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Angiotensin type 2 receptors: Blood pressure regulation and end organ damage

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Abstract

In most situations, the angiotensin AT2-receptor (AT2R) mediates physiological actions opposing those mediated by the AT1-receptor (AT1R), including a vasorelaxant effect. Nevertheless, experimental evidence vastly supports that systemic application of AT2R-agonists is blood pressure neutral. However, stimulation of AT2R locally within the brain or the kidney apparently elicits a systemic blood pressure lowering effect. A systemic effect of AT2R stimulation on blood pressure can also be achieved, when the prevailing effect of continuous background AT1R-stimulation is attenuated by low-dose AT1R blockade. Despite a lack of effect on blood pressure, AT2R stimulation still protects from hypertensive end-organ damage. Current data and evidence therefore suggest that AT2R agonists will not be suitable as future anti-hypertensive drugs, but that they may well be useful for end-organ protection in combination with established anti-hypertensives.

Introduction

It is now generally accepted that the renin angiotensin system (RAS) has many more facets than solely the well-known effects of angiotensin II (Ang II) acting on the AT1-receptor (AT1R). In fact, the RAS harbours several other receptors and hormones (Ang II metabolites) which elicit actions opposing those of the AT1R, resulting in tissue protective effects. Currently known components of the so called “Protective arm of the RAS” are the hormones angiotensin-(1-7) [1] and alamandine [2], angiotensin converting enzyme 2

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Conflict of interest

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(ACE2) [the enzyme responsible for Ang-(1-7) synthesis] [1], and the receptors Mas [for angiotensin-(1-7)], Mas-related G-protein coupled receptor D (MrgD; for alamandine) and the AT2-receptor (AT2R; binding Ang II) [3,4].

In case of the AT2R, research in recent years has been facilitated and fostered by the availability of a specific and selective non-peptide AT2R agonist, Compound 21 (C21), which for the first time has allowed stimulation of the AT2R in long-term preclinical studies [5]. Using this new research tool, the role of the AT2R in blood pressure (BP) regulation and hypertensive end-organ damage has been reassessed and studied by several groups [6].

The following article will review studies that have addressed the effects of AT2R stimulation in the periphery, in the brain or the kidney on BP regulation and hypertensive end-organ damage with a focus on findings published during the last two years.

Effects of systemic AT2R stimulation on blood pressure control

Since the AT2R is known to counteract actions of the AT1R by direct (through dimerisation) [7] or indirect (through dephosphorylation) interference with AT1R-coupled signalling [8], it has been assumed that AT2R-stimulation would result in a lowering of BP. This conclusion is supported by multiple studies showing a weak, but consistent vasorelaxant effect of AT2R-stimulation *ex vivo* in isolated vessels originating from various vascular beds such as mesenteric, renal, coronary, cerebral, cutaneous, and uterine arteries [reviewed in 6]. Moreover, AT2R-stimulation has been shown to elicit a strong natriuretic effect [9–11]. Nevertheless, the vast majority of studies looking at short- or long-term effects of AT2R-stimulation did not observe any anti-hypertensive effect. This holds true for models of genetic hypertension (spontaneously hypertensive rats, SHR; stroke-prone SHR, SHR-SP) [12–16], hypertension induced by inhibition of NO synthesis [17], by Na^+- or volume-overload [11], for renal hypertension [18] and for lean or obese normotensive animals [10,19,20]. Since in some of these models the activity of the RAS (plasma Ang II levels) is suppressed (Na^+- and volume overload), while in others it is rather unchanged (genetic hypertension) or even activated (renal hypertension), the state of activity of the RAS seems to play no role with regard to the efficacy of AT2R-agonists in lowering blood pressure.

However, there are few exceptions, which are as follows (Fig. 1):

- AT2R-stimulation in the CNS seems to have a BP lowering effect as discussed in more detail later in this review.

- The BP lowering effect of AT2R-agonists administered peripherally appears to be unmasked when co-administered with a low dose of an AT1R-blocker (ARB), which by itself has no or only a marginal BP lowering effect. This phenomenon has been shown by the groups of Robert Widdop and Robert Carey using peptide or non-peptide AT2R-agonists [16,21–23]. These data can be interpreted in a way that a constant angiotensinergic tone acting via the AT1R normally dominates over the vasodilatory effect of the AT2R. When an ARB is applied at a high dose, there is no additive effect of AT1R-blockade and AT2R-stimulation on BP.

- The group of Robert Carey recently reported that in various animal models (volume expansion in rats; Na^+-loaded male and female rats; normal C57BL/6 and AT2R-KO mice) systemic infusion of the AT2R-agonist C21 did not alter BP despite a strong natriuretic and diuretic effect [11]. However, in female rats chronically (7 days) and systemically infused with Ang II, the resulting elevated BP was markedly reduced when C21 (60 ng/kg/min) was concomitantly infused intrarenally, supporting the existence of an independent, functional, intrarenal RAS [24]. Since in this latter experimental setup several parameters were changed in comparison to the experiments, in which C21 had no effect on BP, it became not entirely clear from this study, what the actual cause for this rather unexpected BP-lowering effect of C21 was. Potential causes could be a) the model of Ang II induced hypertension, b) the much longer duration of C21 application (7 days versus 3 × 30 minutes), c) the intrarenal route of application, d) the fact that these experiments were performed in female rats, or a combination of some of these parameters. The assumption that female sex is essential is supported by a series of experiments performed by Kate Denton's group, which showed that adult females express more AT2Rs than males, which leads to lower baseline levels of MAP, but also to a leftward shift of chronic pressure-natriuresis compared to males [12,25].

- C21 may have an impact on BP, depending upon whether blood pressure measurements are made in conscious or anaesthetised rats, because there are at least two examples of AT2R-mediated reductions in blood pressure in anaesthetised rats of strains/models (SHR, obese Zucker rats), in which in the conscious state blood pressure was not affected by AT2R-stimulation [13,16], while under anaesthesia it was [5,26].

Central nervous system effects of AT2R stimulation on blood pressure control

The overriding view of blood pressure regulation via the RAS within the central nervous system (CNS) begins and ends with pressor and hypertensive effects of Ang II mediated by AT1R [27–29]. This is perhaps not surprising as, according to traditional receptor binding and autoradiography techniques, CNS cardiovascular control areas such as the paraventricular nucleus of the hypothalamus (PVN), rostral ventrolateral medulla (RVLM) and solitary tract nucleus (NTS) within the brainstem are rich in AT1R but are either devoid of or express only low levels of AT2R [30,31]. Nonetheless, a limited number of functional studies have implicated or suggested that AT2R in the brain influence cardiovascular regulation. For example, AT2R knockout mice display elevated basal blood pressure [32], and increased susceptibility to develop DOCA-salt hypertension [33]. Electrophysiological studies examining the CNS cardiovascular control centres of AT1Ra knockout mice suggest that AT2R in the RVLM play an antagonistic role against AT1R-mediated actions of Ang II within this nucleus [34]. Furthermore, virally-mediated over expression of AT2R within the RVLM [35] or NTS [36] elicits respective decreases in mean arterial blood pressure of normotensive rats and in 2 kidney-1 clip hypertensive rats. In the latter study, it was also demonstrated that AT2R over expression in the NTS restores baroreflex sensitivity [36]. The recent availability of C21, has enabled Gao et al to demonstrate that infusion of this agent into the cerebroventricles lowers blood pressure in normotensive rats [37,38], and more recently to show that similar infusions into rats with heart failure suppresses sympathetic

outflow by improving baroreflex sensitivity [39]. While these collective studies have indicated blood pressure lowering and anti-hypertensive actions of AT2R, the mechanisms underlying these effects were, for the most part, unknown due to an inability to localize AT2R to specific cell types within the brain.

The recent development of a novel transgenic model (Agtr2-eGFP BAC reporter mouse) that provides higher sensitivity for localizing AT2R, has allowed for identification of the discrete cellular localization of AT2R in the brain [40], and may explain the results of the functional studies referenced above. This approach has revealed that AT2R reside or are in close proximity to CNS cardiovascular control centres, and are associated with neuronal phenotypes that are known to influence blood pressure. For example, there is a major concentration of AT2R containing neurons within the intermediate region of the NTS (intNTS), a nucleus that is essential for blood pressure control and baroreflex regulation. Further, these AT2R in the intNTS are primarily localized to GABA neurons [40], as illustrated by the fluorescence micrographs in Figure 2.

When considering that GABA acts within the intNTS to exert a powerful pressor action [41,42], an effect that is exacerbated in hypertension [43–45], the localization of AT2R on GABA neurons in the intNTS raises the possibility that they can exert depressor effects by influencing GABA activity. In contrast to the intNTS, localization of AT2R within the PVN and the RVLM, areas important in controlling sympathetic outflow, is restricted to neuronal fibres and terminals but not cell bodies [40]. Within the PVN, these AT2R-containing neuron fibres and terminals appear to synapse onto pre-autonomic neuron cell bodies [40], raising the possibility that AT2R can influence sympathetic outflow and blood pressure through these connections.

In summary, the role of CNS AT2R in the control of blood pressure is not well established. However, recent functional studies in conjunction with data that indicate localization of AT2R within brain cardiovascular control centres, strongly suggest that AT2R contribute to the neural control of the circulation. The therapeutic relevance of this contribution, such as whether selective activation of CNS AT2R can efficaciously reduce blood pressure and offset hypertensive mechanisms, including AT1R activation, and alleviate end organ damage, can only be determined by investigating whether specific neuronal circuits that regulate cardiovascular function are indeed influenced by AT2R activation.

AT2R stimulation and hypertensive end organ damage

Stimulation of peripheral AT2R has been tested in models of hypertension-induced kidney damage [14], cerebral haemorrhage [14], and vascular remodelling [15,17].

Remarkably, in all of these studies performed in hypertensive animals, systemic AT2R-stimulation had no anti-hypertensive effect, i.e. all protective effects of AT2R-stimulation as determined on molecular level or in terms of organ function were not secondary to BP-lowering effects, but independent of BP levels.

The first evidence for a protective effect of AT2R-stimulation with C21 in a model of hypertensive end-organ damage came from a study by Luigi Sironi’s group performed in

SHR-SP on a high-salt diet [14]. This study investigated both renal and cerebral hypertension-induced pathology. With respect to the kidneys, oral administration of C21 ameliorated hypertensive nephropathy as shown by an inhibition of renal inflammation, vimentin overexpression and collagen accumulation. Brain pathology was assessed by MRI and by analysing survival rates, since these rats usually die of stroke. Survival as well as time until occurrence of brain pathology was extended in SHR-SP treated with C21. These effects of C21 could be inhibited by the AT2R-antagonist PD123319 indicating that they were AT2R-specific. The protective effect of C21 became only apparent at the highest dose used, which was 10 mg/kg/day p.o. This dose is much higher than that normally used in rats, which can be explained by the fact that in this study C21 was applied in 0.5% sodium carboxymethylcellulose as vehicle, which is known to delay drug release.

So far, the study reviewed above is the only one that has investigated application of an AT2R-agonist in hypertension-induced hemorrhagic stroke. However, there are a number of publications reporting reduced infarct size and improved neurological outcome by AT2R-stimulation in ischemic stroke (the main risk factor for which is hypertension), no matter whether treatment was started prior to or up to 6 hours after stroke, and no matter whether the AT2R-agonist was applied peripherally or into the cerebroventricles [46–51].

In 2012, the group of Ernesto Schiffrin and our group published two studies, which both investigated the effect of AT2R-stimulation by C21 on vascular remodelling [15,17]. While the Schiffrin group used SHR-SP (fed a diet with normal salt content) for their study, our study was performed in Wistar rats treated with the inhibitor of nitric oxide synthase, N-Nitro-L-Arginine-Methyl Ester (L-NAME). In both studies, over a 6 week period rats gradually developed hypertension of up to 190 mmHg systolic blood pressure (SBP). AT2R-stimulation had no effect on SBP, while an AT1R-blocker (ARB), which was used in both studies for comparison (losartan in the Schiffrin study, Olmesartan in our study), completely prevented the gradual rise in BP. Both studies were in agreement that despite the lack of BP reduction, C21 significantly reduced collagen accumulation within the vascular wall to a similar extent as the ARB, which normalised BP. Using different techniques, i.e. measurement of stress/strain relationship in mesenteric arteries *ex vivo* or pulse wave velocity *in vivo*, respectively, both studies further revealed a reduction in vascular stiffness by treatment with C21. They also conform in terms of an additive effect of AT2R-stimulation and AT1R-blockade on the prevention of vascular collagen accumulation – again despite a lack of an additive BP lowering effect.

Conclusions

In conclusion, current evidence indicates that systemic application (intravenous, intraperitoneal or oral) of AT2R-agonists has no BP-lowering effect *in vivo*, no matter whether they are applied to normotensive or hypertensive animals, despite a vasodilatory effect *ex vivo*. However, a blood pressure lowering effect of AT2R-stimulation *in vivo* can be unmasked through dampening the prevailing angiotensinergic tone (mediated via AT1R) by low-dose AT1R-blockade. It may also be unmasked by anaesthesia through unknown mechanisms, whereas the state of activation of the RAS seems to play no role.

In contrast to systemic application, application of AT2R-agonists into the brain and possibly also into the kidney does exert a blood pressure lowering effect in normotensive animals, as does overexpression of AT2R within CNS cardiovascular control centres of hypertensive rats.

Despite the lack of an antihypertensive effect in most instances, AT2R-stimulation is still able to attenuate hypertensive end-organ damage in kidneys, vasculature and the brain.

According to current knowledge, AT2R-stimulation will most probably not become a future therapeutic approach for the treatment of hypertension – at least as long as AT2R-agonists that are able to cross the blood-brain-barrier and have central effects after peripheral application are unavailable. Nevertheless, AT2R-agonism may be therapeutically effective in preventing hypertensive end-organ damage when used in combination with established anti-hypertensive drugs.

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Highlights

- The AT2-receptor (AT2R) mediates vasodilation
- Systemic application of AT2R-agonists does not lower blood pressure
- Low-dose blockade of AT1-receptors unmasks the BP lowering effects of AT2Rs
- Intracerebroventricular or intrarenal application of AT2R-agonists reduces BP.
- AT2R-stimulation protects from hypertensive end-organ damage without BP lowering

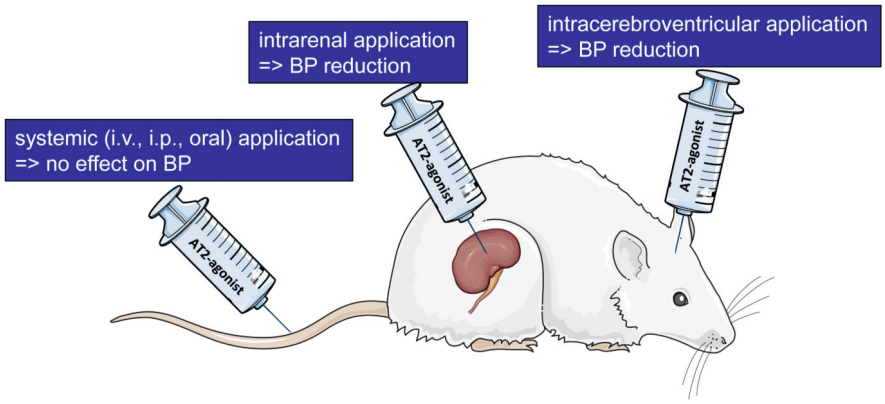

Figure 1. The effect of AT2-receptor stimulation on blood pressure is dependent on the way of application

While systemic application (i.v., i.p. or oral) of AT2-receptor agonists is blood pressure neutral (unless the effect is unmasked by low-dose AT1R blockade), application into the kidney or the brain has a blood pressure lowering effect. The figure was created using Servier Medical Art (http://www.servier.com/Powerpoint-image-bank).

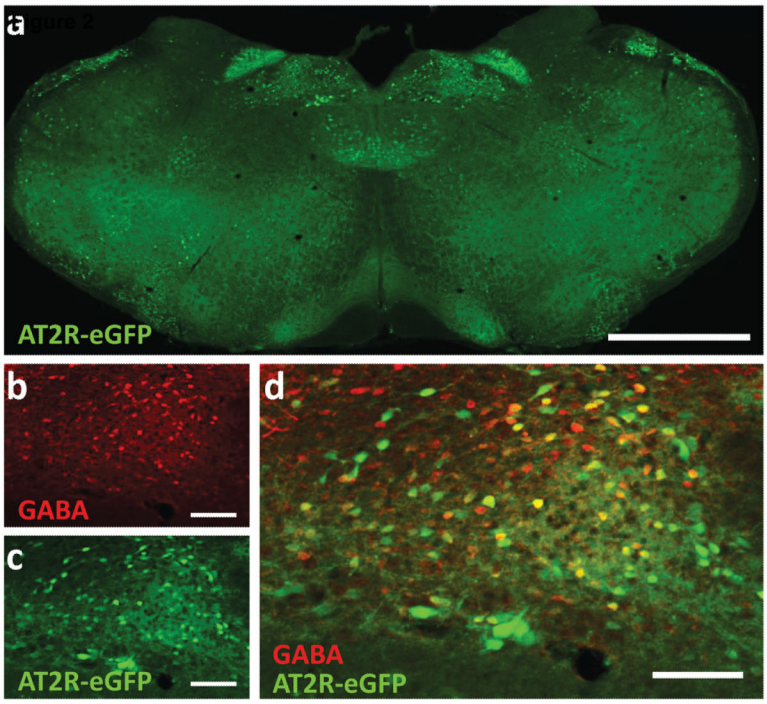

Figure 2. Co-localization of AT2R-eGFP and GABA immunoreactivity in NTS of an AT2R-eGFP reporter mouse

Mice were generated and brains processed for immunostaining as described recently [39]. (a) Low magnification (2.5×) image of a coronal section through the NTS of an AT2R-eGFP mouse. High magnification (10×) images through the NTS depicting (b) eGFP immunoreactivity in green, (c) GABA immunoreactivity in red, and (d) the merged image. Scale bars = 1 mm (a) and 100 μm (b - d)