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Moderate additive effects of endothelin receptor A blockade in Ren-2 transgenic rats subjected to various types of RAS blockade

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ABSTRACT

Aims: Chronic endothelin receptor A (ET_A) blockade lowered blood pressure (BP) by decreasing angiotensin-dependent vasoconstriction and attenuating calcium influx. We tested whether the addition of ET_A blockade to renin–angiotensin system (RAS) blockade would have further effects on the principal vasoactive systems contributing to BP maintenance in Ren-2 transgenic rats (TGR).
Methods: Four-week-old TGR rats were fed with normal-salt diet and given either different renin–angiotensin system (RAS) blockers [angiotensin receptor blocker losartan, angiotensin converting enzyme inhibitor captopril, direct renin inhibitor aliskiren], or ET_A blocker (atrasentan) alone, or a combination of atrasentan with RAS blockers for 4 weeks. At the end of the study, basal BP and acute BP responses to sequential blockade of renin–angiotensin (RAS), sympathetic nervous (SNS), and nitric oxide (NO) systems were determined in conscious rats. Thereafter, BP responses to acute inhibition of nifedipine-sensitive calcium influx through voltage-dependent calcium channels (L-VDCC) were measured.
Key findings: All RAS blockers similarly decreased BP to normotension, their effects being mediated through substantially attenuated RAS-dependent and moderately decreased SNS-dependent vasoconstriction. Atrasentan alone partially lowered BP, while BP was normalized by combination of atrasentan with either RAS blocker. In combination therapies, BP lowering effects resulted from the attenuation of both RAS- and SNS-dependent vasoconstriction. Moreover, atrasentan-treated groups had substantially reduced NO-dependent vasodilation and significantly decreased calcium influx through L-VDCC.
Conclusions: Although the BP-lowering effect of combined ET_A and RAS blockades in TGR is predominantly dependent on the effects exerted by RAS blockade, further effects are attributable to decreased calcium influx due to chronic ET_A blockade.

1. Introduction

Hypertension is one of the major risk factors for cardiovascular diseases. The hypertension epidemic due to aging has substantial health and economic consequences. Although there are many effective therapies, including blockers of renin–angiotensin system (RAS), diuretics, calcium channel blockers and beta blockers, there still are controversies about their usage in different forms of hypertension. Moreover, there are also debates about the preferential usage of different classes of RAS blockers, their mutual combinations and also about their combination with other classes of antihypertensives in both human [1–3] and animal studies [4]. In a model of angiotensin II-dependent hypertension-Ren-2 transgenic rats, ambiguous effects on BP and proteinuria were demonstrated: either similar effects of AT_1 receptor blockade and direct renin inhibition [5,6], or better results of combined therapy in a model of cardiac failure [7], or even beneficial effects of aliskiren [8]. However, the mechanisms underlying the contribution of major vasoactive systems to blood pressure lowering effects of these drugs have not been explored.

Many clinical studies convincingly demonstrated the beneficial effects of the RAS inhibition using angiotensin converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARB) [9]. Moreover, there are considerable data showing antihypertensive and additional nephroprotective effects of RAS blockade also in rat. However, there is now a growing body of evidence against the dual (combined) therapy consisting either of angiotensin converting enzyme inhibitor (ACEi) and angiotensin receptor blocker (ARB) [10] or a combination of ARB or ACEI with direct renin inhibitor aliskiren [11]. A better understanding of the mechanisms contributing to BP lowering and the involvement of particular vasoconstrictor/vasodilator systems in antihypertensive effects of different types of RAS blockers should be therefore valuable for clinical practice. Nevertheless, many experimental studies have also shown late antihypertensive and organoprotective effects of RAS blockade [12–14], although

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our studies in heterozygous TGR did not support such findings [15]. On the other hand, in our previous study we have demonstrated the efficacy of RAS blockade with angiotensin receptor blockade or direct renin inhibitor in both young and adult TGR rats, showing that antihypertensive and antiproteinuric effects of aliskiren persisted for 2 weeks after the treatment withdrawal [8]. In addition, when aliskiren was compared with losartan, at similar reduction of BP and renal angiotensin II and ET-1 levels, only aliskiren decreased proteinuria [16]. Moreover, central infusion of aliskiren was shown to decrease sympathetic hyperactivity in Dahl salt-sensitive rats [17] and oral aliskiren diminished sympathetic activity in patients with chronic kidney disease, suggesting possible beneficial effects through the reduction of sympathetic outflow [18].

Angiotensin II and endothelin-1 are the two most potent vasoconstrictors. There is crosstalk between RAS and ET systems which potentiate each other. While angiotensin II stimulates renal and vascular formation of ET-1 [19], ET-1 increases the production of angiotensin II [20], thus performing a positive feedback loop. Since they both promote inflammation, cell proliferation, hypertrophy, and extracellular matrix accumulation, they have been identified as modifiable molecular mediators [21]. Their combined inhibition was used in a model of renal failure induced by subtotal nephrectomy but conflicting results were reported. While Cao and coworkers demonstrated no beneficial effects of additional ET_A blockade on top of existing RAS blockade [22], our group has reported additional renoprotective effects of combined treatment; however, these effects were seen only when the treatment was very long, i.e., 11 months [23], with no effect after a 5-month treatment [24]. In this study, BP was partially decreased by atrasentan while it was fully normalized during the combined RAS and ET_A treatment. In addition, in other models of renal damage, such as membranous nephropathy [25] or in diabetic rats [26], the benefits of combined RAS and ET_A blockade have been documented. Beneficial effects of combined RAS and ET_A therapy have been demonstrated also in clinical studies, such as DORADO in resistant hypertension [27] or ASCEND in chronic kidney disease [28]. Unfortunately, due to serious adverse effects (retention of fluid), this trial was prematurely terminated [29].

Our previous studies performed with different models of experimental hypertension [genetic — SHR, salt — Dahl salt-sensitive, nitric oxide-deficient — L-NAME treated] [30–32] demonstrated that augmented sympathetic hyperactivity is the major contributor to BP increase in these experimental models. In contrast, models with increased participation of renin–angiotensin system such as pertussis-toxin treated SHR, L-NAME treated SHR or Ren-2 transgenic rats are dependent on both sympathetic vasoconstriction and RAS-dependent (captopril-sensitive) vasoconstriction [33]. Interestingly, in young homozygous TGR rats, RAS-dependent vasoconstriction is dominant, whereas in adult rats sympathetic vasoconstriction prevails [34].

Therefore, we were interested whether i) different contributions of major vasoactive systems participate in BP-lowering effects of various classes of RAS blocking agents, and ii) whether the combination of ET_A blockade would have some additional effects on vasoconstrictor/vasodilator systems in young 4-week-old heterozygous Ren-2 transgenic rats fed a normal-salt diet which were also used in our previous studies [35,36].

2. Materials and methods

All procedures and experimental protocols were approved by the Ethical Committee of the Institute of Physiology, Czech Academy of Sciences, and conform to the European Convention on Animal Protection and Guidelines on Research Animal Use.

Male Hannover Sprague Dawley (HanSD) and Ren-2 transgenic (TGR) rats were housed at 23 °C under a 12 h light/dark cycle, fed a normal-salt diet (0.45% NaCl) and given tap water ad libitum.

2.1. Series 1: the effects of RAS blockade on principal vasoactive systems contributing to BP maintenance

Chronic treatment with aliskiren (direct renin inhibitor; 10 mg/kg/day via osmotic minipumps; Alzet, type 2004), captopril (angiotensin converting enzyme inhibitor; 20 mg/kg/day in the drinking fluid) and losartan (angiotensin receptor blocker; 10 mg/kg/day in the drinking fluid) was started at the age of 4 weeks and lasted 4 weeks. The dosage of all drugs was adjusted weekly. The doses of drugs were based on our previous studies [8,16].

The following groups of experimental animals were studied:

1. Control untreated HanSD rats (n = 8)
2. Control untreated TGR group (n = 8)
3. TGR treated with aliskiren (n = 7)
4. TGR treated with captopril (n = 8)
5. TGR treated with losartan (n = 7)

2.2. Series 2: the effects of ET_A blockade added to RAS blockade on principal vasoactive systems contributing to BP maintenance

RAS blockers were administered at the same concentrations as in Series 1. Atrasentan treatment (5 mg/kg/day in the drinking water) was started simultaneously with the RAS treatment, i.e. beginning at 4 weeks of age and lasting 4 weeks. The dosage was adjusted weekly. The doses of atrasentan were based on our previous studies [35,36].

The following groups of experimental animals were added:

1. HanSD treated with atrasentan (n = 6)
2. TGR treated with atrasentan (n = 8)
3. TGR treated with atrasentan and aliskiren (n = 8)
4. TGR treated with atrasentan and captopril (n = 6)
5. TGR treated with atrasentan and losartan (n = 6)

2.3. Blood pressure measurement

Each week, the BP was measured in conscious animals using a tail plethysmography system (Hatteras, North Carolina, USA). The animals became accustomed to the cages in several sessions prior to the start of the experiment. In order to exclude the influence of circadian BP variations, the measurements were always done between 8 AM and 12 noon.

2.4. Surgery

One day prior to the experiment, two polyethylene cannulas were implanted into the rats under light isoflurane anesthesia (PE 50 for BP measurement in the left carotid artery, PE 10 for the infusion of drugs to the jugular vein); the cannulas were exteriorized in the interscapular region.

Blood pressure and its changes after acute blockade of particular vasoactive systems were recorded using a pressure transducer and a multichannel recorder (ADInstruments, Bella Vista, Australia) in *conscious* animals 24 h later.

2.5. Vasoactive balance

The subsequent steps of the blockade of distinct vasoactive systems were performed in *conscious* animals according to the modified protocol of Minami et al. [37], which is regularly used in our laboratory [34,38]. Briefly, after a 30-minute recovery period (which is sufficient for calming the animals after placing them in plastic measuring cages), baseline levels of systolic, diastolic, and mean arterial pressures were recorded for 15 min. Then, a sequential blockade of the renin–angiotensin system (RAS — 10 mg/kg BW captopril), sympathetic nervous system (SNS — 5 mg/kg BW pentolinium), and nitric oxide synthase (30 mg/kg BW L-NAME) was performed (Fig. 1). Finally, calcium influx through L-VDCC was blocked by the calcium channel blocker, nifedipine

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