction (MI), obesity, hypertension, and HSD feeding.[105-110]

Pharmacological Studies

The first study to analyze the effect of direct stimulation of AT_2R by C21 on cardiac function and molecular events was performed in Wistar rats subjected to left coronary artery ligation for the induction of MI.[108] The results revealed that C21 treatment (for 7 days) reduced the scar size and improved systolic and diastolic ventricular functions. These changes were associated with reduced inflammation and apoptosis in C21 treated groups (particularly, reduction in the expressions of Fas ligand and caspase-3 in the peri-infarct area), and components of the mechanism associated with the regulation of cell survival (a rescue in MI-induced decrease in phosphorylation of p44/42 and p38 mitogen-activated protein kinase) in C21 treated animals. In another similar study, long-term (6 weeks) treatment of rats with MI showed improvements in arterial stiffness parameters and reduced collagen content in peri-infarct myocardium associated with improved heart function in terms of ejection fraction and fractional shortening. These results suggest a long-term beneficial effect of AT_2R activation in preventing cardiac remodeling.[111] Similar cardiac remodeling protective effects were reported in stroke-prone spontaneously hypertensive rat model and HSD-fed animals.[106,110] C21 treatment administered for 6 weeks showed a reduction in myocardial fibrosis, as evidenced by a decrease in myocardial interstitial collagen content and the expression of cardiac hypertrophy-related genes, namely, myosin heavy chain β, and α-SMA (α-skeletal muscle actin). In addition to cardiac protection, vascular injury, indicated by a reduction in vascular stiffness, collagen content, and fibronectin, was reduced by C21 treatment. In addition, C21 prevented the development of cardiac hypertrophy in HSD-fed animals.[106]

Genetic Studies

Genetic studies also support the cardioprotective effects associated with AT_2R. For instance, lack of AT_2R during acute MI exacerbated heart failure and decreased the survival of mice compared with that in WT within 7 days post-MI.[105] Lack of AT_2R was associated with increased expression of myocardial inflammatory prostaglandins and reduced collagen deposition, which chronically resulted in cardiac rupture.[107] In contrast, AT_2R overexpressing transgenic mice showed improved baseline LV systolic function and preservation of systolic function after MI compared with WT mice.[112] This conclusion is further supported by another study showing that the moderate overexpression of AT_2R in the myocardium either before or after induction of MI protected against ischemic injury by attenuating the decrease in fractional shortening and increase in LV end-diastolic pressure.[109] In contrast, chronic expression of AT_2R reduced myocardial contractility[113] and high ventricular-specific expression of AT_2R caused heart failure and dilated cardiomyopathy compared with that observed with low levels of AT_2R expression and that in WT mice.[114] Hence, it seems that the level of AT_2R overexpression may be important to mediate protective versus opposing effects on cardiac function after MI.

Role of Immune and Stem Cells

Chronic inflammation is an important biological process that triggers tissue injury, resulting in tissue/organ remodeling. AT_2R is expressed in immune cells, particularly T lymphocytes. In a rat model of MI, the infarcted myocardium was infiltrated with $CD_4^+AT_2R^+$ T cells and $CD_8^+AT_2R^+$ T cells, which were characterized by forkhead box protein P3 expression, upregulated IL (interleukin) 10 levels and downregulated IL-2 and interferon γ expression.[115,116] These T cells have been implicated in the improvements in cardiac function post-MI by reducing ischemic injury, thus offering a promising approach for regenerative cardiac therapy via myocardial transplantation of these cells. A study focusing on mesenteric arteries reported that AT_2R activation resulted in a release of IL-17 by memory T cells surrounding the high-flow arteries, suggesting that AT_2R could play a possible therapeutic role in ischemic disorders by allowing flow-mediated outward remodeling of resistance arteries that are involved in revascularization.[117] The experimental proof of this effect on the outward remodeling of coronary vessels is still lacking. Evidence also indicates the role of stem cells and their modulation by AT_2Rs in cardiac repair. The preconditioning of bone marrow mononuclear cells by AT_2R stimulation either by agonist CGP42112A or a combination of the Ang II and AT_1R antagonist valsartan increased the activation of ERK and endothelial NOS expression, thereby enhancing the generation of NO. This increase in NO enhanced cardiomyocyte function in vitro as well as improved survival of transplanted heart tissue in the ischemic region after intramyocardial transplantation of preconditioned bone marrow mononuclear cells. This suggests a new therapeutic strategy for the efficient utilization of stem cells in cardiac repair.[118] Furthermore, AT_2R stimulation of $CD117^+$ stem cells showed improvements of cardiac cell morphology in vitro, but it did not translate to the beneficial effect of $CD117^+$ stem cells.[119]

In contrast to the antihypertrophic effect of AT_2R in cardioprotection, some studies have shown that AT_2R may be responsible for or may not have any effect on cardiac hypertrophy and fibrosis. AT_2R agonism mediated by 0.3 mg/kg per day C21 and receptor upregulation in MI mouse models was not beneficial against LV remodeling, but rather increased the LV end-systolic and diastolic volume, suggesting an adverse effect of direct AT2R stimulation post-MI.[120] AT_2R deletion prevented

HFD-induced hypertrophy in mice. This observation was further supported by an in vitro study showing that blocking AT_2R with the antagonist PD123319 prevented leptin-induced cardiomyocyte hypertrophy.[121] This study is consistent with the finding that AT_2R deletion protects against obesity.

Overall, only a few studies indicate no effect or adverse effects related to AT_2R during MI or cardiac tissue remodeling. However, an overwhelming number of studies, both genetic and pharmacological, suggest that AT_2R exerts cardiovascular protective effects in various animal models. However, an explanation of the opposing role of AT_2R in cardiovascular protection would be helpful to ascertain what experimental conditions, if any, are responsible for the diverse effects.

Renoprotection

Obesity, hypertension, and diabetes are independent major risk factors for chronic kidney injury/disease.[122] AT_2R activation reduces adiposity/obesity, improves insulin resistance and glycemia, and lowers BP, as discussed in the previous sections of this review. Recent evidence suggests that AT_2R activation protects the kidneys against injury in various animal models. Our group has shown that treatment of HSD-fed OZR with C21 improved the kidney conditions, including glomerulosclerosis, visceral epithelial cell hypertrophy, tubular atrophy, and interstitial fibrosis, along with a reduction in protein-to-creatinine ratio, as well as fractional excretion of protein and albumin in these animals. In addition to improvements in proteinuria, a decline in GFR, a hallmark of chronic kidney diseases, was prevented by C21 treatment.[50] Another study performed in younger OZR (7 weeks old) without HSD administration showed that AT_2R protective effects were independent of BP changes.[123] In this study, C21 protected the kidney from early renal damage caused by obesity-linked renal pathology associated with enhanced mesangial matrix expansion in these animals. In addition, AT_2R-knockout mice exhibited increased mesangial matrix expansion and albuminuria, which worsened upon HFD feeding for 16 weeks,[124] suggesting a protective role of AT_2R against HFD-induced kidney injury.

In models of type 1 diabetes, AT_2R plays a protective role under various experimental conditions. For instance, C21 treatment of $ApoE^{-/-}$ mice with type 1 diabetic nephropathy demonstrated protection effects by decreasing the mesangial area, glomerular injury, α-SMA expression, and collagen content in the ECM.[125] C21 treatment was effective in lowering albuminuria in mouse models of diabetic nephropathy at 15 weeks of age; this effect was lost around 20 weeks of age. However, C21 in combination with losartan was more efficacious in attenuating tubulointerstitial fibrosis and albuminuria during the progression of diabetic nephropathy.[126] In addition to pharmacological studies, genetic studies also support the protective effects of AT_2R in diabetic nephropathy. For example, transgenic overexpression of mitochondrial AT_2R in the renal tubular cells of streptozotocin-induced diabetic rats resulted in renoprotection in the early stages of diabetes by inhibiting the decrease in mitochondrial bioenergetics efficiency, increase in superoxide production, and increased cell proliferation.[127] In contrast, mice lacking AT_2R showed features of early diabetic nephropathy, including renal hypertrophy, tubular apoptosis, progressive ECM protein accumulation, and decreased GFR, indicating impaired kidney structure and function.[128]

Renal Anti-Inflammation Effects

Similar to the anti-inflammatory activity of AT_2Rs in cardiac hypertrophy, several reports have shown the anti-inflammatory role of AT_2R in various kidney injury models, including obesity and diabetes. The activation of AT_2R by C21 treatment attenuated albuminuria and early inflammation by decreasing renal TNF-α and IL-6 levels and increasing NO and cGMP levels in renovascular hypertension and diabetes, independent of BP.[129,130] In our 2 studies, treatment of OZR with the AT_2R agonist C21 or CGP42112A for 2 weeks reduced the levels of proinflammatory cytokines TNF-α, IL-6, and chemokine monocyte chemoattractant protein-1 but increased the levels of anti-inflammatory cytokine IL-10 in the kidney and plasma along with a reduction in oxidative stress (heme oxygenase-1, $gp\text{-}91^{phox}$) markers.[24,123] These changes in cytokine levels were associated with a reduction in the renal infiltration of $CD68^+$ cells, which may also differentiate into macrophages.[123] Further in vitro studies performed in human kidney proximal tubule epithelial cells and monocytes confirmed that C21 decreased the LPS-induced increase in IL-6 and TNF-α; however, it increased the levels of IL-10. Furthermore, the studies revealed that the NO/IL-10 pathway may mediate the anti-inflammatory effect of AT_2R activation on the production of TNF-α and IL-6. In support of this, neutralizing IL-10 antibody or the NO synthase inhibitor L-arginine methyl ester abrogated the C21-mediated reduction in TNF-α and IL-6 production.[13] However, the involvement of the NO/IL-10 pathway in AT_2-mediated anti-inflammatory effects in animals is still unknown. The mechanism might be more complex as AT_2R is expressed in various cell types, including immune and nonimmune cells, which are involved in an interplay to mediate a net anti-inflammatory response.

In light of the monocytes studies,[13] we performed an acute study in an LPS-induced acute kidney injury mouse model.[131] We have observed that prior, not concomitant, treatment with C21 prevented the LPS-induced renal infiltration of $CD11b^+$ immune cells and ameliorated the increased levels of cytokines IL-6 and monocyte chemoattractant protein-1, while preserving IL-10 levels, indicating that AT_2R exerts prophylactic effects in preventing renal injury and dysfunction. The changes

in cytokine profile occurred in parallel with the changes in kidney structure and function. Hence, C21 treatment prevented LPS-induced vacuolization and reduction in blood urea nitrogen and albuminuria.[131]

Overall, it is clear that the aforementioned pharmacological and genetic studies suggest that AT_2R plays a renoprotective role under conditions of obesity, salt-induced injury, diabetic injury, and 5/6 kidney model of chronic kidney diseases[132,133] as well as in a mouse model of acute kidney injury.[131] These renoprotective effects are associated with the anti-inflammatory activity of AT_2R activation. However, significant research is required to understand the initial steps of immune cell infiltration and repair mechanisms associated with AT_2R activation in immune and kidney cells.

AT_2R-MASR INTERACTION AND SEVERE ACUTE RESPIRATORY SYNDROME-CORONAVIRUS-2 (SARS-CoV-2)

As mentioned earlier, AT_2R is a GPCR with low expression. However, its pharmacological activation significantly affects various pathophysiological processes, some of which are reviewed in this article. Recently, we published a review[37] showing that AT_2R interacts with MasR and other GPCRs, namely bradykinin BK1, dopamine D1, and angiotensin AT_1 receptors, and proposed that such an interaction could constitute a potential mechanism by which AT_2 amplifies its cellular signaling and biological responses. AT_2 and MasR are components of the protective arm of RAS and demonstrate similar beneficial effects in various renal-cardiovascular conditions, such as vasodilatation, natriuresis, and lowering of BP.[37] Our group[6,37]and others[134] have shown that AT_2R and MasR are colocalized, dimerized, and functionally interdependent. The AT_2R antagonist abolishes the Ang (1–7) activation of MasR and vice versa. This AT_2R-MasR functional interdependence is relevant to SARS-CoV-2 virus infection, which has been recently reviewed thoroughly.[135] The pharmacological activation of AT_2R via interaction with MasR may compensate for Ang (1–7) deficiency induced by the inhibition of ACE2 mediated by binding to SARS-CoV-2. Currently, it is known that ACE2 is a receptor for SARS-CoV-2 for its entry into the cell, leading to an attenuation of ACE2 activity.[136,137]

ACE2 is a major enzyme that converts Ang II into Ang (1–7), and the inhibition/attenuation of ACE2 leads to an increase in the Ang II accumulation and reduction in Ang (1–7), which is an endogenous ligand for MasR. This change shifts the balance between deleterious effects associated with Ang II/AT_1R and beneficial effects of Ang (1–7)/MasR.[138] We have reported that chronic treatment of OZR with AT_2R agonist C21 caused changes in various components of the kidney RAS, such as a reduction in AT_1R expression and increase in MasR expression and ACE2 activity, with a net decrease in Ang II levels and increase in Ang (1–7) levels in the kidney.[52] Moreover, despite the reduced production of Ang (1–7) during SARS-CoV-2-mediated downregulation of ACE2, the AT_2R may activate MasR and sustain MasR signaling without the need of Ang (1–7) owing to its physical dimerization and functional interdependence with MasR. AT_2R also exerts beneficial effects by reducing Ang II/AT_1R signaling. Based on this rationale, the AT_2R agonist C21 has been used in double-blind, randomized, placebo-controlled phase 2 clinical trials conducted in patients with COVID-19. The outcome of this study demonstrated that oral doses of 100 mg of C21 administered twice daily for 7 days reduced the requirement of supplemental oxygen by 90% on day 14 after the start of treatment. One patient in the C21 group required mechanical ventilation as compared with 4 in the placebo group, and 1 death was observed in the C21 group compared with 3 deaths in the placebo group.[139] This may indicate a scope of C21 in the potential treatment strategies for patients with COVID-19.

CONCLUSIONS

Sufficient reports suggest that the pharmacological activation of AT_2R shows significant therapeutic benefits in natriuresis, vasorelaxation, insulin sensitivity, and inflammation, which results in antihypertensive, antiobesity, and organ protective effects in various preclinical models. Moreover, the modulation of immune cells and stem cells by AT_2R shows further promise for tissue repair and regeneration. The long-term and sex-specific responses to direct AT_2R stimulation and whether estrogen therapy is amenable to enhance AT_2R-mediated benefits in the postmenopausal period require further examination to reveal AT_2R's therapeutic potential. Since AT_2R signaling is still understudied, it is important to explore how various pathways linked to AT_2R may lead to various protective effects and, in some cases, opposite effects. The crystal structure of AT_2R and its conformation in the presence of various agonists and antagonists could provide a lead to harness the full potential of AT_2R activation in various pathologies. The beneficial effects of the AT_2R agonist C21 in patients with COVID-19 provide a direct rationale for further exploration of the therapeutic effects of receptor agonism.

ARTICLE INFORMATION

Affiliation

From the Department of Pharmacological and Pharmaceutical Sciences, College of Pharmacy, University of Houston, TX.

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Disclosures

None.

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