

Impact of the AT(2) Receptor Agonist C21 on Blood Pressure and Beyond

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Impact of the AT₂ Receptor Agonist C21 on Blood Pressure and Beyond

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Abstract It is now widely accepted that the angiotensin AT₂ receptor (AT₂R) plays an important protective role during pathophysiologic conditions, acting as a repair system. The development of the first selective nonpeptide AT₂R agonist C21 accelerated our understanding of AT₂R-mediated protective signaling and actions. This article reviews the impact of C21 on blood pressure in normotensive and hypertensive animal models. Although C21 does not act as a classical antihypertensive drug, it could be useful in preventing hypertension-induced vascular and other end organ damages *via* anti-apoptotic, anti-fibrotic and anti-inflammatory actions. In particular, a strong body of evidence started to emerge around its anti-inflammatory feature. This property should be further investigated for potential clinical indications in cardiovascular diseases and beyond.

Keywords Hypertension · Blood pressure · Angiotensin · AT₂R · Renin-angiotensin system · RAS · Compound 21 · C21 · Inflammation · Antihypertensive drug therapy

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Introduction

The renin angiotensin system (RAS) plays a key role in blood pressure regulation. Its actions have been described as mostly dependent on stimulation of the Ang II receptor type 1 (AT₁R). The development of AT₁R blockers (ARB) and other receptor subtype-specific ligands in the 1990s revealed a second Ang II receptor subtype, the angiotensin AT₂ receptor (AT₂R) [1]. Since this discovery, several research groups have contributed to improving our understanding of this ‘enigmatic’ receptor [2]. It is now widely recognized that the AT₂R receptor exerts actions opposing those of the AT₁R, such as vasodilation, anti-proliferation, cell differentiation and anti-inflammation [3–7]. Although all mechanisms are still not fully deciphered, a picture of AT₂R signaling has emerged with three major transduction mechanisms: (1) activation of the NO/cGMP pathway, (2) activation of a series of protein phosphatases and (3) activation of phospholipase A2 inducing the release of arachidonic acid [8]. All or some of these signaling cascades seem to be initiated by various proteins binding to the C-terminal end of the AT₂-R such as the “AT₂-R binding proteins” (ATIP or ATBP, [9, 10•]), the homeodomain enhancer protein and Zfphep, and the transcription factor PLZF (promyelocytic leukemia zinc finger, [11, 12]). These pathways have to be further investigated, especially with regard to their importance in anti- or pro-proliferative and cellular differentiation actions of the AT₂R (see [6, 13•] for review of AT₂R signaling).

The AT₁R-opposed actions of the AT₂R are not only determined by specific signaling pathways but also by the levels of AT₂R expression in a given tissue. Early evidence for this was provided by Gohlke et al. in 1998 [14] and Pees et al. [15]. These authors demonstrated that AT₂R stimulation in the aorta of adult SHR-SP was responsible for activation of the bradykinin/NO/GMPc pathway [14]. In contrast, they did not find any evidence for such

AT₂R-mediated NO production in the vascular wall of WKY rats [15]. What seems to be a discordance between these two rat strains can indeed be explained by a significantly higher expression level of AT₂R in SHR compared to WKY [16]. In fact, the AT₁R/AT₂R ratio seems to be modified according to a given pathophysiologic state [17, 18]. In particular, AT₂R expression is upregulated in tissue injury [19, 20], suggesting this could constitute a protective system during pathophysiologic processes.

In this context, exploiting the therapeutic potential of the ‘protective arm’ of the RAS, to which the AT₂R as well as ACE2 and the Ang1-7/Mas receptor system belong [21], became a focus of interest with respect to drug development. The AT₂R thus became a potential therapeutic target, and synthesis of compounds stimulating the AT₂R was initiated. Compound 21 (C21) evolved from these efforts as the first selective nonpeptide AT₂R agonist [22].

Before the advent of C21, research on AT₂R functions was conducted either *via* indirect stimulation with Ang II in the presence of an ARB, *via* blockade of AT₂R function with an AT₂R antagonist or by elimination of AT₂R function using AT₂R knockout animals. The only early compound to directly stimulate the AT₂R was the peptide CGP42112A. Unfortunately, due to its peptidic nature, its use *in vivo* was limited. Moreover, CGP42112A also features antagonistic properties at low concentrations [4]. The synthetic AT₂R agonist, C21, thus constitutes the first pharmacokinetically unproblematic tool for the direct study of AT₂R functions and the first AT₂R agonist with drug-like properties. As a result, several experimental studies using C21 have been performed. With the background provided by preclinical studies since 2004 [22] and a currently ongoing toxicological program, it is anticipated that C21 is soon going to enter a clinical phase I study. In this article, we will summarize the important properties of C21 identified up to now.

Compound 21 and Blood Pressure

Since stimulation of AT₂R induces vasodilation in isolated vessels and production of NO, one might reasonably think that administration of C21 would generate a decrease in blood pressure (BP). However, as summarized in Table 1, stimulation of AT₂R *in vivo* does not yield a hypotensive effect – at least not acutely.

In fact, C21 alone did not decrease blood pressure when administered in normotensive animals [22, 23•, 24, 25]. Moreover, in animal models of hypertension, regardless of the type of hypertension (genetic or induced), C21 did not provide any antihypertensive action either [23•, 26•, 27•, 28•, 29]. A decrease in BP was only described in the original publication about C21 synthesis and design [22]. In this study, a decrease in BP was observed following an

acute i.v. infusion of C21 in SHR, but this was observed in anesthetized animals in which anesthesia may have hampered baroreflex control of BP. Thus, current evidence does not reveal any acute antihypertensive action of C21 in conscious animals, despite the diversity of treatment dosage, duration and route of administration tested (Table 1).

However, a potential antihypertensive effect of C21 alone may not be easily detectable *in vivo* because of a predominant AT₁R-dependent angiotensinergic tone. Thus, in order to observe effects resulting from AT₂R stimulation, it may be necessary to block AT₁R. In fact, when combined with a low-dose ARB that does not modify BP, C21 exerted an antihypertensive action [23••]. This is in agreement with previous results obtained with CGP42112A [30–32]. When C21 was combined with a high dose of an ARB, which induces an antihypertensive effect, no additive effect of C21 on BP was observed [27•, 28•].

Although stimulation of AT₂R with the AT₂ agonist C21 does not engender direct, acute antihypertensive effects, a secondary reduction of BP may occur because of favorable effects of chronic C21 administration on vascular remodeling and kidney function. A trend towards a reduction of BP has indeed been observed in models of renal hypertension [26•], L-NAME induced hypertension [28•] and diabetic SHR-SP (unpublished observation), but these effects did not reach statistical significance.

Since the decrease in BP by AT₂R-stimulation is only minor or in some studies not detectable at all, favorable effects of C21 in models of hypertensive end organ damage can be regarded as mainly or entirely BP independent. For example, Rehman et al. highlighted a reduction in vascular stiffness by C21 treatment in the aorta but also in mesenteric resistance arteries from hypertensive rats (SHR-SP) independently of any BP reduction [27•]. Moreover, our group has recently demonstrated that C21 treatment prevents the development of hypertension-induced aortic remodeling and accelerated pulse-wave velocity in L-NAME hypertensive rats without significantly changing BP [28•]. The C21-induced reduction of stiffness of mesenteric resistance arteries observed in the study by Rehman et al. as well as attenuated aortic stiffness in our study could in both cases be explained by a decrease in extracellular matrix components, collagen content and fibronectin. This may limit the increase in vascular resistance and, subsequently, the progression of hypertension as well as end-organ damage concomitant with chronic hypertension. Furthermore, at the kidney level, AT₂R stimulation with C21 produced vasodilatory and natriuretic effects and may therefore also improve renal function [24]. Despite the absence of direct antihypertensive action, C21 could thus be a useful additional tool in the long-term management of hypertension.

Table 1 Effect of C21 on blood pressure. Effect of C21 alone, associated with a high antihypertensive dose or a low non-antihypertensive dose of angiotensin receptor blocker (ARB) on blood pressure of normotensive, hypertensive or other pathologic animal models. C21 dosage, route and treatment duration are detailed

Animal model	C21 dosage	Route	Duration	Anesthetized animal	Effect on BP	Ref.
C21 alone						
S-D	0.05 mg/kg	i.v.	acute	Yes	No	[22]
SHR	0.05 mg/kg	i.v.	acute	Yes	↓	[22]
WKY or SHR, 16-18 w.o.	50 to 1000 ng/kg/min	i.v.	4 h	No	No	[23••]
S-D, 11-12 w.o.	100 to 300 ng/kg/min	i.v.	acute	Yes	No	[24]
S-D, 2K1C	0.3 mg/kg/day	i.p.	4 days	No	No	[26•]
Wistar rats with myocardial infarction	0.01, 0.03, or 0.3 mg/kg	i.p.	6 days	Yes	No	[33••]
C57BL/6 mice, 10-12 w.o.	1, 3, and 10 mg/kg/day	i.p.	2 weeks	No	No	[25]
SHR-SP, 6 w.o.	1 mg/kg/day	<i>per os</i>	6 weeks	No	No	[27•]
Wistar, 10 w.o. + L-NAME	0.3 mg/kg/day	<i>per os</i>	6 weeks	No	No	[28•]
SHR-SP, 4-5 w.o. + 1 % NaCl	0.75, 5 or 10 mg/kg/day	<i>per os</i>	until death	No	No	[29]
C21 associated with a high-dose ARB						
SHR-SP, 6 w.o. + losartan (10 mg/kg/day <i>per os</i>)	1 mg/kg/day	<i>per os</i>	6 weeks	No	No	[27•]
Wistar, 10 w.o. + L-NAME + olmesartan medoxomil (10 mg/kg/day <i>per os</i>)	0.3 mg/kg/day	<i>per os</i>	6 weeks	No	No	[28•]
C21 associated with a low-dose ARB						
SHR, 16-18 w.o. + candesartan (0.1 mg/kg bolus i.v.)	50 ng/kg/min	i.v.	4 h	No	↓	[23••]

Compound 21 and Anti-inflammation

Recent investigations, reviewed in the following, have revealed other beneficial effects beyond those for hypertension provided by C21.

In a model of myocardial infarction (MI) performed in Wistar rats, treatment with C21 improved cardiac function and decreased scar size after 7 days of treatment [33••]. The underlying mechanisms may include the strong anti-inflammatory effects of C21. Several inflammatory markers, increased following MI, were indeed lowered by C21, such as plasma monocyte chemoattractant protein-1 (MCP-1) and several proinflammatory cytokines. Moreover, in the peri-infarct zone, C21 attenuated the rise of apoptosis markers. These effects were blocked by the AT₂R antagonist PD123319, supporting that specific AT₂R stimulation by C21 exerted anti-inflammatory and anti-apoptotic actions in the context of MI.

In contrast, in a recent study with MI induced in mice, the authors did not observe a reduction of left ventricular remodeling following AT₂R stimulation with C21 [34]. A potential reason for the lack of benefit of C21 in this study was the fact that the same dose of C21 used successfully as a bolus injection in a MI model in rats [33••] was applied in this study as a continuous infusion by minipumps, thus—considering a plasma half-life of C21 of 4 h—presumably not reaching effective plasma levels. Moreover, the authors themselves discussed that the larger

infarct sizes obtained in their study could have masked beneficial effects of C21.

In a model of hypertension-induced renal dysfunction (salt-loaded SHR-SP), C21 delayed the occurrence of brain damage and reduced proteinuria [29]. These beneficial effects were specifically related to AT₂R stimulation since they were abolished by PD123319. The authors observed an attenuation of inflammatory and fibrotic processes in the kidneys, pointing again to the anti-inflammatory properties of C21. In a two-kidney-one-clip (2K1C) rat model of hypertension, in which the inflammatory status is upregulated as highlighted by the increase of TNF- α tumor necrosis factor- α), IL-6 (interleukin 6) and TGF- β 1 expression in the clipped kidneys, C21 significantly decreased these inflammatory markers [26•]. However, these effects were not completely blocked by PD123319. One possible explanation may rely on differences between these two AT₂R ligands concerning the choice of their administration route or their affinity for AT₂R [26•].

These recent studies highlighted that C21 affords anti-inflammatory properties *via* direct stimulation of AT₂R. Underlying mechanisms were investigated by Rompe et al. [35••]. Human primary dermal fibroblasts were incubated with TNF- α in order to induce IL-6 expression. C21 treatment decreased IL-6, MCP-1 and TNF- α expressions, thus arguing for its anti-inflammatory action. This effect was also reported in endothelial cells from the human umbilical vein. Pre-incubation of cells with PD123319 abolished this effect,

providing the evidence that these actions were AT_2R -dependent. The authors further observed that the inhibitory effect of C21 on IL-6 expression was suppressed under inhibition of serine/threonine or tyrosine phosphatases, thus demonstrating that the anti-inflammatory cascade below AT_2R stimulation implies stimulation of phosphatases. Moreover, the effect of C21 on IL-6 generation was also suppressed when cells were preincubated with a selective inhibitor of arachidonate epoxygenation, indicating that the arachidonic acid metabolite 11,12- epoxyeicosatrienoic acid (EET) constitutes a second messenger in the AT_2R -dependent anti-inflammation pathway. This is in agreement with EET acting as an anti-inflammatory mediator in vascular inflammation [36]. Rompe et al. also investigated whether the changes in IL-6 expression by C21 were related to a change in NF- κ B (nuclear factor- κ B) activity as IL-6 transcription is under the control of this transcription factor. This was achieved by monitoring nuclear translocation of the NF- κ B p50 subunit and also by measuring NF- κ B-dependent IL-6 promoter transcriptional activity *via* a luciferase reporter assay. C21 indeed reduced NF- κ B activity and translocation. Moreover, in a mouse model of cutaneous inflammation, in which IL-6, MCP-1 and TNF- α mRNA are upregulated, C21 induced a significant reduction of these inflammatory markers, providing further *in vivo* evidence of C21's anti-inflammatory properties. Thus, AT_2R -dependent anti-inflammation seems to involve activation of protein

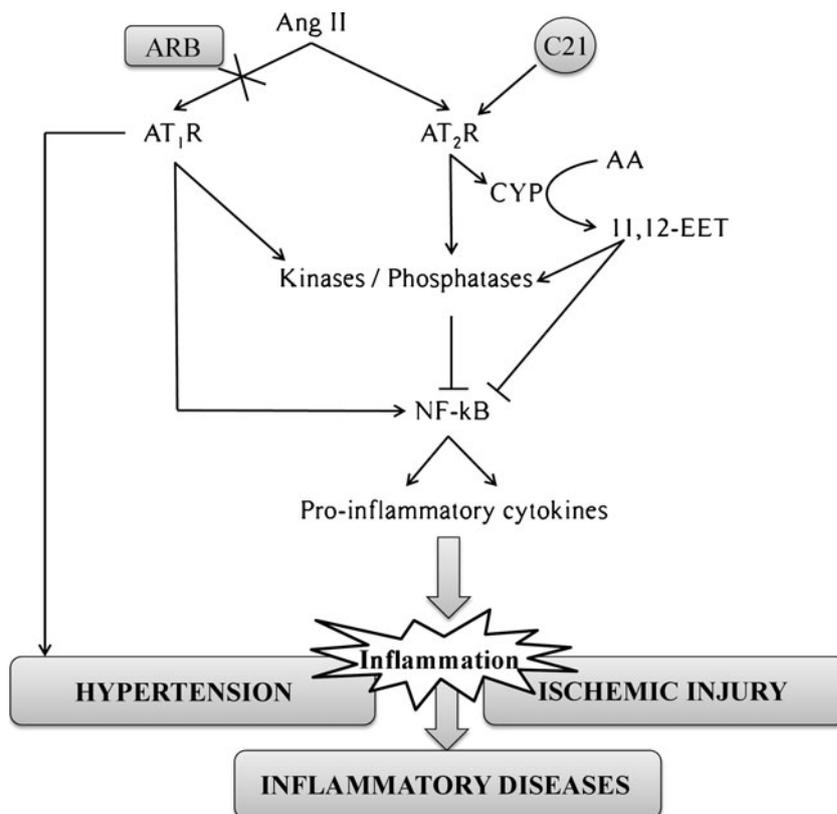
phosphatases, epoxidation of arachidonic acid to EETs and inhibition of NF- κ B reducing pro-inflammatory cytokines (Fig. 1) [35••]. It is important to note that, on the IL-6 promoter level, the NF- κ B inhibition in this study was comparable in strength to inhibition provided by hydrocortisone, thus supporting the idea that C21 should be studied as a potential drug candidate in inflammatory diseases [35••].

Finally, as inflammatory processes also contribute to the pathophysiology of hypertension, their inhibition may potentiate the benefit of blood pressure reduction [37]. The AT_2R agonist C21, partly *via* its anti-inflammatory properties, may thus be the ideal partner of ARBs in the context of hypertension and related cardiovascular complications (Fig. 1).

Perspectives

Although stimulation of AT_2R by C21 seems to generate numerous actions such as anti-apoptosis and anti-fibrosis [33••], several recent studies reviewed above highlight anti-inflammation as a major function of C21. Therapeutically, C21 could therefore be used as a tool in order to counteract pathologic inflammatory processes. Beyond cardiovascular diseases, in which inflammation is only a part of the pathophysiological process, C21 could enlarge

Fig. 1 Schematic overview of the AT_2R -dependent anti-inflammation signaling pathway, counteracting the proinflammatory action of AT_1R activation. Due to its anti-inflammatory properties, the AT_2R agonist C21 may be the ideal partner of ARBs in the context of hypertension and related cardiovascular complications. C21 could also enlarge its therapeutic perspectives to non-cardiovascular inflammatory diseases. *Ang II*, angiotensin II; *AT₁R*, angiotensin II receptor subtype 1; *AT₂R*, angiotensin II receptor subtype 2; *ARB*, angiotensin receptor blocker; *C21*, compound 21; *CYP*, cytochrome P450; *AA*, arachidonic acid; *11, 12-EET*, 11, 12-epoxyeicosatrienoic acid; *NF- κ B*, nuclear factor κ B



its therapeutic perspectives to non-cardiovascular inflammatory diseases.

Beyond classical treatments, stem cells constitute promising candidates for future therapies [38]. Moreover, there is a growing body of evidence suggesting that RAS, and AT₂R in particular, are implied in the proliferation and differentiation of hematopoietic and mesenchymal stem cells (see [39•] for review). In their recent review, Durik et al. addressed the impact of RAS modulation, and in particular AT₂R stimulation, on tissue regeneration by progenitor cells [39•]. Indirect evidence suggests that AT₂R stimulation may improve the therapeutic effects of MSC grafts in myocardial infarction [40] and brain ischemia [41].

The cellular mechanisms involved in the cardioprotective role of AT₂R have been further investigated following MI. The expression of AT₂R has been indeed observed in human cardiac stem cells as well as in CD8-positive T cells (CD8⁺ AT₂R⁺ T cells) in the peri-infarct area [42••, 43]. AT₂R⁺ are increased in human cardiac stem cells after MI, and their stimulation with C21 attenuated apoptosis of cardiomyocytes [43]. Besides, AT₂R⁺ are also expressed in a fraction of CD8-positive T cells in the peri-infarct area [42••]. Contrary to CD8⁺ AT₂R⁻ T cells, CD8⁺ AT₂R⁺ T cells did not induce cytotoxicity to cardiomyocytes and exhibited a decreased expression of proinflammatory cytokines. Intramyocardial transplantation of these cells after MI reduced the infarct size, thus providing *in vivo* evidence of cardioprotection *via* CD8⁺ AT₂R⁺ T cells. These studies highlighted an AT₂R-mediated cellular mechanism protecting the heart from injury at least in part *via* anti-apoptotic and anti-inflammatory actions. This could contribute to the beneficial effects observed in post-MI following an acute [33••] or a chronic C21 treatment [44].

Otherwise, the neuroprotective action of AT₂R has in particular been previously investigated after transient cerebral ischemia by unilateral middle cerebral artery occlusion in the rat [19]. In this model, AT₂R⁺ were upregulated in neuronal cells of the peri-ischemic area, and this was associated with cerebroprotective actions. In order to explore the underlying mechanisms, the authors focused on the role of AT₂R⁺ in primary neuronal cells. They highlighted that AT₂R stimulation promotes neurite outgrowth and neuronal survival [19]. This may support the AT₂R-dependent neuroprotection provided by MSC grafts during brain ischemia [41]. Stimulation of AT₂R of progenitor cells seems to improve the effects of cell therapy treatments in the context of cardiovascular and neural injury, acting as a repair system [39•].

Conclusion

Although the AT₂R agonist C21 does not act as a classical antihypertensive drug, it could be useful in preventing

hypertension-induced organ damage. Moreover, a body of evidence emerges around its anti-inflammatory feature: this should be further investigated for a potential clinical indication.

Considering the AT₂R expression levels in healthy (low expression) and injured tissues (upregulated expression), direct AT₂R stimulation with C21 could constitute a selective repair therapy directed at the injury site, with a limited occurrence of adverse events. AT₂R agonists are the first agonists of the RAS developed with a therapeutic goal. Up to now, the therapeutic goal of interfering with the RAS was slowing down the renin/ACE/AT₁ axis. In contrast to this approach, AT₂R agonists, with the lead compound C21, may afford new therapeutic options *via* promotion of the “protective RAS.”

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References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of outstanding importance

1. Chiu AT, Herblin WF, McCall DE, et al. Identification of angiotensin II receptor subtypes. *Biochem Biophys Res Commun.* 1989;165:196–203.
2. Unger T. The angiotensin type 2 receptor: variations on an enigmatic theme. *J Hypertens.* 1999;17:1775–86.
3. Vincent J-M, Kwan YW, Chan SL, et al. Constrictor and dilator effects of angiotensin II on cerebral arterioles. *Stroke.* 2005;36:2691–5.
4. Stoll M, Steckelings UM, Paul M, et al. The angiotensin AT₂-receptor mediates inhibition of cell proliferation in coronary endothelial cells. *J Clin Invest.* 1995;95:651–7.
5. Meffert S, Stoll M, Steckelings UM, et al. The angiotensin II AT₂ receptor inhibits proliferation and promotes differentiation in PC12W cells. *Mol Cell Endocrinol.* 1996;122:59–67.
6. Steckelings UM, Kaschina E, Unger T. The AT₂ receptor—a matter of love and hate. *Peptides.* 2005;26:1401–9.
7. Wu L, Iwai M, Li Z, et al. Regulation of inhibitory protein-κB and monocyte chemoattractant protein-1 by angiotensin II type 2 receptor activated Src homology protein tyrosine phosphatase-1 in fetal vascular smooth muscle cells. *Mol Endocrinol.* 2004;18:666–78.
8. Nouet S, Nahmias C. Signal transduction from the angiotensin II AT₂ receptor. *Trends Endocrinol Metab.* 2000;11:1–6.
9. Wruck CJ, Funke-Kaiser H, Pufe T, et al. Regulation of transport of the angiotensin AT₂ receptor by a novel membrane-associated Golgi protein. *Arterioscler Thromb Vasc Biol.* 2005;25:57–64.
10. • Rodrigues-Ferreira S, Nahmias C: An ATIPical family of angiotensin II AT₂ receptor-interacting proteins. *Trends Endocrinol Metab.* 2010, 21: 684–690. *This article reviews recent discoveries*

- concerning a family of AT_2 receptor-interacting proteins involved in the AT_2R signaling, regulation and function.
11. Stoll M, Hahn AWA, Jonas U, et al. Identification of a zinc finger homeodomain enhancer protein after AT_2 receptor stimulation by differential mRNA display. *Arterioscler Thromb Vasc Biol.* 2002;22:231–7.
 12. Senbonmatsu T, Saito T, Landon EJ, et al. A novel angiotensin II type 2 receptor signaling pathway: possible role in cardiac hypertrophy. *EMBO J.* 2003;22:6471–82.
 13. • Funke-Kaiser H, Reinemund J, Steckelings UM, Unger T: Adapter proteins and promoter regulation of the angiotensin AT_2 receptor—implications for cardiac pathophysiology. *J Renin Angiotensin Aldosterone Syst* 2010, 11: 7–17. *This article reviews new identified pathways implied in the modulation of signaling, expression and function of AT_2R , especially via AT_2R adapter proteins and heterodimer formation.*
 14. Gohlke P, Pees C, Unger T. AT_2 receptor stimulation increases aortic cyclic GMP in SHRSP by a kinin-dependent mechanism. *Hypertension.* 1998;31:349–55.
 15. Pees C, Unger T, Gohlke P. Effect of angiotensin AT_2 receptor stimulation on vascular cyclic GMP production in normotensive Wistar Kyoto rats. *Int J Biochem Cell Biol.* 2003;35:963–72.
 16. Touyz RM, Endemann D, He G, et al. Role of AT_2 receptors in angiotensin II-stimulated contraction of small mesenteric arteries in young SHR. *Hypertension.* 1999;33:366–72.
 17. Widdop RE, Vinh A, Henrion D, Jones ES. Vascular angiotensin AT_2 receptors in hypertension and ageing. *Clin Exp Pharmacol Physiol.* 2008;35:386–90.
 18. Foulquier S, Dupuis F, Perrin-Sarrado C, et al. High salt intake abolishes AT_2 -mediated vasodilation of pial arterioles in rats. *J Hypertens.* 2011;29:1392–9.
 19. Li J, Culman J, Hörtnagl H, et al. Angiotensin AT_2 receptor protects against cerebral ischemia-induced neuronal injury. *FASEB J.* 2005;19:617–9.
 20. Makino I, Shibata K, Ohgami Y, et al. Transient upregulation of the AT_2 receptor mRNA level after global ischemia in the rat brain. *Neuropeptides.* 1996;30:596–601.
 21. Steckelings UM, Paulis L, Unger T, Bader M. Emerging drugs which target the renin-angiotensin-aldosterone system. *Expert Opin Emerg Drugs.* 2011;16:619–30.
 22. Wan Y, Wallinder C, Plouffe B, et al. Design, synthesis, and biological evaluation of the first selective nonpeptide AT_2 receptor agonist. *J Med Chem.* 2004;47:5995–6008.
 23. •• Bosnyak S, Welungoda IK, Hallberg A, et al.: Stimulation of angiotensin AT_2 receptors by the non-peptide agonist, Compound 21, evokes vasodepressor effects in conscious spontaneously hypertensive rats. *Br. J. Pharmacol.* 2010, 159: 709–716. *This is the first study demonstrating a C21-induced vasorelaxation in vitro that is translated into a vasodepressor response in vivo when combined with a low dose of AT_1R blocker.*
 24. Hilliard LM, Jones ES, Steckelings UM, et al. Sex-specific influence of angiotensin type 2 receptor stimulation on renal function: a novel therapeutic target for hypertension. *Hypertension.* 2012;59:409–14.
 25. Jing F, Mogi M, Sakata A, et al. Direct stimulation of angiotensin II type 2 receptor enhances spatial memory. *J Cereb Blood Flow Metab.* 2012;32:248–55.
 26. • Matavelli LC, Huang J, Siragy HM: Angiotensin AT_2 receptor stimulation inhibits early renal inflammation in renovascular hypertension. *Hypertension* 2011, 57: 308–313. *This study provides evidence for the anti-inflammatory actions of C21 in clipped kidney of the Goldblatt hypertension model.*
 27. • Rehman A, Leibowitz A, Yamamoto N, et al.: Angiotensin Type 2 Receptor Agonist Compound 21 Reduces Vascular Injury and Myocardial Fibrosis in Stroke-Prone Spontaneously Hypertensive Rats. *Hypertension* 2012, 59: 291–299. *This is the first study reporting effects of chronic C21 treatment on the vascular wall in genetic hypertensive rats. C21 reduced the stiffness of mesenteric resistance arteries independently of any blood pressure reduction.*
 28. • Paulis L, Becker STR, Lucht K, et al.: Direct angiotensin II type 2 receptor stimulation in $N\omega$ -nitro-L-arginine-methyl ester-induced hypertension: the effect on pulse wave velocity and aortic remodeling. *Hypertension* 2012, 59: 485–492. *This is the first study reporting the effects of chronic C21 treatment on the vascular wall in a hypertension-induced model. C21 prevented aortic stiffening independently of any blood pressure reduction.*
 29. Gelosa P, Pignieri A, Fändriks L, et al. Stimulation of AT_2 receptor exerts beneficial effects in stroke-prone rats: focus on renal damage. *J Hypertens.* 2009;27:2444–51.
 30. Li XC, Widdop RE. AT_2 receptor-mediated vasodilatation is unmasked by AT_1 receptor blockade in conscious SHR. *Br J Pharmacol.* 2004;142:821–30.
 31. Barber MN, Sampey DB, Widdop RE. AT_2 receptor stimulation enhances antihypertensive effect of AT_1 receptor antagonist in hypertensive rats. *Hypertension.* 1999;34:1112–6.
 32. Carey RM, Howell NL, Jin XH, Siragy HM. Angiotensin type 2 receptor-mediated hypotension in angiotensin type-1 receptor-blocked rats. *Hypertension.* 2001;38:1272–7.
 33. •• Kaschina E, Grzesiak A, Li J, et al.: Angiotensin II type 2 receptor stimulation: a novel option of therapeutic interference with the renin-angiotensin system in myocardial infarction? *Circulation* 2008, 118: 2523–2532. *This is the first in vivo study using C21. A short treatment with C21 improved cardiac function and decreased scar size after myocardial infarction. This was observed in association with a decrease in inflammation and apoptosis markers.*
 34. Jehle AB, Xu Y, Dimaria JM, et al. A nonpeptide angiotensin II type 2 receptor agonist does not attenuate postmyocardial infarction left ventricular remodeling in mice. *J Cardiovasc Pharmacol.* 2012;59:363–8.
 35. •• Rompe F, Artuc M, Hallberg A, et al.: Direct angiotensin II type 2 receptor stimulation acts anti-inflammatory through epoxyeicosatrienoic acid and inhibition of nuclear factor kappaB. *Hypertension* 2010, 55: 924–931. *This is the first study showing anti-inflammatory effects of direct AT_2R stimulation by C21 in vitro and in vivo. Underlying signaling mechanisms are reported.*
 36. Node K, Huo Y, Ruan X, et al. Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. *Science.* 1999;285:1276–9.
 37. Savoia C, Schiffrin EL. Inflammation in hypertension. *Curr Opin Nephrol Hypertens.* 2006;15:152–8.
 38. Sánchez PL, Villa A, Sanz R, et al. Present and future of stem cells for cardiovascular therapy. *Ann Med.* 2007;39:412–27.
 39. • Durik M, Sevá Pessôa B, Roks AJM: The renin-angiotensin system, bone marrow and progenitor cells. *Clin. Sci.* 2012, 123: 205–223. *This review addresses the impact of renin-angiotensin-system modulation on tissue regeneration by progenitor cells. In particular, they highlight the potential importance of AT_2R stimulation with agonists in the future of stem cell therapy.*
 40. Numasawa Y, Kimura T, Miyoshi S, et al. Treatment of human mesenchymal stem cells with angiotensin receptor blocker improved efficiency of cardiomyogenic transdifferentiation and improved cardiac function via angiogenesis. *Stem Cells.* 2011;29:1405–14.
 41. Iwanami J, Mogi M, Li J-M, et al. Deletion of angiotensin II type 2 receptor attenuates protective effects of bone marrow stromal cell treatment on ischemia-reperfusion brain injury in mice. *Stroke.* 2008;39:2554–9.
 42. •• Curato C, Slavic S, Dong J, et al.: Identification of noncytotoxic and IL-10-producing $CD8^+$ AT_2R^+ T cell population in response

- to ischemic heart injury. *J. Immunol.* 2010, 185: 6286–6293. *This study revealed the expression of AT₂R in CD8⁺ T cells in the peri-infarct myocardium, thus revealing an AT₂R-mediated cellular mechanism in regulating immune response during ischemic heart injury.*
43. Altarache-Xifró W, Curato C, Kaschina E, et al. Cardiac c-kit + AT₂+ cell population is increased in response to ischemic injury and supports cardiomyocyte performance. *Stem Cells.* 2009;27:2488–97.
44. Lauer D, Slavic S, Sommerfeld M, et al. AT₂ receptor stimulation improves cardiac function 6 weeks after myocardial infarction in the rat. [Abstract ESH 8 C.03] Presented at the 22nd European Meeting on Hypertension and Cardiovascular Prevention. London, Great Britain, April 26-29, 2012.