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ROLE OF BRAIN ALDOSTERONE AND MINERALOCORTICOID RECEPTORS IN ALDOSTERONE-SALT HYPERTENSION IN RATS

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Abstract—Central blockade of mineralocorticoid receptors (MRs) or angiotensin II type 1 receptors (AT_1Rs) attenuates aldosterone (aldo)-salt induced hypertension. We examined the role of the subfornical organ (SFO), aldo synthesized locally in the brain, and MR and AT_1R specifically in the paraventricular nucleus (PVN) in aldo-salt hypertension. Wistar rats were treated with subcutaneous aldo (1 μg/h) plus saline as drinking fluid, and gene expression was assessed by real-time qPCR. Other sets of rats received chronic intra-cerebroventricular (icv) infusion of aldo synthase (AS) inhibitor FAD286, MR blocker eplerenone or vehicle, electrolytic or sham lesions of the SFO, or intra-PVN infusion of AAV-MR-siRNA or AAV-AT_{1a}R-siRNA. Infusion of aldo had no effect on 11βHSD2, MR and AT_1R mRNA in different nuclei but increased CYP11B2 mRNA in the SFO, and serum and glucocorticoid-kinase 1 (Sgk1) and epithelial sodium channel (ENaC) γ subunit mRNA in the SFO and supraoptic nucleus (SON). MR-siRNA decreased both MR and AT_1R mRNA in the PVN by ~60%, but AT_{1a}R-siRNA only decreased AT_1R mRNA. SFO lesion, blockade of brain AS or MR, or knockdown of MR or AT_1R in the PVN similarly attenuated aldosterone-induced saline intake by ~50% and hypertension by ~70%. These results suggest that an increase in circulating aldosterone may via MR and AT_1R in the SFO increase local aldosterone production in hypothalamic nuclei such as the SON and PVN, and via MR enhance AT_1R signaling in the PVN. This central aldosterone-MR-AT_1R neuro-modulatory pathway appears to play a major role in the progressive hypertension.
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Key words: aldosterone, aldosterone synthase, brain, mineralocorticoid receptor, angiotensin II type 1 receptor, gene expression knockdown.

INTRODUCTION

Central mechanisms appear to play a major role in both deoxycorticosterone acetate (DOCA)-salt and aldosterone-salt induced hypertension. Central blockade of mineralocorticoid receptors (MRs) with intracerebroventricular (icv) infusion of an MR blocker or recombinant adeno-associated virus carrying siRNA against MR (AAV-MR-siRNA) attenuates aldosterone-salt hypertension by 70–80% (Gomez-Sanchez et al., 1990; Xue et al., 2011). Central MR blockade also markedly attenuates DOCA-salt hypertension (Janiak et al., 1990; Rahmouni et al., 2002).

Because of the high reflection coefficient at the blood–brain barrier (BBB) (Pardridge and Mietus, 1979; Funder and Myles, 1996; Parker et al., 2006), circulating aldosterone shows poor penetration into most brain areas related to cardiovascular regulation, such as the hypothalamus. In rats, subcutaneous (sc) infusion of aldosterone at 1.5 and 7.5 μg/kg/h for 2 weeks increases plasma aldosterone levels by five- to eightfold without significant changes in hypothalamic aldosterone content (Huang et al., 2010). The subfornical organ (SFO) lacks the BBB, and MR are densely expressed in the SFO (Amin et al., 2005), and may sense an increase in circulating aldosterone. In cultured SFO neurons, aldosterone enhances Ang II-induced increase in intracellular calcium (Xue et al., 2012). Electrolytic lesioning of the SFO does not affect the blood pressure (BP) increase in uni-nephrectomized DOCA-salt rats (Osborn et al., 2006), but in DOCA-salt mice, knockdown of angiotensin type 1 receptors (AT_1Rs) with icv administration of adenovirus encoding cre-recombinase reduces AT_1R mRNA in the SFO and attenuates increases in BP and water and salt intake by 50–60% (Hilzendeger et al., 2013). In rats, icv infusion of an AT_1R blocker prevents aldosterone-salt hypertension (Xue et al., 2011) and reverses DOCA-salt hypertension (Park and Leenen, 2001). In DOCA-salt mice, (pro) renin receptor (PRR) and Ang II formation in the brain are increased, and neuron-specific PRR knockout prevents DOCA-salt hypertension and activation of cardiac and vasomotor sympathetic tone (Li et al., 2014). Thus, central angiotensinergic pathways appear to play a major role.

The downstream mechanisms/pathways following SFO activation have not yet been identified. Enhanced

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Abbreviations: Aldo, aldosterone; Ang II, angiotensin II; AS, aldosterone synthase; AT_1R, angiotensin II type 1 receptor; BBB, blood–brain barrier; BP, blood pressure; DEPC, diethylpyrocarbonate; DOCA, deoxycorticosterone acetate; EDTA, ethylenediaminetetraacetic acid; eGFP, enhanced green fluorescent protein; ENaC, epithelial sodium channel; EO, endogenous ouabain; icv, intra-cerebroventricular; MAP, mean arterial pressure; MR, mineralocorticoid receptor; PGK1, phosphoglycerate kinase 1; PRR, (pro) renin receptor; PVN, paraventricular nucleus; ROS, reactive oxygen species; RVLM, rostral ventrolateral medulla; sc, subcutaneous; SCM, scrambled; SFO, subfornical organ; Sgk1, serum and glucocorticoid-regulated kinase 1; SON, supraoptic nucleus.

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AT_1R signaling in the SFO and the organum vasculosum of the lamina terminalis (OVLT) may activate angiotensinergic pathways to the paraventricular nucleus (PVN) and the rostral ventrolateral medulla (RVLM) (Wright et al., 1993; Cato and Toney, 2005), as well as to magnocellular, vasopressinergic neurons in the supraoptic nucleus (SON) (Johnson et al., 1999). In addition this may activate a hypothalamic aldosterone-MR-AT_1R neuro-modulatory pathway (Huang et al., 2010; Gabor and Leenen, 2012; Chen et al., 2014a). An increase in aldosterone via MR may increase endogenous ouabain (EO) in magnocellular neurons (Yoshika et al., 2011) and thereby enhance AT_1R expression and signaling in the PVN and SON (Huang et al., 2011), which may increase vasopressin release and sympathetic activity. Whether this pathway also contributes to aldosterone-induced inhibition of oxytocin neurons in the PVN and increase in sodium appetite (Stricker and Verbalis, 1996; Grafe et al., 2014), has not yet been studied. We hypothesized that an increase in circulating aldosterone primarily causes AT_1R activation in the SFO, which is directly relayed to the PVN (Gabor and Leenen, 2012) and in addition is relayed to magnocellular neurons in the SON or PVN causing local production of aldosterone. If so, central infusion of an MR blocker or aldosterone synthase (AS) inhibitor should be similarly effective in preventing circulating aldosterone-induced central actions.

In the present study, we assessed in Wistar rats drinking saline: (1) effects of sc infusion of aldosterone at 1 μg/h (Huang et al., 2010; Xue et al., 2011) on BP, hypothalamic aldosterone and corticosterone levels, and expression of relevant genes in several brain nuclei such as the SFO, PVN and SON; (2) effects of electrolytic lesion of the SFO on aldosterone-salt hypertension; (3) effects of chronic icv infusion of the MR blocker eplerenone or AS inhibitor FAD286 on aldosterone-salt hypertension and hypothalamic aldosterone and corticosterone; and (4) effects of intra-PVN infusion of AAV-MR-siRNA or AAV-$AT_{1a}R$-siRNA on MR and AT_1R expression in the PVN and aldosterone-salt hypertension.

EXPERIMENTAL PROCEDURES

Animals and surgeries

Male Wistar rats weighing 200–250 g were obtained from Charles River Breeding Laboratories (Montreal, Quebec, Canada), housed on a 12-h light/dark cycle at constant room temperature, and provided with a standard laboratory chow (120 μmol Na^+/g) and tap water *ad libitum*. For all surgeries, rats were anesthetized with 2% isoflurane in oxygen. Effective levels of anesthesia were maintained by observing reactions to physical stimulation such as toe-pinch, as well as monitoring the pattern of respiration. For pain relief, buprenorphine (0.04 mg/kg) was injected sc ½ hour before and twice daily for 3 days following surgeries. All experiments 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 Institutes of Health (8th ed., 2011).

Protocol I: effects of aldosterone-salt on aldosterone levels, BP and gene expression. A telemetry probe (DSI model TA11PA-C40) was placed into the abdominal cavity and secured to the ventral abdominal wall with the catheter inserted into the abdominal aorta. The telemetry signal was obtained using an analog adapter and data acquisition system, which was set to calculate and store the mean values of resting BP and HR. The rats were housed in regular cages placed on the telemetry receivers. Regular diet and tap water was provided. After 3 days, collection of BP and HR data was started with scheduled sampling mode with duration of 1 min and interval of 1 h.

After resting BP and HR were recorded for 2–3 days, rats were divided into four groups (4–6 per group) for the following treatments: (1) sc infusion of aldosterone at 1 μg/h plus tap water as drinking fluid; (2) sc infusion of aldosterone at 1 μg/h plus saline (0.9% NaCl) as drinking fluid; (3) saline as drinking fluid without aldosterone; and (4) tap water as drinking fluid without aldosterone. Osmotic minipumps (Model 2004, ALZET) were used for sc infusion for 3 weeks. On day 21 of sc infusion, all rats stayed overnight in a quiet room, and the next morning non-stressed, undisturbed rats were decapitated and trunk blood and brain tissue were collected for aldosterone and corticosterone measurement.

Two additional sets of rats were divided into three groups (n = 5–7/group): (1) control drinking tap water; (2) drinking 0.9% NaCl; and (3) drinking 0.9% NaCl and sc infusion of aldosterone at 1 μg/h. After 10 days or 3 weeks of treatments, rats were anesthetized with intraperitoneal injection of pentobarbital and transcardially perfused with chilled diethylpyrocarbonate (DEPC, Sigma–Aldrich Canada Ltd, Oakville, ON, Canada) treated PBS (pH 7.4). Brain and adrenal tissues were collected for assessment of gene expression.

Protocol II: effects of electrolytic lesion of the SFO on aldosterone-salt hypertension. Rats instrumented with telemetry probes were divided into two groups, and electrolytic lesion or sham lesion of the SFO was performed according to Collister and Hendel (2005). Briefly, the rat was mounted on a stereotaxic apparatus, and a dorsal midline incision was made through the skin of the skull. A 3-mm hole was drilled 1.5 mm posterior to the bregma. A Teflon-coated tungsten electrode (A-M Systems Inc., WA, USA) with 0.010 inch exposed at the tip was passed into the brain at four predetermined coordinates caudal and ventral to bregma, respectively (in mm): −0.8 and −5.2, −1.0 and −5.1, −1.2 and −4.9, and −1.4 and −4.7. At each location, a 1-mA current was passed for 10 s to make an anodal lesion using a lesion-making device (Model 53500, Biological Research Apparatus, Varese, Italy). Surgery for sham rats was identical to the lesioning, except ventral coordinates were 1.5 mm less and no current was passed through the electrode. At least 7 days after the brain surgery, osmotic pumps (Model 2004) were implanted for sc infusion of aldosterone at 1 μg/h for 3–4 weeks and all rats started on 0.9% NaCl as drinking fluid. At the end of sc infusion,

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