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Commentary

Vasopressor meets vasodepressor: The AT1–B2 receptor heterodimer ☆☆

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ABSTRACT

The AT1 receptor for the vasopressor angiotensin II is one of the most important drug targets for the treatment of cardiovascular diseases. Sensitization of the AT1 receptor system is a common feature contributing to the pathogenesis of many cardiovascular disorders but underlying mechanisms are not fully understood. More than a decade ago, evidence was provided for control of AT1R activation by heterodimerization with the B2 receptor for the vasodepressor peptide, bradykinin, a physiological counterpart of the vasoconstrictor angiotensin II. AT1–B2 receptor heterodimerization was shown to enhance AT1R-stimulated signaling under pathophysiological conditions such as experimental and human pregnancy hypertension. Notably, AT1R signal sensitization of patients with preeclampsia hypertension was attributed to AT1R–B2R heterodimerization. Vice versa, transgenic mice lacking the AT1–B2 receptor heterodimer due to targeted deletion of the B2R gene showed a significantly reduced AT1R-stimulated vasopressor response compared to transgenic mice with abundant AT1R–B2R heterodimerization. Biophysical methods such as BRET and FRET confirmed those data by demonstrating efficient AT1–B2 receptor heterodimerization in transfected cells and transgenic mice. Recently, a study on AT1R-specific biased agonism directed the focus to the AT1–B2 receptor heterodimer again. The β-arrestin-biased [Sar1,Ile4,Ile8]-angiotensin II promoted not only the recruitment of β-arrestin to the AT1R but also stimulated the down-regulation of the AT1R-associated B2 receptor by co-internalization. Thereby specific targeting of the AT1R–B2R heterodimer became feasible and could open the way to a new class of drugs, which specifically interfere with pathological angiotensin II-AT1 receptor system activation.

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1. Introduction

The angiotensin II AT1R system is a key regulator of cardiovascular functions and one of the most important cardiovascular drug targets [1,2]. Angiotensin II is released by angiotensin-converting enzyme (ACE)-dependent cleavage of angiotensin I, which is generated from the precursor angiotensinogen by enzymatic activity of renin [3,4]. Angiotensin II binds to two different receptors, AT1R and AT2R [5]. Numerous studies documented that the AT1 receptor is the responsible receptor involved in cardiovascular pathologies of the vasculature, heart, kidney and brain [6–9] whereas the pathophysiological role of AT2R is still under investigation, and drugs targeting AT2R are not approved for clinical use to date [10,11].

Since its discovery, the bradykinin B2 receptor system emerged as the physiological antagonist of the angiotensin II AT1R system by acting as potent vasodepressor [12–14]. Due to its blood pressure-lowering capacity, activation of the bradykinin B2 receptor system has long been considered as potential approach for prevention and treatment of cardiovascular diseases [15]. But B2R-selective agonists with sufficient in vivo stability are not yet available and potential side effects are a concern [16].

Nevertheless, the B2 receptor is an existing cardiovascular drug target that is activated by the class of ACE-inhibiting drugs. Notably, beneficial bradykinin B2 receptor activation in vivo is achieved by application of an ACE inhibitor because the angiotensin-converting

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enzyme not only cleaves angiotensin I to release angiotensin II, but also executes degradation and inactivation of bradykinin peptides [17]. Consequently, inhibition of ACE prevents not only the generation of the vasopressor angiotensin II but also increases the availability of the vasodepressor peptide bradykinin [17]. Patient studies with a B2R-selective antagonist confirmed that bradykinin B2 receptor activation contributes indeed to the blood pressure-lowering activity of an ACE inhibitor [18].

In light of those discoveries, numerous studies with ACE inhibitors and AT1 receptor antagonists documented physiological antagonism between the vasopressor AT1 receptor and the bradykinin-activated vasodepressor B2 receptor system [19]. The more surprising was the finding that the vasodepressor B2 receptor protein enhanced signaling of the vasopressor angiotensin II AT1 receptor by formation of a heterodimeric receptor protein complex [20,21]. Signal enhancement of the AT1 receptor relied solely on the B2R protein and did not require the agonist bradykinin [20,21]. The concept that the biological role of a receptor protein was different from the agonist-stimulated receptor was provocative and is still under discussion [22,23]. However, recent studies supported the existence of AT1R–B2R heterodimerization by biophysical techniques such as fluorescence resonance energy transfer, FRET [24], and bioluminescence resonance energy transfer, BRET [25]. Moreover, specific targeting of the functional interaction between those two receptor proteins by a small molecule compound was approached [25]. The AT1–B2 receptor heterodimer could thus constitute a novel "druggable" target within the renin–angiotensin–aldosterone system, RAAS.

2. The vasopressor angiotensin II AT1 receptor system

The angiotensin II AT1 receptor system was discovered more than 100 years ago as potent vasopressor-generating cascade [3,4]. Angiotensin II is released from the precursor angiotensinogen after sequential cleavage with renin and the angiotensin-converting enzyme, ACE [3]. By stimulation of the AT1 receptor and subsequent aldosterone release, angiotensin II controls vascular tone, salt and water homeostasis, cardiomyocyte growth, and smooth muscle proliferation [6–9]. The available spectrum of drugs targeting that system encompasses the class of ACE inhibitors and AT1-specific receptor antagonists as largest drug families. Renin–angiotensin–aldosterone system-targeting drugs also include mineralocorticoid receptor antagonists, which block aldosterone-mediated effects of RAAS. Finally, the class of renin inhibitors is the most recent addition of approved drugs modifying biological functions of the angiotensin II system [26]. Indications of angiotensin II AT1R-targeting drugs include essential hypertension, congestive heart failure, ischemic heart disease, atherosclerosis, diabetic nephropathy and cerebrovascular stroke. Cardioprotection, atheroprotection and renoprotection are key features of all drugs, which were documented by a wealth of clinical and experimental studies [1,2]. The pharmacological profile of angiotensin II-AT1R blockade was extended by recent observations regarding neuroprotective activities of centrally active ACE inhibitors and AT1R antagonists, which could be beneficial in retarding neurodegeneration, memory loss and other symptoms associated with dementing diseases such as Alzheimer's disease [27,28].

A panoply of experimental and clinical studies elucidated pathomechanisms triggered by stimulation of the angiotensin II AT1 receptor, which could account for beneficial actions of angiotensin II AT1R-inhibiting drugs. Activation of the AT1 receptor of vascular smooth muscle cells exerts vasoconstriction, mainly by activation of a classical Gq/11-coupled signal transduction pathway. In concert with the generation of ROS, AT1R stimulates cell growth and proliferation, which leads to vascular hypertrophy and smooth muscle phenotype transformation from synthetic to proliferative [29,30]. In the heart, signal transduction pathways stimulated by AT1R promote cardiac hypertrophy, cardiac fibrosis and lipid overload, culminating finally in cardiomyocyte death [31,32]. In addition to vasculature-driven pressure overload, those pathomechanisms are major contributors to the pathogenesis of heart failure [33]. In the kidney, AT1R controls water and sodium retention, directly by stimulating water and sodium reabsorption in the proximal tubule and indirectly by stimulating the generation of aldosterone [2,34]. The latter hormone further acts on mineralocorticoid receptors of the cardiovascular system and thereby exerts a key role in the development of heart failure [35]. In addition to peripheral actions, stimulation of the AT1 receptor in the central nervous system has an important role in blood pressure control by promoting the release of sympathetic neurotransmitters such as adrenaline and noradrenaline, or the stress response-related corticosteroids [36,37]. By an action on bone marrow progenitor cells, angiotensin II AT1R activation stimulates lympho- and erythropoiesis [38]. Those effects of the angiotensin AT1R system on bone marrow-derived and circulating blood cells are considered to contribute to pro-inflammatory responses and the pathogenesis of atherosclerosis [39,40].

3. The bradykinin B2 receptor system – a physiological antagonist of the angiotensin II AT1 receptor system

Bradykinin and the related peptide kallidin (Lys^0-bradykinin) were discovered as potent vasodepressor peptides, which are released by proteolytic cleavage of the precursor high-molecular-weight (HMW) and low-molecular-weight (LMW) kininogen by the action of kallikrein [12,13,41]. Bradykinin exerts vasorelaxation and related physiological functions by stimulating the constitutively expressed B2 receptor [14,42,43]. The vasodepressor activity is due to the generation of nitric oxide (NO) by endothelial B2 receptors activating a Gq/11-coupled signal transduction cascade, and by the release of eicosanoids and EDHF [44]. With those vasodepressor actions, the bradykinin B2 receptor system is a physiological antagonist of the angiotensin II AT1R system [16].

At the level of ACE, the angiotensin II and bradykinin systems are interconnected (Fig. 1). ACE not only generates the vasopressor angiotensin II but also accounts for the proteolytic degradation of B2 receptor-stimulating vasodepressor peptides, bradykinin and

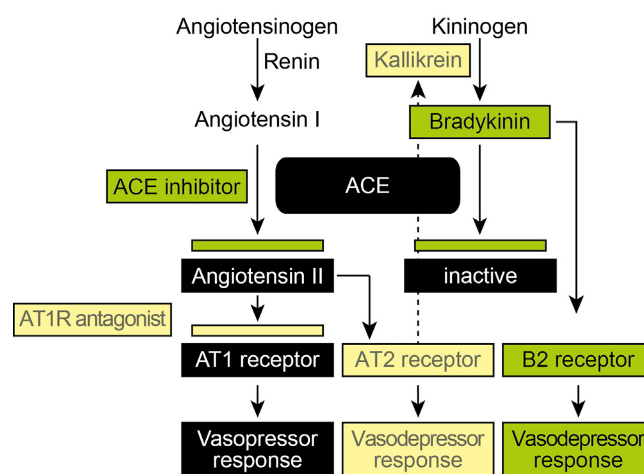

Fig. 1. The vasoactive peptide systems for angiotensin II and bradykinin are interconnected. ACE inhibition blocks the generation of the vasopressor peptide angiotensin II and reduces the degradation of the vasodepressor bradykinin (green). Upon AT1R antagonism, bradykinin level could also increase, most likely by AT2 receptor-stimulated kallikrein activation (light yellow).

kallidin (Fig. 1). Thus, ACE promotes a vasopressor response by reciprocally controlling the physiological availability of the antagonistic peptides angiotensin II and bradykinin [16]. Vice versa, inhibition of ACE by an ACE inhibitor blocks the generation of angiotensin II and enhances the availability of the vasodepressor, bradykinin (Fig. 1). Studies with the B2R-specific antagonist HOE140 (Icatibant) demonstrated that such an effect is relevant in various models of cardiovascular diseases and patients where B2 receptor stimulation contributes to the blood pressure lowering activity of ACE inhibitors and the beneficial effect of ischemic preconditioning [17–19].

In comparison to ACE inhibition, the profile of AT1 receptor antagonists is different and bradykinin exerts only a minor role [45]. In agreement with that notion, AT1R antagonists have a documented lower frequency of potential and proven bradykinin-mediated side effects such as cough and angioedema [46]. Nevertheless, circulating bradykinin levels were also shown to increase upon AT1R blockade [47]. That effect could be mediated by angiotensin II AT2 receptor-mediated bradykinin generation [48–50]. As a result, the bradykinin B2 receptor could support the AT2R-dependent NO generation and vasodilation upon AT1R inhibition, at least in experimental models [51–53]. Taken together, the bradykinin B2 receptor acts as the physiological counterpart of the AT1 receptor either directly or in concert with the AT2 receptor (Fig. 1).

4. Vasopressor meets vasodepressor: the AT1–B2 receptor heterodimer

4.1. Discovery of AT1–B2 receptor heterodimerization by biochemical and biophysical methods

In view of the physiological interconnections between bradykinin B2 receptor-mediated and angiotensin II AT1 receptor-mediated effects, we sought to determine whether there was a direct interaction between the angiotensin and bradykinin systems at the level of the receptor proteins. Those investigations led to the discovery of a physical interaction between the AT1 and B2 receptor proteins by formation of a high molecular mass protein complex [20]. The AT1 and B2 receptor complex was not only detected with AT1–B2 receptor-transfected HEK cells but also with endogenously expressed AT1 and B2 receptors of aortic smooth muscle cells and omental vessels by co-immunoenrichment studies [20,21]. According to the molecular mass of the protein complex stabilized by a cleavable cross-linker, the complex was identified as AT1–B2 receptor heterodimer [20,21].

In 2011, AT1–B2 receptor hetero(di)merization was confirmed by a biophysical method, using fluorescence resonance energy transfer (FRET), which detects protein–protein interactions in close proximity, at a distance of less than 10 nm [24]. The FRET study applied fusion proteins of the AT1 and B2 receptor, respectively, with variants of the enhanced green fluorescent protein fused to the receptor's C-terminus, i.e. AT1R-Cerulean and B2R-eYFP [24]. The low cell surface delivery of the AT1 receptor-Cerulean fusion protein was specifically enhanced by a cleavable signal peptide attached to the AT1 receptor's N-terminus [24]. A FRET efficiency of more than 24% indicated that the AT1 receptor and B2 receptor were in close proximity and formed a complex on the cell membrane of intact HEK cells [24].

A recent study confirmed those FRET studies and demonstrated the physical AT1–B2 receptor interaction in intact cells by the related bioluminescence resonance energy transfer ($BRET_{50}$) method using an AT1 receptor tagged at the C-terminus with Venus fluorescent protein (AT1R-Venus) and a B2 receptor C-terminally tagged with Renilla luciferase (B2-RLuc) [25].

4.2. Difficulties in the detection of AT1–B2 receptor heterodimerization

Although the initial discovery of AT1–B2 receptor heterodimer formation seemed straightforward [20,21], several groups failed to detect AT1–B2 receptor heterodimerization in subsequent years [22,23]. Reported difficulties in the detection of AT1–B2 receptor heterodimerization in transfected cells under conventional cell culture conditions [22,23] could be due to the lack of essential chaperones required for receptor–heterodimer folding [54–56]. In agreement with that notion, an in vivo cell expansion model was capable to restore the expression of proteins required for heterodimer folding, which were susceptible to down-regulation during in vitro cell cultivation [55]. Apart from AT1R–B2R heterodimer folding [55], that in vivo cell expansion model has a documented efficacy to restore other essential proteins of GPCR signaling pathways as well, which were down-regulated during in vitro cell culture [57].

In addition to the limited availability of heterodimer-specific chaperones, calcium overload-induced apoptosis triggered by heterodimer-mediated over-stimulation of the Gq/11-signaling cascade (cf. Section 4.3) could selectively eliminate cells with abundant AT1–B2 receptor heterodimerization. Likewise, cells with AT1R–B2R heterodimers are highly susceptible to apoptosis and/or cell death triggered by angiotensin II/bradykinin derived, e.g. from culture medium supplemented with serum additive. An insufficient number of cells with co-expression of AT1R and B2R results, and could be responsible for the failure to detect AT1R–B2R heterodimerization and also AT1R–B2R heterodimer-mediated signaling [22,23]. A definite conclusion why several groups failed to detect AT1–B2 receptor heterodimerization, is difficult to draw because of the lack of precise biochemical and/or histological control experiments such as co-immunoenrichment studies and/or (immuno-)fluorescent microscopy of receptor proteins [22,23]. Specifically, those groups determined the number of receptor binding sites and inositol phosphate levels with a batch of cells but did not control for co-expression of AT1R and B2R at the single cell level [22,23].

Apart from insufficient receptor co-expression, the transiently transfected AT1 receptor was reported to form constitutive AT1 receptor aggregates under the applied experimental conditions [22]. Extensive AT1R protein aggregation could also reduce/prevent the interaction of AT1R with B2R since the balance between receptor homomerization and heteromerization could be shifted toward homomers [56]. As an example, the COS cell transfection system could favor the formation of such homomeric receptor aggregates, because of a high cellular expression level due to SV40 large T-antigen-driven DNA replication at high copy number. Again, control experiments are lacking, such as immunoblot detection of receptor proteins to visualize the extent of receptor protein aggregation and/or immature receptor protein folding [22,23].

While a definite answer to the key question why several groups failed to detect the AT1R–B2R heterodimer cannot be provided due to lack of essential control experiments, detection of AT1R–B2R heterodimerization in transfected cells by biochemical and biophysical methods is feasible, as was demonstrated by different groups and in different laboratories independently [20,21,24,25].

4.3. The AT1–B2 receptor heterodimer shows enhanced receptor G-protein activation and altered receptor sequestration

In addition to the sole interaction of two related GPCRs, experiments with transfected cells also revealed functional differences between the homomeric AT1 and B2 receptor, respectively, and the heterodimeric AT1–B2 receptor complex. Notably, the

formation of the AT1R–B2R complex was accompanied by a substantial increase in angiotensin II AT1R-stimulated G-protein activation of Gq/11 and Gi/o proteins. Enhancement of AT1R-stimulated G-protein activation was independent of bradykinin B2 receptor stimulation because a B2 receptor mutant with a defective bradykinin-binding site (B2R-F297A) also mediated an enhanced angiotensin II AT1R response [20]. In contrast, a B2 receptor mutant with intact bradykinin-binding site but with impaired G-protein activation due to mutation of the conserved DRY-motif in the connecting loop of membrane domains III–IV (B2R-Y157A) did not enhance the AT1R response [20]. Those findings suggested that a functional receptor–G-protein interface of the B2 receptor was required for AT1R signal enhancement by the B2R protein. Such a characteristic of the AT1R–B2R heterodimer is analogous to the GABA-B receptor heterodimer, which requires GABA-B1/B2 heterodimerization for efficient G-protein coupling [58]. However, in contrast to the GABA-B heterodimer, AT1R–B2R heterodimerization is not a prerequisite for plasma membrane translocation, and individual AT1 and B2 receptor homomers are also functional.

In addition to the receptor-G-protein interaction, AT1R–B2R heterodimerization also changed the mode of receptor sequestration. The sequestration of individual AT1 or B2 receptors, respectively, was largely dynamin-/clathrin-independent whereas a dominant-negative dynamin K44A mutant suppressed the angiotensin II- and/or bradykinin-induced receptor sequestration of the AT1–B2 receptor heterodimer [20]. This observation indicated that the intracellular interface of the AT1–B2 receptor heterodimer was different from that of individual AT1 and B2 receptor homomers. In agreement with altered receptor sequestration, angiotensin II stimulation of the AT1–B2 receptor heterodimer promoted the co-internalization of the B2 receptor [24]. Vice versa, bradykinin stimulation of the AT1–B2 receptor heterodimer promoted AT1 receptor co-internalization [24]. These findings further confirmed that AT1 and B2 receptors formed a stable complex on intact cells, which could be activated by stimulation with angiotensin II or bradykinin. Taken together, receptor heterodimerization generates a novel receptor entity, which has clearly distinguished features from the individual AT1 and B2 receptor mono-/homomers regarding G-protein interaction/activation and receptor internalization.

5. Pathophysiological relevance of AT1–B2 receptor heterodimerization

5.1. The AT1–B2 receptor heterodimer contributes to angiotensin II hypersensitivity in preeclampsia hypertension

The finding that heterodimerization enhanced the activation of the AT1 receptor by angiotensin II, was of major interest in view of several hypertensive cardiovascular disorders with increased angiotensin II responsiveness. Notably, preeclampsia hypertension is characterized by an increased pressor response to angiotensin II [59–61]. Since preeclampsia/eclampsia is the most common complication of late pregnancy and still a leading cause of pregnancy-induced death worldwide, we investigated whether AT1–B2 receptor heterodimerization was altered in pregnant women complicated with preeclampsia. We could show that patients with preeclampsia hypertension had a significantly increased level of AT1–B2 heterodimeric receptors on circulating blood cells and omental vessels [21]. The finding that a site-directed antibody to the connecting loop between membrane domains III–IV of the B2 receptor was capable to reduce the angiotensin II AT1R-stimulated G-protein activation on omental vessels isolated from preeclamptic women, provided strong evidence for a causal involvement of AT1–B2 receptor heterodimerization in the increased angiotensin II responsiveness and pressor response of preeclampsia [21,62]. These observations were the first report of altered GPCR dimerization in human disease and the potential involvement of AT1R–B2R heterodimerization in an important cardiovascular complication. Nevertheless, the question is still not solved whether the AT1R–B2R heterodimer could be used as a basis to develop a preeclampsia-specific treatment approach without causing severe developmental defects of the fetus such as oligohydramnios and renal failure as do other RAAS inhibitors.

5.2. Increased AT1–B2 receptor heterodimerization in experimental hypertension

An increased AT1R-stimulated response to the vasopressor angiotensin II is also a characteristic feature of experimental models and patients with hypertensive cardiovascular disease [63–67]. To investigate AT1–B2 receptor heterodimerization in experimental hypertension, we chose the spontaneously hypertensive rat model. We found an increased level of the AT1–B2 receptor complex on mesangial cells isolated from spontaneously hypertensive rats compared to normotensive rats [68]. The AT1–B2 receptor heteromeric complex contributed to an enhanced angiotensin II-stimulated G-protein activation and increased endothelin-1 secretion of mesangial cells as demonstrated by the sensitivity of the angiotensin II-stimulated responses to inhibition by site-directed antibodies against the B2 receptor intracellular interface [68]. Taken together, increased AT1–B2 receptor heterodimerization is also a characteristic feature of experimental hypertension and contributes to an enhanced angiotensin II responsiveness of renal mesangial cells. Since mesangial cells contribute to the development of hypertension-induced glomerulosclerosis, those data could suggest the potential involvement of the AT1–B2 receptor heterodimer in the development of hypertensive nephropathy.

6. Decreased AT1R-stimulated vasopressor response in mice lacking the AT1–B2 receptor heterodimer due to targeted deletion of the B2 receptor gene

To analyze the impact of the B2 receptor on the angiotensin II AT1R-stimulated vasopressor response in vivo, B2 receptor-deficient mice with targeted deletion of the *Bdkrb2* gene were applied [43]. To obtain a control strain with identical genetic background as *Bdkrb2*$^{-/-}$ mice, we restored the constitutive expression of the ubiquitously expressed B2 receptor in *Bdkrb2*$^{-/-}$ mice by transgenic B2R expression under control of the CMV immediate-early promoter/enhancer, which directs ubiquitous expression of a transgene [69]. Transgenic Tg-B2R^{+} mice were generated by pronuclear injection of the *CMV-B2R-eYFP* transgene into *Bdkrb2*$^{-/-}$ embryos as described [57,69]. Radioligand binding and immunoblot detection demonstrated transgenic B2R protein expression in the aorta of Tg-B2R^{+} mice whereas B2R binding sites, the B2 receptor protein, and thus consequently the AT1–B2 receptor heterodimer were absent in the Tg-B2$^{-/-}$ strain (Fig. 2A and B; [69]).

To determine the impact of the B2 receptor on the angiotensin II AT1R-stimulated vasopressor response, we performed invasive hemodynamic measurements of transgenic mice with and without B2 receptor expression. Compared to Tg-B2R^{+} mice with restored B2 receptor protein and identical genetic background, the aortic pressor response to angiotensin II stimulation of B2 receptor-deficient (Tg-B2$^{-/-}$) mice was significantly reduced (Fig. 2C; [69]). Significant differences were observed at angiotensin II doses of 100 ng and 1 μg/kg body weight (Fig. 2C). As a control, the angiotensin II-stimulated increase in mean arterial pressure (MAP) was mediated by the AT1 receptor because pre-treatment with the AT1R-specific antagonist, losartan, completely blunted the angiotensin II response

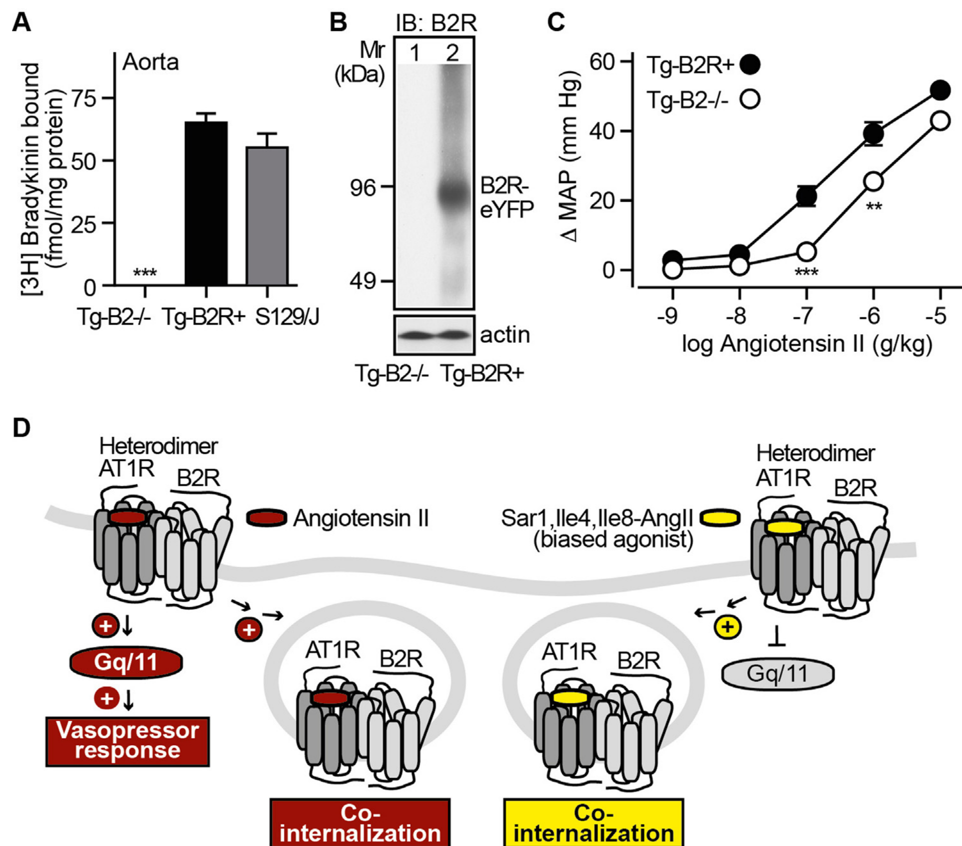

Fig. 2. Decreased AT1R-stimulated vasopressor response in mice lacking the AT1–B2 receptor heterodimer due to targeted deletion of the B2 receptor gene (adapted from Ref. [69]). (A) Transgenic mice with *B2R-eYFP* expression under control of the ubiquitous CMV promoter/enhancer (Tg-B2R$^+$) were generated by nuclear injection of the transgene into embryos from *Bdkrb2*$^{-/-}$ mice. Radioligand binding showed comparable number of aortic [^{3}H]bradykinin binding sites of Tg-B2R$^+$ mice and S129/J controls whereas B2R binding sites were absent in Tg-B2$^{-/-}$ mice. (B) Immunoblot detection with B2R-specific antibodies confirmed the absence of the B2 receptor protein on aortic membranes of Tg-B2$^{-/-}$ mice. (C) Angiotensin II (10 μl bolus injection into the jugular vein) caused a smaller increase in mean arterial pressure (MAP) in Tg-B2$^{-/-}$ mice than in Tg-B2R$^+$ mice. Data are mean ± s.e.m., n = 4 male mice/group, $^{**}P < 0.01$, $^{***}P < 0.001$ (ANOVA and Tukey's multiple comparison test). (D) Schematic illustration of AT1R–B2R heterodimer activation by angiotensin II or a biased agonist (Sar1,Ile4,Ile8-AngII). The biased agonist promotes receptor co-internalization but inhibits G-protein activation.

[69]. For comparison, the aortic AT1 receptor number was not different between Tg-B2$^{-/-}$ and Tg-B2R$^+$ mice (91.7 ± 4.1 fmol/mg and 89.3 ± 3.4 fmol/mg of Tg-B2$^{-/-}$ and Tg-B2$^+$ mice, respectively; n = 5). Thus, B2 receptor deficiency and consecutive lack of the AT1–B2 receptor heterodimer were accompanied by a reduced angiotensin II-stimulated pressor response [69].

These results with Tg-B2$^{-/-}$ and Tg-B2R$^+$ mice are complementary to a previous study, which detected a significantly decreased pressor response of *Bdkrb2*$^{-/-}$ mice after an angiotensin II dose of 5 ng/mouse [70]. In contrast to our study, that study used SVE S129/J mice as control strain, and analyzed the angiotensin II pressor response of the femoral artery [70].

The observation that *Bdkrb2*$^{-/-}$ mice had a reduced angiotensin II-stimulated pressor response, was not anticipated [70]. In contrast, studies with *Bdkrb2*$^{-/-}$ mice largely focussed on the loss of the B2R-stimulated vasodepressor effect [70,71]. Nevertheless, our transgenic mouse models with and without B2R expression in the same genetic background, confirmed previous findings, i.e. that individuals with deficiency of the vasodepressor B2 receptor and thus consequently the AT1–B2 receptor heterodimer, showed a decreased AT1R-mediated vasopressor response compared to Tg-B2R$^+$ mice with efficient heterodimerization [69,70].

Taken together, data with transgenic mice are compatible with the concept that upon AT1R–B2R heterodimerization, the B2 receptor enhances angiotensin II AT1R-stimulated G-protein signaling and thereby supports the AT1R-mediated vasopressor response (Fig. 2D). That vasopressor-enhancing function of the B2R–AT1R heteromer complements the well-established vasodepressor effects of the bradykinin-stimulated B2R homomer.

7. The AT1–B2 receptor heterodimer as "novel" drug target

7.1. Disruption of the AT1–B2 receptor G-protein interface

Considering the potential pathophysiological relevance, pharmacological targeting of the AT1R–B2R heterodimer by specifically designed ligands is of major interest. Initial experimental approaches applied site-directed antibodies shielding the intracellular receptor G-protein interface of the B2 receptor located within the connecting loop between membrane domains III–IV of the B2 receptor [21,68]. Those antibodies were capable to reduce the angiotensin II-stimulated G-protein activation of Gαq/11 on omental vessels of preeclamptic patients [21]. While that approach provided evidence for the contribution of the AT1–B2 receptor heterodimer to the angiotensin II hypersensitivity of preeclamptic vessels [21], the method is not feasible for whole cell or in vivo studies. Moreover, targeting of the intracellular interface of the B2 receptor is not specific for the AT1R–B2R heterodimer and could also interfere with the bradykinin-stimulated G-protein activation of B2 receptor mono-/homomers. Future research would have to focus on the identification of a heterodimer-specific contact site to the G-protein that could be used for the development of a small molecule inhibitor, which specifically disrupts the interaction of AT1R–B2R with the G-protein.

7.2. A biased angiotensin II AT1 receptor agonist targets the AT1–B2 receptor heterodimer

Recently, another approach to target the AT1–B2 receptor heterodimer was established. Wilson et al. [25] used an AT1R ligand, the [Sar1,Ile4,Ile8]-AngII, with biased agonist activity, which antagonizes the AT1R-stimulated G-protein activation but promotes AT1R-mediated β-arrestin recruitment, and subsequent AT1 receptor internalization and β-arrestin-dependent signaling [72]. The β-arrestin pathway-biased [Sar1,Ile4,Ile8]-AngII was applied to the AT1–B2 receptor heterodimer of vascular smooth muscle cells and transfected HEK cells. Stimulation of the AT1–B2 receptor heterodimer with [Sar1,Ile4,Ile8]-AngII promoted not only AT1 receptor internalization but also B2 receptor co-internalization [25]. As a consequence, B2 receptor-stimulated signaling of cells with AT1–B2 receptor heterodimers was significantly reduced whereas [Sar1,Ile4,Ile8]-AngII had no effect on B2 receptor signaling in the absence of AT1R [25]. Those findings are complementary to previous studies, which showed co-internalization of the AT–B2 receptor heterodimer upon stimulation with angiotensin II or bradykinin [24], and a bradykinin-stimulated recruitment of β-arrestin to the B2 receptor [57]. Thus, an AT1R-specific agonist with biased activity toward β-arrestin recruitment/signaling concomitantly targets the B2 receptor of the AT1R–B2R heterodimer (Fig. 2D). Future studies will have to investigate whether biased agonism for the AT1R–B2R heterodimer could constitute the prototype for a new class of RAAS-targeting drugs.

8. Outlook

Studies with the AT1R biased agonist, [Sar1,Ile4,Ile8]-AngII, which stimulates β-arrestin recruitment to the AT1R–B2R heterodimer and subsequent down-regulation of AT1R–B2R, demonstrated that pharmacological targeting of the AT1–B2 receptor heterodimer is feasible [25]. Since another member of that class of biased AT1R agonists is currently in phase II clinical trial for the treatment of acute heart failure [73], the in vivo role of the AT1–B2 receptor heterodimer has become a matter of considerable interest.

Evidence from in vitro and in vivo studies demonstrated sensitization of the AT1R response by AT1–B2 receptor heterodimerization. That mechanism could contribute to angiotensin II hypersensitivity under pathophysiological conditions such as preeclampsia hypertension and other hypertensive conditions. Transgenic models with deficient AT1R–B2R heterodimerization due to targeted deletion of the B2R gene compared to transgenic mice with efficient AT1R–B2R heterodimerization further supported the concept that the B2 receptor enhances the vascular AT1R response by heterodimerization.

While those in vitro and in vivo data established a role of the AT1–B2 receptor heterodimer in sensitizing the AT1R-mediated vasopressor activity of the vasculature, more investigations are required to determine the impact of the AT1–B2 receptor heterodimer on the AT1R response of other relevant organs, e.g. heart, kidney and brain. Because the B2 receptor is ubiquitously expressed, additional pathophysiological functions of the AT1–B2 receptor heterodimer can be anticipated, and are suggested by previous data [68,74]. Notably, conditions of excessive angiotensin II AT1 receptor stimulation (e.g. hypertension, heart failure, atherosclerosis) could specifically promote AT1–B2 receptor heterodimerization because the B2 receptor is triggered by AT1 receptor-stimulated signaling [75]. Thus, under conditions of angiotensin II overstimulation, a vicious cycle is suggested consisting of (i) AT1R-induced B2R up-regulation, (ii) AT1R–B2R heterodimerization, and (iii) enhanced AT1R-stimulated signaling, which in turn triggers a new round of B2R up-regulation with subsequent AT1R–B2R heterodimerization and signal sensitization. With the class of biased AT1R agonists as newly established pharmacological tools, the translation of experimental research on AT1–B2 receptor heterodimerization to the clinic could be on the way ahead.

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