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ESTRADIOL AND ANGIOTENSIN II CROSSTALK IN HYDROMINERAL BALANCE: ROLE OF THE ERK1/2 AND JNK SIGNALING PATHWAYS

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Abstract—The angiotensin II (ANGII) receptor AT1 plays an important role in the control of hydromineral balance, mediating the dipsogenic and natriorexigenic effects and neuroendocrine responses of ANGII. While estradiol (E2) is known to modulate several actions of ANGII in the brain, the molecular and cellular mechanisms of the interaction between E2 and ANGII and its physiological role in the control of body fluids remain unclear. We investigated the influence of E2 (40 μg/kg) pretreatment and extracellular-signal-regulated kinase (ERK1/2) and c-Jun N-terminal kinase (JNK) cell signaling on the dipsogenic and natriorexigenic effects, as well as the neuroendocrine responses to angiotensinergic central stimulation in ovariectomized rats (OVX). We showed that the inhibitory effect of E2 on ANGII-induced water and sodium intake requires the ERK1/2 and JNK signaling pathways. On the other hand, E2 pretreatment prevents the ANGII-induced phosphorylation of ERK and JNK in the lamina terminalis. E2 therapy decreased oxytocin (OT) and vasopressin (AVP) secretion and decreased ERK1/2 phosphorylation in the supraoptic and paraventricular nuclei (SON and PVN, respectively). We found that the AVP secretion induced by ANGII required ERK1/2 signaling, but OT secretion did not involve ERK1/2 signaling. Taken together, these results demonstrate that E2 modulates ANGII-induced water and sodium intake and AVP secretion by affecting the ERK1/2 and JNK pathways in the lamina terminalis and ERK1/2 signaling in the hypothalamic nuclei (PVN and SON) in OVX rats.

Key words: sodium intake, water intake, vasopressin secretion, oxytocin secretion, ovariectomized rats.

INTRODUCTION

Angiotensin II (ANGII), a key component in the control of body fluid homeostasis, induces potent dipsogenic and natriorexigenic effects, as well as oxytocin (OT) and vasopressin (AVP) secretion when injected into the brain. These responses are thought to be mediated by ANGII type 1 receptors (AT1 receptor) (Antunes-Rodrigues et al., 1985, 2004; Beresford and Fitzsimons, 1992; Lenkei et al., 1997; Zhu et al., 2005; Reis et al., 2010). The AT1 receptor has seven transmembrane domains and is coupled to the Gq protein (de Gasparo et al., 2000). Its stimulation activates phospholipase C and consequent increase of protein kinase C (PKC) activity. In addition to this classical signaling pathway, AT1 receptor agonism also activates members of the mitogen-activated protein kinase family (MAPK), specifically p44/42 MAPK, also known as ERK1/2 (Sadoshima et al., 1995) and c-Jun N-terminal Kinase (JNK) (Mehta and Griendling, 2007). Although the PKC signaling pathway is associated with the activation of ERK1/2 or JNK in several systems, some studies have shown that stimulation of AT1 receptors activates ERK1/2 or JNK and PKC independently (Hines et al., 2003; Hunyady and Catt, 2006). In this context, Daniels et al. (2005, 2007, 2008) postulated that these intracellular signaling pathways of the AT1 receptor are involved in different behaviors. These authors showed that PKC signaling is involved in water intake control, whereas ERK1/2 signaling modulates the increased sodium intake induced by ANGII central administration.

Estradiol (E2) also modulates water and sodium intake, as shown by studies demonstrating that treatment with E2 reduces ANGII-induced water intake (Jonklaas and Buggy, 1985; Krause et al., 2003; Tanaka et al., 2003) and inhibits the natriorexigenic response induced by sodium depletion in ovariectomized (OVX) rats (Stricker et al., 1991; Fitzsimons, 1998; Almeida-Pereira et al., 2013). However, the role of E2 on ANGII-induced neurohypophysial hormone release has not been elucidated.

While the estrogen receptor (ER) is better known for its classic genomic actions, recent studies have shown that estrogens also activate nongenomic cellular signaling events. Stimulation of ER activates members of the MAPK family, such as ERK1/2 and JNK (Feng

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Abbreviations: ANGII, angiotensin II; AT1, ANGII type 1 receptor; AVP, vasopressin; DMSO, dimethyl sulfoxide; DUSPs, dual-specificity phosphatases; E2, estradiol; ER, estrogen receptor; ERK1/2, extracellular-signal-regulated kinase; GPCRs, G-protein-coupled receptors; icv, intracerebroventricular; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase family; MKP-1, MAPK phosphatase 1; MnPO, median preoptic nuclei; OT, oxytocin; OVLT, organum vasculosum of the lamina terminalis; OVX, ovariectomized; PBS, phosphate-buffered saline; PKC, protein kinase C; PVN, paraventricular nucleus; SFO, subfornical organ; SON, supraoptic nucleus.

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et al., 2001; Sétáló et al., 2002). However, there are no clear evidences on the physiological role of a potential interaction between the ER signaling pathway and ANGII in the maintenance of body fluid homeostasis.

For a better understanding of the physiological relevance of the interaction between E2 and AT1 signaling, we investigated the influence of E2 and MAPK signaling (ERK1/2 and JNK) on the dipsogenic and natriorexigenic effects as well as the neuroendocrine responses induced by angiotensinergic central stimulation in OVX rats. The present experiments represent the first direct test of the following hypotheses: (1) ERK1/2 or JNK signaling is required by E2 to regulate the behavioral effects of ANGII; (2) ERK1/2 signaling is involved in ANGII-induced OT or AVP secretion as well as in sodium appetite control; and (3) E2 regulates the neurohypophysial hormone secretion induced by ANGII central administration through changes in AT1 receptor-dependent MAPK family signaling.

In addition to partially mimicking the changes induced by senescence in females, bilateral ovariectomy is also a good model for studying the specific effects of E2 via E2 replacement, since intact rats exhibit cyclic changes in E2 levels. While menopause is an ovarian failure that is a normal part of the aging process and does not occur suddenly as in the case of ovariectomy (Landgren et al., 2004), its use as a model is still relevant because recently, more women are prematurely entering menopause due to either bilateral oophorectomy or cancer treatments (Lo Presti et al., 2004; Oktem and Oktay, 2009).

Postmenopausal estradiol replacement therapy is usually employed for decreasing the risk of cardiovascular and neurodegenerative diseases (Bold, 1999; Arevalo et al., 2015). However, uncontrolled sodium consumption increases the risk of hypertension (Denton et al., 1995), particularly in women in later menopause, who have a greater risk of developing hypertension (Khalil, 2005). The central and peripheral renin-angiotensin system is one of major regulators of blood pressure (Jeunemaitre et al., 1992; Coble et al., 2014). Therefore, understanding how the crosstalk between E2 and ANGII signaling controls hydromineral balance is crucial, as it can reveal potential pharmacological targets for preventing cardiovascular diseases, as well as mapping out the potential benefits of E2 replacement and its action on the central nervous system.

EXPERIMENTAL PROCEDURES

Animals and ethical approval

Female Wistar rats (~250 g) were obtained from the Animal Care Facility of the University of Sao Paulo, Brazil, and maintained under controlled temperature (25 ± 1 °C) conditions in a daily 12:12-h light–dark cycle (6:00 a.m.: 6:00 p.m.) with free access to tap water and pelleted food. All experiments were performed at night between 6:00 and 9:00 p.m., which is the period when rats are most active and, therefore, the best time to analyze their ingestive behavior. This study was conducted according to the "Guide for the Care and Use of Laboratory Animals" (NIH Publication No. 85-23, revised 1996). Every experimental protocol was approved by the Ethics Committee for Animal Use of the School of Medicine of Ribeirao Preto, University of Sao Paulo (protocol # 017/2012).

Surgeries

All surgeries were performed under 2,2,2-tribromoethanol (250 mg/kg, 2.5%, intraperitoneal, Sigma Aldrich, St Louis, MO, USA) anesthesia, followed by prophylactic doses (intramuscular) of veterinary pentabiotic (Fort Dodge, Campinas, SP, Brazil).

Lateral ventricle cannula implantation. The surgery for implanting a cannula into the right lateral ventricle was performed aseptically, eight days before the day of the experiment, using the following stereotactic coordinates: 0.5 mm (caudal to bregma); 1.5 mm from midline; 3.7 mm ventral to dura mater (Paxinos and Watson, 1997). The cannula was fixed in place with dental cement and screws, and stainless steel guide wires were inserted into the cannula to prevent its obstruction. After this surgery, the rats were subjected to ovariectomy under the same anesthetic conditions and placed in collective cages until their complete recovery.

Ovariectomy and treatment with estradiol. Rats were subjected to bilateral ovariectomy and randomly separated into two groups: OVX rats treated with vehicle (corn oil, 0.1 mL per rat, subcutaneous) or OVX rats treated with estradiol cypionate (E2; Pfizer, New York, NY, USA) at a subcutaneous dose of 10 μg/rat (~40 μg/kg) (OVX E2) (Kisley et al., 1999; Krause et al., 2006; Vilhena-Franco et al., 2011). The administration of vehicle or E2 began 24 h after surgery and was conducted once a day for 8 days between 7:00 and 10:00 a.m. On the eighth day after the OVX surgery the last dose of estradiol or vehicle was administered, and the experiments were performed at night. The efficacy of the surgical procedure and E2 therapy were confirmed based on body weight gain and measurements of the uterine index. On the eighth day, the OVX rats treated with E2 were found to have gained less weight than the OVX rats treated with oil (4.0 ± 1.27 vs. 15.27 ± 2.19 g, $t_{(20)} = 4.45$, $p < 0.001$, $n = 11$) and had a higher uterine index (278.30 ± 14.77 vs. 74.62 ± 3.09 mg/100 g body weight, $t_{(23)} = -12.99$, $p < 0.001$, $n = 12/13$). Thus, these data validate the OVX procedure and E2 therapy.

Experimental protocols

Experiment 1: effect of the mitogen-activated protein kinase (MEK1/2) inhibitor U0126 on ANGII-induced water and NaCl intake in OVX and OVX E2 rats. On the day of the experiment, oil-treated OVX rats and E2-treated OVX rats received an intracerebroventricular (icv) administration of the MEK1/2 inhibitor U0126 (1 mM/2 μL/rat, Calbiochem) or the vehicle (0.9% sterile