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DOCA/salt hypertension alters Period1 and orexin-related gene expression in the medulla and hypothalamus of male rats: Diurnal influences

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ABSTRACT

In peripheral tissues, aldosterone alters expression of multiple genes, including the clock gene Period 1 (Per1), 11 beta-hydroxysteroid dehydrogenase-2 (11-HSD2), and α-ENAC, the epithelial sodium channel subunit. We evaluated the impact of chronic aldosterone exposure (DOCA) and salt intake on nocturnal changes in gene expression in the male Sprague Dawley rat brain. Additionally, genes associated with the orexin (ORX) system were also evaluated based on the role of this neuropeptide in arousal, feeding and hypertension and an interconnection with Per1 expression. DOCA/salt treatment increased saline intake primarily at night, elevated arterial pressure and lowered heart rate. In the medulla oblongata, DOCA/salt upregulated Per1, 11-HSD2, and α-ENAC expression independent of time of day, but did not change ORX receptor type 1 (ORX-R1) or type 2 (ORX-R2) expression. ORX-R1, and ORX-R2 expression in the medulla did however correlate with Per1 expression following DOCA/salt treatment but not in controls. In the hypothalamus, DOCA/salt treatment upregulated Per1, ORX-A, and ORX-R2 expression, in general, and Per1 and ORX-A expression at night. ORX-A, ORX-R1 and ORX-R2 expression in the hypothalamus correlated with Per1 expression following DOCA/salt but not in controls. These findings demonstrate for the first time that DOCA/salt hypertension modulates ORX gene expression in the brain and suggest that changes in expression in the ORX system may occur directly or indirectly via aldosterone-induced changes in Per1 expression. Our findings also build on emerging evidence that monitoring gene expression during both the day and night is critical to understanding the role of specific genes in hypertension.

1. Introduction

Cortisol and aldosterone have equal affinity for mineralocorticoid receptors (MRs). Thus, selective activation of MRs by aldosterone, requires the presence of the intracellular enzyme 11 beta-hydroxysteroid dehydrogenase-2 (11-HSD2) which inactivates cortisol (or corticosterone in rats). In peripheral tissues, 11-HSD2 is expressed selectively in epithelial cells associated with sodium transport (Fukushima et al., 2005) and 11-HSD2 expression is upregulated in response to increases in circulating aldosterone, suggesting that 11-HSD2 is a biomarker of cells involved in sodium homeostasis. In 1995 11-HSD2 mRNA was identified in the brain, including neurons in the nucleus of the solitary tract (NTS) in the dorsal medulla, the subcommissural circumventricular organ in the forebrain, and some cells in the ventromedial hypothalamus (Roland et al., 1995). Similar to the periphery, increases in circulating aldosterone was identified to trigger an increase in both the number of neurons expressing 11-HSD2 and the number of 11-HSD2 positive neurons in the NTS, expressing c-Fos, a biomarker of neuronal excitation (Geerling and Loewy, 2006a; Geerling et al., 2006b). Alternatively, c-Fos expression was in 11-HSD2 positive neurons was shown to decrease following sodium intake (Geerling and Loewy, 2006a; Geerling et al., 2006c). These observations suggested 11-HSD2 neuronal activation is linked to sodium appetite while sodium ingestion may be linked to 11-HSD2 neuronal inhibition. In agreement with these findings, conditional deletion of 11-HSD2 in the brainstem of mice was recently shown to trigger heightened salt intake (Evans et al., 2016), confirming the importance of these neurons regulating sodium appetite. Additionally, the loss of 11-HSD2 was associated with impaired baroreflex function, suggesting that activation of these neurons in response to circulating aldosterone is also involved in the central control of blood pressure.

In the kidney, aldosterone also stimulates expression of the clock gene, Period 1 (Per1). Per1 is a circadian-related immediate early response gene, which regulates renal sodium-channel gene expression (Gumz et al., 2003 and Gumz et al., 2009). Mice with reduced levels of the Per1 gene have attenuated sodium channel expression, increased

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sodium excretion, and low blood pressure (Richards et al., 2013). Interestingly, Per1 knockout mice develop hypertension when given a long-lasting mineralcorticoid and a high salt diet, while wild type mice do not, suggesting that Per1 gene expression may also be involved in sodium appetite and central control of blood pressure (Solocinski et al., 2017). In fact, Per1 gene expression in the NTS fluctuates over 24 hour period in both mice and rats (Herichova et al., 2007; Kaneko et al., 2009) and Per1 signaling in the brain has been linked to ingestive behavior (Bainier et al., 2017; Mendoza et al., 2010). Since changes in sodium appetite also follow daily changes in circulating aldosterone (Fujimura et al., 1992; Tordoff and Okiyama, 1996), it is possible that Per1 expression in the NTS fluctuates across the day/night cycle and contributes to the regulation of 11-HSD2 expression, similar to its role in the regulation of sodium-channel expression in the kidney.

To gain a better understanding of how Per1 and 11-HSD2 expression fluctuates in the brain under normal conditions and in response to chronically elevated aldosterone and sodium intake, the present study was undertaken. Per1 and 11-HSD2 gene expression were evaluated during the day, (inactive period for rats), versus the night (active period) in the medulla of both control and deoxycorticosterone acetate (DOCA)/salt treated male Sprague Dawley rats. We hypothesized that DOCA/salt treatment would stimulate a sustained upregulation of Per1 and 11-HSD2 gene expression when compared to control and changes in 11-HSD2 expression would correlate with Per1 expression. Changes in Per1 gene expression were also evaluated in the hypothalamus. The hypothalamus, similar to the medulla, plays a prominent role in regulating blood pressure and salt-appetite, well as receiving dense projections from the suprachiasmatic nucleus (SCN), the primary regulator of circadian rhythms in the brain (Belle et al., 2014; Kinsman et al., 2017; Kohsaka et al., 2012). Additionally, the hypothalamus is the location of the orexin system which is involved in both system arousal, feeding behavior, and cardiovascular control (Alexandre et al., 2013; Bonnavion et al., 2016; Johnson et al., 2012; Shirasaka et al., 2002), as well as playing a role in the regulation of Per1 positive cells in the SCN. Alternatively, ORX positive neurons rhythmically express Per1 via polysynaptic input from the SCN (Mahoney et al., 2013). The impact of DOCA/salt hypertension on ORX expression is currently undefined.

2. General Methods

All experimental procedures were approved by the University of Florida Institutional Animal Care and Use Committee and followed the National Institutes of Health guidelines for animal use in research. Adult male Sprague Dawley (SD, $n = 39$; Harlan) rats aged between 12 and 22 weeks and weighing between 330 and 550 g were used in this study. Animals were randomly assigned to one of two experimental groups: telemetry (Group #1; $n = 7$) or gene/protein expression (Group #2; $n = 32$). Within each experimental group, animals were randomly assigned to receive DOCA treatment (subcutaneous pellet) or no treatment (control). All animals were weighed before entering a treatment group and at the end of the experimental treatment.

Prior to undergoing surgery, all animals were given a subcutaneous injection of Rimadyl (0.1 mg/kg) and buprenorphine (0.05 mg/kg) and then anesthetized to a surgical level of anesthesia with isoflurane (2–4% in 100% O_2). Surgeries were done under aseptic conditions and following closure, all incisions were treated with triple antibiotic ointment. The animals then received a subcutaneous injection of saline (3–5% body weight) for fluid replacement and recovered on a heating pad. An additional injection of buprenorphine (0.02–0.05 mg/kg) was given before returning the animal to its home cage. Supplemental analgesics and anti-inflammatory drugs were given, as needed the following days. After the initial surgery, all animals were singly housed in standard cages under standard housing conditions with 12 h: 12 h light/dark cycle (lights on at 6 AM) and constant room temperature of 22 ± 2 °C. All animals received ad libitum access to rat chow and tap water.

In Group #1, animals were instrumented with a radio telemetry probe (TA11PA-C40; Data Sciences, St. Paul, MN) in the descending aorta for continuous blood pressure measurement. Following 2 weeks of recovery, four of the seven rats underwent a second surgery for subcutaneous placement of a pellet containing DOCA (50 mg, 21 day release, Innovative Research of America) between the scapulae. Following recovery from surgery, DOCA animals returned to their home cages and allowed free access to a saline solution (1% NaCl, 0.2% KCl). Three of the seven rats served as untreated controls. For all animals in Group #1, mean arterial pressure (MAP), heart rate (HR), and activity were recorded for 5 min every 3 h (A/D sampling rate 1000 Hz). MAP, HR, activity data were collected for a total of 16 days, including 3 days prior and 13 days after DOCA or no pellet placement. Animals were left undisturbed except during cage cleaning and drinking bottle exchange. Saline intake was measured daily in the DOCA animals. All animals were sacrificed on day 17 (intraperitoneal injection of sodium pentobarbital, 200 mg/kg) during the day between 9 and 11 AM. Brains were rapidly removed and snap frozen (isopentane/dry ice) and stored at − 80 °C for gene expression analysis.

In Group #2, 15 of 32 rats underwent an initial surgery for the subcutaneous placement of a DOCA pellet (50 mg). All DOCA animals were given free access to the saline solution for 14 days and intake was monitored daily; including three DOCA animals for which saline intake was monitored twice daily for 3 consecutive days (at 9 AM and again at 5 PM, between days 8–10 following pellet placement). After 13 days, animals were randomly assigned to be sacrificed either during the day between 9 and 11 AM ($n = 16$) or at night between 9 and 11 PM ($n = 15$). Animals were either sacrificed for gene expression analysis as described above or transcardially perfused with saline followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer. Perfused brains were stored in 4% paraformaldehyde for immunohistochemical processing.

2.1. Tissue Processing

2.1.1. Gene Expression

Frozen brain sections ~2 mm in width on the transverse axis were cut from each animal, including one section from the medulla (between 14.60 and 11.60 mm caudal from bregma) and one from the hypothalamus (between 3.5 and 1.5 mm caudal from bregma and ± 2.4 mm medial/lateral). The medulla was targeted because it contains both the NTS and critical circuitry involved in baroreflex regulation and autonomic drive, including the rostral and caudal ventrolateral medulla and nucleus ambiguous. The hypothalamus, including the paraventricular nucleus, lateral hypothalamus, dorsomedial hypothalamus, and arcuate nucleus, was targeted for analysis due to its role in ingestive behavior, sleep-wake behavior, arousal and autonomic control. The frozen samples from each region were rapidly homogenized in 1–1.5 ml of Trizol® and centrifuged according to manufacturer's instructions (Invitrogen, Carlsbad, CA). RNA concentration and purity was determined with spectrophotometry (RINs ~9–10). RNA samples were then converted to cDNA with a high capacity cDNA archive kit (Applied Biosystems, Foster City, CA) according to manufacturer's instructions, as previously described (Boychuk et al., 2011; Hayward et al., 2015). All quantitative PCR (qPCR) reactions were run in triplicate in either an Applied Biosystems Prism 7500 Sequence Detection System (medulla) or Roche LightCycler 480II (hypothalamus). 18 s ribosomal RNA and all other mRNA sequences were determined by qPCR using Taqman probes and primers (ThermoFisher Scientific) and Taqman one-step qPCR master mix. 18 s (Rn03928990_g1) expression was used as internal controls in the medulla and hypothalamus, respectively. Other genes evaluated included: 11-HSD2: Rn00492539_m1; Per1: Rn01496757_m1; orexin A (ORX-A): Rn00565995_m1; ORX-receptor type 1 (ORX-R1): Rn00565032_m1; ORX-R2: Rn00565155_m1; and epithelial sodium channel subunit 1α (Scnn1a or α-ENAC): Rn00580652_m1. α-ENAC expression was evaluated in the medulla due to the link between