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The role of estradiol in adrenal insufficiency and its interaction with corticosterone on hydromineral balance

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ABSTRACT

Estradiol (E2) plays an important role in controlling the homeostasis of body fluids. Several studies have reported the involvement of the hypothalamic pituitary adrenal axis (HPA) in the homeostatic control of hydromineral balance and the influence of estrogens on the modulation of this system. Nevertheless, until now, the physiological relevance of HPA axis activity on the hydromineral balance in females has not yet been fully elucidated. Therefore, the objective of the present study was to evaluate the effects of E2 (20 μg/animal) pretreatment on neuroendocrine and hydroelectrolyte changes induced by adrenalectomy (ADX) with or without glucocorticoid hormone replacement (corticosterone, CORT; 10 mg/kg) in ovariectomized rats (OVX). The results show that sodium appetite, natriuresis and the elevated plasma angiotensin II (ANG II) concentration induced by ADX were attenuated by E2 pretreatment. Additionally, a reduction of AT1 mRNA expression in the subfornical organ (SFO) and an increase in plasma atrial natriuretic peptide (ANP) concentrations by E2 pretreatment were observed. E2 pretreatment reversed the reduction in water intake induced by ADX in ADX CORT-replaced rats. Moreover, E2 pretreatment attenuated corticotropin releasing factor (CRF) mRNA expression in the paraventricular nucleus (PVN) induced by ADX. In contrast, E2 pretreatment increased CRF mRNA expression in the PVN in ADX CORT-replaced rats. Taken together, these results suggest that E2 has an important role in the modulation of behavioral and neuroendocrine responses involved in the maintenance of body fluid homeostasis in ADX rats with or without glucocorticoid replacement therapy.

Introduction

Appetite for sodium and thirst are important homeostatic behaviors that contribute to the maintenance of body fluid homeostasis. Adrenal insufficiency induced by bilateral adrenalectomy (ADX) is known to alter water and sodium balance due to the inability of the renal to maintain sodium and water balance (Fitzsimons, 1998) in response to an aldosterone deficit (Krause and Sakai, 2007). Sodium depletion and circulating volume reduction induce an increase in renin activation and, consequently, an increase in the circulating levels of angiotensin II (ANG II). ANG II mediates the dipsogenic and natriorexigenic responses elicited by hypovolemic and hyponatremic stimuli (Fitzsimons, 1998), and these ANG II effects have been postulated to be primarily mediated by its type AT1 receptor (Beresford and Fitzsimons, 1992). Additionally, ANG II exerts important renal effects such as antidiuresis and antinatriuresis, which greatly contribute to the hydroelectrolyte balance (Hollenberg, 1984).

Several studies have investigated the involvement of the hypothalamic–pituitary–adrenal (HPA) network in the control of body fluid homeostasis. Experimental evidence shows that thirst and sodium appetite are stimulated by systemic administration of adrenocorticotropic hormone (ACTH; Li and Whitworth, 1992; Denton et al., 1999), whereas other studies indicate that CRF also participates in the regulation of sodium intake, but the mechanisms influenced by CRF in the control of sodium appetite have not been elucidated (Watts, 1992).

Gonadal hormones, especially estrogen, play important roles in the control of body fluid homeostasis. It is well established in the literature that estradiol (E2) regulates thirst and sodium appetite in females, although the precise nature of their control has not been fully elucidated (Covian and Antunes-Rodrigues, 1963; Danielsen and Buggy, 1980; Jonklaas and Buggy, 1984; Fitzsimons, 1998). Additionally, E2 has an important antinatriuretic effect, which induces an increase in renal sodium tubular reabsorption (Brunette and Leclerc, 2001). The neuroendocrine control of the secretion of vasopressin (AVP), oxytocin (OT) and atrial natriuretic peptide (ANP) is also modulated by estrogen. Several studies have shown that E2 has a stimulatory effect on the secretion of OT, ANP and AVP (Forsling et al., 1982; Jankowski et al., 2001; Mecawi et al., 2011; Vilhena-Franco et al., 2011; Yamaguchi et al., 1979). The secretion of AVP and OT occurs in response to osmotic, volemic and non-osmotic stimuli (for review see Antunes-Rodrigues et al. (2004)). In addition to its function in controlling blood pressure, ANP also plays an important role in the control of hydromineral homeostasis, where

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it exerts opposite effects to ANG II (Antunes-Rodrigues et al., 1985, 1986; Quirion et al., 1988).

HPA axis activity is also influenced by gonadal steroids as illustrated by the elevated plasma levels of glucocorticoids observed in females (Critchlow et al., 1963; Kitay, 1961). In parallel with corticosterone (CORT) hypersecretion, estradiol was shown to increase ACTH secretion and mRNA expression of CRH in the PVN (Silva et al., 2010; Viau and Meaney, 1991). However, other studies have shown that E2 decreases or does not change the plasma levels of ACTH and CORT as well as mRNA expression of CRF in the PVN (Ferreira-Silva et al., 2009; Figueiredo et al., 2002; Gerrits et al., 2005). In this context, the physiological relevance of HPA axis activity related to the control of body fluid homeostasis as well as the mechanisms related to reproductive function in females are not yet fully elucidated. Therefore, this study aims to elucidate the influence of E2 and their interaction with corticosterone on behavioral, endocrine and molecular changes involved in the control of body fluid homeostasis under adrenal insufficiency, using the experimental model of adrenalectomy with or without glucocorticoid replacement in ovariectomized rats.

Materials and methods

Animals

Female Wistar rats (~250 g) obtained from the animal facility located on the Campus of Ribeirao Preto, University of Sao Paulo, Brazil, were maintained under controlled temperature (23 ± 1 °C) and were exposed to 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 experimental procedures were performed in the morning between 08:00 and 11:00 A.M. This study was conducted according to the "Guide for the Care and Use of Laboratory Animals" (NIH Publication No. 85-23, revised 1996), and the experimental protocols were approved by the Ethical Committee for Animal Use of the School of Medicine of Ribeirao Preto, University Sao Paulo (protocol # 034/2010).

Surgeries

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

Ovariectomy and treatment with estradiol

Rats were subjected to bilateral ovariectomies (OVX) and randomly separated into OVX groups treated with vehicle (corn oil, 0.2 mL per rat, subcutaneous) or OVX rats treated with estradiol cypionate (E2; Pfizer, New York, NY, USA) at a dose of 20 μg/animal, subcutaneous (OVX-E2). The administration of vehicle or E2 began 24 h after surgery and was conducted once a day for 14 days between 07:00 and 10:00 A.M. The last administrations of estradiol or vehicle were performed 24 h before euthanasia. The efficiency of the surgical procedure and treatment with E2 was confirmed by body weight gain during the first 7 days and the uterine index at the end of the experiments (after 14 days). On the seventh day of treatment with E2, the rats had a lower weight gain compared to the OVX rats (21.62 ± 1.58 vs. 33.56 ± 2.14 g, $t_{(47)} = 4.45$, $p < 0.001$, $n = 24/25$). At the end of the experiment, after 14 days of treatment with E2, the treatment increased the uterine index of the animals ($F_{7,93} = 737.89$, $df = 1$, $p < 0.001$); however, the uterine index was not affected by adrenalectomy or replacement with corticosterone.

Adrenalectomy and corticosterone replacement

Seven days after treatment with E2 or vehicle, the OVX and OVX-E2 rats were submitted to bilateral adrenalectomies (ADX) or sham operations and randomly separated into groups treated with vehicle (corn oil in 5% ethanol, 0.2 mL per rat, sc, OVX-SHAM, OVX-E2-SHAM, OVX-ADX, OVX-E2-ADX) or corticosterone (B; Sigma, St Louis, MO, USA) at 10 mg/kg, sc (OVX-SHAM-B, OVX-E2-SHAM-B, OVX-ADX-B, OVX-E2-ADX-B). The administration began immediately after ADX surgery and was conducted once a day for 7 days between 5:00 and 6:00 P.M. The last administrations of corticosterone or vehicle were performed on the day before euthanasia. At the end of each study, the success of the surgical procedure was verified by post-mortem inspection to verify the absence of the adrenal glands. The last administrations of vehicle, estradiol and corticosterone were performed 24 h before euthanasia.

Water and sodium intake

Four days after treatment with E2 or vehicle, the animals were placed in individual metabolic cages to appropriately adapt and were provided with two bottles per animal filled with hypertonic saline solution (1.8% NaCl) or tap water, and they were provided with food ad lib. Fluid intake was evaluated daily for 6 days following ADX. The ratio of water to sodium intake was calculated as: (1.8% NaCl intake/water intake + 1.8% NaCl intake) × 100 (Frankmann et al., 1991).

As body weight gain (Fig. 1C) varied significantly in function of treatments (oil vs. estradiol, $F_{7,93} = 8.22$, $df = 1$, $p < 0.01$; sham vs. ADX, $F_{7,93} = 8.77$, $df = 1$, $p < 0.01$; between the three factors, $F_{7,93} = 4.67$, $df = 1$, $p < 0.05$) the measures were analyzed both as absolute and as body weight-adjusted values. The results for absolute and body weight-adjusted measures did not show differences in the pattern of response. Thus only the adjusted results are presented. Therefore, the values are expressed as mL/100 g body weight. In addition, for better visualization the data were presented in the figures as water and sodium intakes accumulated during six days and are expressed as mL/100 g body weight/6 days. Similarly, this procedure was performed with the ratio of water to sodium intake analysis. Table 1 showed the daily values of the analyzed responses.

Determination of urinary sodium

The urine samples were collected on days 1, 3, and 6 after ADX for urinary sodium concentration determined by flame photometry (Flame Photometer, Micronal Model B-262, Sao Paulo, SP, Brazil). The results are expressed as mEq/100 g body weight. In addition, for better visualization the data were presented in the figure as accumulated during three days and are expressed as mL/100 g body weight/3 days. Table 1 showed the daily values of the analyzed responses.

Determination of plasma sodium concentration and hematocrit

Plasma sodium concentration was analyzed using flame photometry (Micronal B-262, Sao Paulo, SP, Brazil). Plasma volume was indirectly inferred from the hematocrit values that were determined using small aliquots of trunk blood collected in capillary tubes and was expressed as the percentage of cells in the blood. To determine these parameters, the animals were decapitated on the fourteenth day of treatment with estradiol or vehicle.

Blood collection, hormonal extractions and immunoassays

After fourteen days of E2 treatment or seven days of replacement with CORT, the animals were decapitated, and their trunk blood was collected for OT, AVP, ANG II, ANP and CORT analysis. Blood collection was performed in chilled plastic tubes containing heparin (10 μL of heparin per mL of blood) to measure plasma OT, AVP and CORT or ethylenediaminetetraacetic acid (2 mg/mL) and proteolytic enzyme inhibitors (10 μL of 1 mM phenylmethylsulfonyl fluoride, 10 μL of 500 mM pepstatin A and 20 μL of p-hydroxymercuribenzoate per mL of blood) for plasma ANP and ANG II determination. Plasma was obtained after