Neuropeptides xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Neuropeptides

journal homepage: www.elsevier.com/locate/npep

Overexpression of AT2R in the solitary-vagal complex improves baroreflex in the spontaneously hypertensive rat

Prashant J. Ruchaya [a], Guilherme F. Speretta [a], Graziela Torres Blanch [a], Hongwei Li [b], Colin Sumners [c], José V. Menani [a], Eduardo Colombari [a,*,1], Débora S.A. Colombari [a,*,1]

[a] Department of Physiology and Pathology, School of Dentistry, São Paulo State University, Araraquara, SP, Brazil
[b] School of Biotechnology, Southern Medical University, Guangzhou, China
[c] Department of Physiology and Functional Genomics and McKnight Brain Institute, College of Medicine, University of Florida, Gainesville, FL, USA

ARTICLE INFO

Article history:
Received 11 March 2016
Received in revised form 20 May 2016
Accepted 5 June 2016
Available online xxxx

Keywords:
Hypertension
ACE2
Baroreflex
Angiotensin II
Angiotensin receptor
NTS

ABSTRACT

The aim of this study was to investigate the physiological effects of increased angiotensin II type 2 receptor (AT2R) expression in the solitary-vagal complex (nucleus of the solitary tract/dorsal motor nucleus of the vagus; NTS/DVM) on baroreflex function in non-anaesthetised normotensive (NT) and spontaneously hypertensive rats (SHR). Ten week old NT Holtzman and SHR were microinjected with either an adeno-associated virus expressing AT2R (AAV2-CBA-AT2R) or enhanced green fluorescent protein (control; AAV2-CBA-eGFP) into the NTS/DVM. Baroreflex and telemetry recordings were performed on four experimental groups: 1) NTeGFP, 2) NTAT2R, 3) SHReGFP and 4) SHRAT2R (n = 4–7/group). Following in-vivo experimental procedures, brains were harvested for gene expression analysis. Impaired bradycardia in SHReGFP was restored in SHR rats overexpressing AT2R in the NTS/DMV. mRNA levels of angiotensin converting enzyme decreased and angiotensin converting enzyme 2 increased in the NTS/DMV of SHRAT2R compared to SHReGFP. Increased levels of pro-inflammatory cytokine mRNA levels in the SHReGFP group also decreased in the SHRAT2R group. AT2R overexpression did not elicit any significant change in mean arterial pressure (MAP) in all groups from baseline to 4 weeks post viral transfection. Both SHReGFP and SHRAT2R showed a significant elevation in MAP compared to the NTeGFP and NTAT2R groups. Increased AT2R expression within the NTS/DMV of SHR was effective at improving baroreflex function but not MAP. We propose possible mediators involved in improving baroreflex are in the ANG II/ACE2 axis, suggesting a potential beneficial modulatory effect of AT2R overexpression in the NTS/DMV of neurogenic hypertensive rats.

1. Introduction

Neurogenic hypertension is a chronic disease involving a myriad of factors resulting in the phenotypic elevation in sympathetic nerve activity (SNA) and blood pressure (BP) (Primatesta and Poulter, 2006). Hypertension is a condition that leads to the predisposition of cardiovascular diseases such as heart failure and stroke. The spontaneously hypertensive rat (SHR) displays an increased sympathetic tone (Trippodo and Frohlich, 1981) making it an appropriate model for investigating essential hypertension. Characteristically, SHR exhibit a hyperactive renin-angiotensin system (RAS), including higher angiotensin II (ANG II) levels and turnover in several brain areas [reviewed by (Veerasingham and Raizada, 2003)] and also an impairment of the baroreflex (Judy and Farrell, 1979).

The current standardised therapy for treating hypertension is the use of angiotensin II type I receptor (AT1R) blockers together with angiotensin converting enzyme (ACE) inhibitors (Fisher and Fadel, 2010; Grassi et al., 2010; Mann, 2003). Microinjecting angiotensin II (ANG II) centrally can target AT1Rs in cardiovascular regulating nuclei that stimulate cardiovascular pressor responses. This has been demonstrated in the rostral ventrolateral medulla (RVLM) (Ito et al., 2002; Mayorov et al., 2004), the nucleus of the solitary tract (NTS) (Casto and Phillips, 1984; Rettig et al., 1986), and the paraventricular nucleus of hypothalamus (Zhu et al., 2002).

However, ANG II can also act on the angiotensin II type 2 receptor (AT2R). Similar to AT1R, AT2R is a seven-transmembrane receptor showing over 90% homology across humans, mice and rats (Mukoyama et al., 1993). Considering the discrete localization of AT2R and surrounding important cardiovascular control regions of the adult rodent brain (de Kloet et al., 2014), an intriguing question is the role of AT2R in cardiovascular regulation. In fact, Ichiki and colleagues have

* Corresponding authors at: Department of Physiology & Pathology, School of Dentistry of Araraquara, UNESP, Rua Humaitá, 1680, Araraquara 14801-903, SP, Brazil.
E-mail addresses: eduardo.colombari@foar.unesp.br (E. Colombari), deborac@foar.unesp.br (D.S.A. Colombari).
[1] DSAC and EC are co-senior and co-corresponding authors.

http://dx.doi.org/10.1016/j.npep.2016.06.006
0143-4179/

found that basal systolic and diastolic BP is increased in mice lacking the AT2R (Ichiki et al., 1995). However, discrepancies in the literature have been highlighted by different studies showing that basal BP was unaffected in the absence of AT2R in mice (Hein et al., 1995; Li et al., 2003). Despite these differences in basal arterial BP, all of these studies show that the pressor effects of peripheral or central ANG II infusion were significantly enhanced in AT2R knockout mice compared to wild type mice (Hein et al., 1995; Li et al., 2003). Moreover, recently, we demonstrated using the 2-kidney, 1-clip (2K1C) hypertensive rat model that increased expression of AT2R in the solitary-vagal complex [NTS/dorsal motor nucleus of the vagus (DMV); NTS/DMV] restores normal baroreflex function and attenuated hypertension (Blanch et al., 2014).

The NTS is integral to the CNS control of cardiovascular function, receiving baroreceptor and chemoreceptor afferents from the periphery and having direct and indirect pathways to the RVLM to modulate SNA and BP (Guyenet, 2006). Therefore, the possibility that AT2R may modulate the altered vagal/sympathetic tone in neurogenic hypertension has come to the forefront of our interest. In the present study, we investigated; 1) baroreflex function in conscious, non-anesthetized normotensive (NT) and SHRs; 2) the chronic effects of overexpressing AT2R in the NTS/DMV complex on BP and heart rate (HR) of freely moving, non-anesthetized NT and SHRs; and 3), given that studies show crosstalk between renin-angiotensin system (RAS) components and pro-inflammatory cytokines (PICs) in central cardiovascular regulatory nuclei (Guggilam et al., 2008; Shi et al., 2010; Sriramula et al., 2013), we also assessed the gene expression profile of different components of the RAS and PICs within the NTS/DVM complex in these rats.

2. Methods

2.1. Animals

Ten week old Holtzman rats and SHR were used for all experimental procedures. Post recovery, animals were housed individually with access to water and rat chow (BioBase Rat Chow, Águas Frias, Brazil) ad libitum. Room temperature was maintained at 23 ± 2 °C, humidity at 55 ± 10% and on a 12:12 h light-dark cycle. Experimental protocols were approved by the Ethical Committee in Animal Use (CEUA) in the School of Dentistry- UNESP (Proc. CEUA 07/2011). In addition, the principles governing the care and treatment of animals, as stated in the Guide for the Care and Use of Laboratory Animals published by the National Academy of Sciences (eighth ed., 2011), were followed at all times during this study.

2.2. Viral transduction of AT2R in the NTS/DVM

Adeno-associated viral vectors expressing either AT2R or eGFP (AAV2-CBA-AT2R, 3.6×10^{11} genome copies [gc]/injection; AAV2-CBA-eGFP, 1.5×10^{12} gc/injection) were used to induce the overexpression of the respective protein in the NTS/DVM. Rats were anesthetized using ketamine (80 mg/kg body wt., IP) and xylazine (7 mg/kg body wt., IP) and placed in a stereotaxic head frame (David Kopf Instruments, Tujunga, CA). A partial craniotomy of the occipital bone was implemented, to expose the dorsal surface of the brainstem. A total of five microinjections were made along the entire rostral-caudal axis with a distance of 0.5 mm anterior from the calamus scriptorius and 0.5 mm lateral from the midline using a glass micropipette coupled to a Picospritzer microinjection system (Parker, Cleveland, OH, USA). The volume microinjected (100 nl/site) was determined by viewing the movement of the meniscus through a binocular microscope fitted with a precalibrated eyepiece reticule with a 10 min interval between each injection. Four groups were used for the experiments as follows; 1) normotensive rats injected with AAV2-CBA-eGFP (NTeGFP), 2) normotensive rats injected with AAV2-CBA-AT2R (NTAT2R), 3) SHRs injected with AAV2-CBA-eGFP (SHReGFP) and 4) SHRs injected with AAV2-CBA-AT2R (SHRATR2). Proceeding the surgical procedure, rats were administered with antibiotic IM, (benzylpenicillin – 80,000 IUs plus streptomycin – 33 mg; Pentabiótico Veterinário –Pequeno Porte, Fort Dodge Saúde Animal Ltda, Campinas, Brazil) and analgesic/anti-inflammatory SC, (ketoprofen 1%; 0.03 ml/rat; Ketoflex, Mundo Animal, São Paulo, Brazil). AAV2-CBA-eGFP or AAV2-CBA-AT2R were constructed as described (Li et al., 2005) and these vectors elicit gene transduction primarily in neurons of all different phenotypes (Zhang et al., 2013). Our previous studies have demonstrated that AAV2-CBA-AT2R microinjected as above elicits a significant increase in AT2R protein in the NTS/DVM, based on receptor binding analyses (Blanch et al., 2014).

2.3. Baroreflex test

Rats were anesthetized using ketamine associated with xylazine as described previously, and a polyethylene tube (PE-10 connected to a PE-50) was inserted into the abdominal aorta through the femoral artery and another catheter was implanted into the femoral vein. Arterial and venous catheters were tunneled subcutaneously and exposed on the back of the rat. To record pulsatile arterial pressure, mean arterial pressure (MAP) and heart rate (HR) in conscious unrestrained, freely moving animals, the arterial catheter was connected to a Statham Gould (P23 Db; El Segundo, CA, USA) pressure transducer coupled to a preamplifier (model ETH-200 Bridge Bio Amplifier, Chicago, IL, USA) that was connected to a Powerlab computer data acquisition system (model Powerlab 16SP, ADInstruments, Colorado Springs CO, USA). After a baseline period of cardiovascular recordings, rats received iv injections of phenylephrine (5 μg/kg, body wt.) or sodium nitroprusside (SNP; 30 μg/kg, body wt.) to test the HR reflex responses to pressor and depressor stimuli, respectively. We analysed the one second mean HR values in response to 10 mm Hg incremental changes in MAP, starting at 5 mm Hg up to a maximal change of 35 mm Hg (Blanch et al., 2014). The values were plotted, a linear regression was performed for each animal, and the slope of each linear regression was used to calculate the differences between groups.

2.4. Radio-telemetry recording of arterial pressure

Under ketamine associated with xylazine as described previously, a laparotomy was made to expose the descending aorta, proceeded by the implantation of a telemetry device (model TA11PA-C40; Data Sciences Int., St. Paul, MN, USA) to record arterial pressure. Dataquest 4.31 software (Data Sciences Int.) was used to analyse raw data that were expressed as MAP and HR. Recordings of arterial pressure were programmed with the following conditions; 24 h/d, 5 min/h and 20 s segments at a frequency of 1000 Hz. Post surgical treatment was administered as described above.

2.5. Histology of injection sites

Rats were deeply anesthetized with sodium thiopental (70 mg/kg b·wt, IP) and perfused transcardially with 0.1 M PBS (pH 7.4) followed by 4% (w/v) paraformaldehyde. Brains were extracted and post fixed in 10% (w/v) sucrose for 24 h. Brains were snap frozen and 40 μm thick sections were cut using a cryostat (Leica CM1900, Wetzlar, Germany). The sections were visualized using a Leica fluorescence microscope (Leica, DM5500 B, Wetzlar, Hesse, Germany) with the appropriate filter to visualize eGFP expression.

2.6. qRT–PCR analyses in the NTS/DMV

Rats were deeply anesthetized with isoflurane (5% in 100% O_2), decapitated and the brains extracted for dissection. The NTS/DMV was identified using the obex and central canal as landmarks for dissection. The tissue was snap frozen at −80 °C until required for further use. mRNA was extracted by homogenising the tissue in 2-mercaptoethanol

Please cite this article as: Ruchaya, P.J., et al., Overexpression of AT2R in the solitary-vagal complex improves baroreflex in the spontaneously hypertensive rat, Neuropeptides (2016), http://dx.doi.org/10.1016/j.npep.2016.06.006