Neuroscience 329 (2016) 112–121

MINERALOCORTICOID AND ANGIOTENSIN II TYPE 1 RECEPTORS IN THE SUBFORNICAL ORGAN MEDIATE ANGIOTENSIN II – INDUCED HYPOTHALAMIC REACTIVE OXYGEN SPECIES AND HYPERTENSION

HONG-WEI WANG,[†] BING S. HUANG,[†] ROSELYN A. WHITE, AIDONG CHEN, MONIR AHMAD AND FRANS H. H. LEENEN[*]

University of Ottawa Heart Institute, Ottawa, Canada

Abstract—Activation of angiotensinergic pathways by central aldosterone (Aldo)-mineralocorticoid receptor (MR) pathway plays a critical role in angiotensin II (Ang II)-induced hypertension. The subfornical organ (SFO) contains both MR and angiotensin II type 1 receptors (AT_1R) and can relay the signals of circulating Ang II to downstream nuclei such as the paraventricular nucleus (PVN), supraoptic nucleus (SON) and rostral ventrolateral medulla (RVLM). In Wistar rats, subcutaneous (sc) infusion of Ang II at 500 ng/min/kg for 1 or 2 weeks increased reactive oxygen species (ROS) as measured by dihydroethidium (DHE) staining in a nucleus – specific pattern. Intra-SFO infusion of AAV-MR or $AT_{1a}R$-siRNA prevented the Ang II-induced increase in AT_1R mRNA expression in the SFO and decreased MR mRNA. Both MR- and $AT_{1a}R$-siRNA prevented increases in ROS in the PVN and RVLM. MR- but not $AT_{1a}R$-siRNA in the SFO prevented the Ang II-induced ROS in the SON. Both MR- and $AT_{1a}R$-siRNA in the SFO prevented most of the Ang II-induced hypertension as assessed by telemetry. These results indicate that Aldo-MR signaling in the SFO is needed for the activation of Ang II-AT_1R-ROS signaling from the SFO to the PVN and RVLM. Activation of Aldo-MR signaling from the SFO to the SON may enhance AT_1R dependent activation of pre-sympathetic neurons in the PVN. © 2016 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: brain, angiotensinergic pathways, reactive oxygen species, mineralocorticoid receptors, angiotensin II type 1 receptor.

INTRODUCTION

A modest increase in circulating angiotensin II (Ang II) by e.g. renal artery stenosis or chronic infusion of Ang II causes a progressive increase in blood pressure (BP) (Leenen and De Jong, 1975; Huang et al., 2010). Both peripheral and central mechanisms may contribute to Ang II-induced hypertension. Chronic intracerebroventricular (icv) infusion of an aldosterone synthase inhibitor (Huang et al., 2010), a mineralocorticoid receptor (MR) blocker (Huang et al., 2010; Xue et al., 2011), or an angiotensin II type 1 receptor (AT_1R) blocker (Zimmerman et al., 2004) prevents most of the progressive increase in BP induced by circulating Ang II. Knockdown of either MR or AT_1R in the paraventricular nucleus (PVN) markedly attenuates the Ang II-induced hypertension (Chen et al., 2014), and icv infusion of an MR blocker normalizes enhanced AT_1R responses in the PVN (Gabor and Leenen, 2013) suggesting that MR activation contributes to AT_1R activation in the PVN. We proposed that MR-AT_1R signaling in the PVN is essential for the central nervous system (CNS) component of the progressive hypertension by circulating Ang II (Huang et al., 2010, 2013; Gabor and Leenen, 2012).

The forebrain circumventricular organs, the subfornical organ (SFO) and organum vasculosum of the lamina terminalis (OVLT), contain AT_1R (Wei et al., 2009; Lu et al., 2009; Huang et al., 2010) and via glutamatergic and angiotensinergic efferents can relay the signals of circulating Ang II to important regulatory downstream nuclei such as the PVN (Collister and Hendel, 2005; Ferguson, 2009) and rostral ventrolateral medulla (RVLM) (Vieira et al., 2010; Shinohara et al., 2015). Intra-SFO injection of Ang II increases BP, which is prevented by intra-SFO injection of an Ang II receptor blocker (Mangiapane and Simpson, 1980; Tiruneh et al., 2013). Electrolytic lesion of the SFO attenuates ~60% of the hypertension induced by subcutaneous (sc) infusion of Ang II (Collister and Hendel, 2005). In mice, knockdown of NADPH oxidase subunit p22 by SFO-targeted injection of an adenovirus encoding cre-recombinase prevents the BP increase by sc Ang II indicating that NADPH oxidase induced reactive oxygen species (ROS) in the SFO mediate Ang II-induced hypertension (Lob et al., 2013).

MR are also present in the SFO (Amin et al., 2005; Chen et al., 2014). In cultured SFO neurons, aldosterone enhances Ang II-induced increase of Ca^{2+} influx (Xue

*Corresponding author. Address: Hypertension Unit, University of Ottawa Heart Institute, H3238 – 40 Ruskin Street, Ottawa, Ontario K1Y 4W7, Canada. Tel: +1-613-761-4521; fax: +1-613-761-5105.
E-mail address: fleenen@ottawaheart.ca (F. H. H. Leenen).
[†] Joint first authors.

Abbreviations: AAV, adeno-associated virus; AAV-siRNA, adeno-associated virus carrying small interfering RNA; aldo, aldosterone; Ang II, angiotensin II; AS, aldosterone synthase; AT_1R, angiotensin II type 1 receptor; BP, blood pressure; CNS, central nervous system; DEPC, diethylpyrocarbonate; eGFP, enhanced green fluorescent protein; HR, heart rate; ICV, intra-cerebroventricular; MAP, mean arterial pressure; MR, mineralocorticoid receptor; OVLT, organum vasculosum of the lamina terminalis; PBS, phosphate-buffered saline; PGK 1, phosphoglycerate kinase1; PVN, paraventricular nucleus; RAAS, renin–angiotensin–aldosterone system; ROS, reactive oxygen species; RVLM, rostral ventrolateral medulla; SCM, scrambled; SFO, subfornical organ; SON, supraoptic nucleus; SC, subcutaneous.

http://dx.doi.org/10.1016/j.neuroscience.2016.04.050

et al., 2012). However, no studies have yet assessed the role of MR in the SFO for Ang II-induced hypertension. We hypothesized that MR activation by plasma aldosterone increases AT_1R signaling in the SFO and contributes to sc Ang II-induced ROS in the PVN and RVLM (Shinohara et al., 2015) and the Ang II-induced hypertension.

In the present study, we assessed in Wistar rats (1) effects of sc infusion of Ang II at 500 ng/kg/min on ROS in the SFO, PVN, supraoptic nucleus (SON) and RVLM; (2) effects of knockdown of MR or AT_1R in the SFO by intra-SFO infusion of AAV-MR-siRNA or AAV-AT_{1a}R-siRNA on sc Ang II induced changes in MR and AT_1R expression in the SFO and PVN and in ROS in above-mentioned nuclei and on Ang II-induced hypertension.

EXPERIMENTAL PROCEDURES

Animals and surgeries

Male, 6–7-wk-old Wistar rats weighing 200–225 g (Charles River Breeding Laboratories, Montreal, Quebec, Canada) were housed on a 12-h light/dark cycle at constant room temperature and fed a standard commercial rat chow (120 μmol Na^+/g) and water *ad libitum*. For all surgeries, rats were anesthetized with 2% isoflurane in oxygen. For pain relief, slow release buprenorphine (1 mg/kg) was injected sc 1 h before surgery. All study protocols were approved by the University of Ottawa Animal Care Committee, and conform to *the Guide for the Care and Use of Laboratory Animals* published by the US National Institute of Health (NIH Publication, 8th Edition, 2011).

Adeno-associated virus (AAV)

AAV-MR-siRNA, AAV-AT_{1a}R-siRNA or control vector AAV-SCM-siRNA were purchased from GeneDetect (Bradenton, FL). For the specific siRNA sequences of MR and AT_{1a}R, see Chen et al. (2014). All 3 viral vectors contain enhanced green fluorescent protein (eGFP). The viral vectors were infused at a rate of 0.1 μl/min for 10 min for intra-SFO infusion of 5.0×10^7 (MR-siRNA and SCM-siRNA) and 25×10^7 (AT_{1a}R-siRNA) genomic particles. Bilateral intra-PVN infusion of AAV-MR-siRNA or AAV-AT_{1a}R-siRNA at these amounts caused 60–70% knockdown of MR or AT_1R mRNA and protein expression in the PVN without affecting expression in other nuclei such as the SFO and SON and prevented most of hypertension caused by sc infusion of Ang II at 500 ng/kg/min (Chen et al., 2014).

Protocol I. Sc Ang II – induced changes in central ROS and BP; Effects of gene knockdown by intra-SFO infusion of AAV-siRNA

Seven day study. Rats were randomly divided into two groups. In one group ($n = 6$) an osmotic minipump (Alzet, model 2001, flow rate 1 μl/h) filled with Ang II (7.5 μg/μl) was implanted sc for continuous infusion at 500 ng/kg/min for 7 days. The other group ($n = 4$) underwent the same surgery without pump implantation. The day before the end of the infusion period, in the morning a cannula was placed in the left femoral artery of each rat. After recovery in a quiet room for several hours, BP was measured in the afternoon. The next morning, rats were anesthetized deeply by sodium pentobarbital, then perfused transcardially with chilled phosphate-buffered saline (PBS, pH 7.4). The whole brain was removed, frozen rapidly in −20 to −30 °C pre-chilled methylbutane (Sigma, St. Louis, MO, USA), and stored at −80 °C.

14 day study. For intra-SFO infusion of AAV, rats were mounted on a stereotaxic frame and a small hole with a diameter of 1 mm was drilled through the top of the skull about 1 mm posterior to the bregma. A 29 G stainless steel cannula was inserted through the hole into the SFO with coordinates of 1.3 mm posterior and 4.7 mm ventral to the bregma. The cannula was connected to a 10 μl Hamilton micro-syringe via PE10 tubings for intra-SFO infusion of: AAV-SCM-siRNA, AAV-MR-siRNA or AAV-AT_{1a}R-siRNA (for details, see Chen et al., 2014).

About 10 days after the AAV infusion, in three groups of rats osmotic minipumps (Alzet, model 2002) were placed for chronic sc infusion of Ang II at 500 ng/kg/min, i.e. (1) SCM-siRNA plus Ang II ($n = 3$); (2) MR-siRNA plus Ang II ($n = 5$); and (3) AT_{1a}R-siRNA plus Ang II ($n = 5$). One group of rats treated with SCM-siRNA ($n = 3$) was treated with sc infusion of saline (SCM-siRNA plus vehicle) and served as control. In the afternoon of day 12 of the Ang II infusions, the right femoral artery was cannulated. The next morning, after a 20-min rest, resting BP was recorded for 20 min. On day 14 of Ang II infusion, rats were anesthetized with pentobarbital and perfused transcardially with PBS (pH 7.4). Whole brains were removed, frozen rapidly in −20 to −30 °C pre-chilled methylbutane, and stored at −80 °C.

Protocol II. Gene knockdown by intra-SFO infusion of AAV and BP measured by telemetry

Under anesthesia, a telemetry probe (DSI model TA11PA-C40) was placed into the abdominal cavity with the catheter inserted into the abdominal aorta. The telemetry signal was obtained using an analog adapter and data acquisition system. After three days, BP and HR data collection began using Scheduled Sampling Mode with duration of 1 min and interval of 1 h. After recording resting BP and HR for three days, AAV-SCM-siRNA ($n = 6$), AAV-AT_{1a}R-siRNA ($n = 5$), or AAV-MR-siRNA ($n = 6$) was infused into the SFO. About 7–10 days after the infusions, osmotic minipumps filled with Ang II were implanted sc for a 2-week infusion at 500 ng/kg/min.

At the end of the experiments, brain tissue was collected, frozen and stored for evaluation of MR, AT_1R and eGFP expression. Using a cryostat, serial 50-μm (for MR and AT_1R mRNA expression) and 10-μm (for eGFP expression) coronal sections were made for the