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MODULATION OF THE ACTIVITY OF VASOPRESSINERGIC NEURONS BY ESTROGEN IN RATS REFED WITH NORMAL OR SODIUM-FREE FOOD AFTER FASTING

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Abstract—Feeding increases plasma osmolality and ovarian steroids may influence the balance of fluids. Vasopressin (AVP) neurons in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) express estrogen receptor type β (ERβ), but not estrogen receptor type α (ERα). The circumventricular organs express ERα and project efferent fibers to the PVN and SON. Our aim was to assess whether interactions exist between food state-related osmolality changes and the action of estrogen on AVP neuron activity and estrogen receptor expression. We assessed plasma osmolality and AVP levels; fos-coded protein (FOS)- and AVP-immunoreactivity (-IR) and FOS-IR and ERα-IR in the median preoptic nucleus (MnPO) and organ vasculosum lamina terminalis (OVLT) in estrogen-primed and unprimed ovariectomized rats under the provision of *ad libitum* food, 48 h of fasting, and subsequent refeeding with standard chow or sodium-free food. Refeeding with standard chow increased plasma osmolality and AVP as well as the co-expression of FOS-IR/AVP-IR in the PVN and SON. These responses were not altered by estrogen, with the exception of the decreases in FOS-IR/AVP-IR in the lateral PVN. During refeeding, estrogen modulates only a subpopulation of AVP neurons in the lateral PVN. FOS-ERα co-expression in the ventral median preoptic nucleus (vMnPO) was reduced by estrogen and increased after refeeding with standard chow following fasting. It appears that estrogen may indirectly modulate the activity of AVP neurons, which are involved in the mechanism affected by hyperosmolality-induced refeeding after fasting. This indirect action of estrogen can be at least in part via ERα in the vMnPO.

Key words: vasopressin, estrogen, estrogen receptor-α, osmolality, fasting, refeeding.

INTRODUCTION

Receptors of the central nervous system that detect changes in osmolality (osmoreceptors) are located mainly in the organum vasculosum of the lamina terminalis (OVLT), subfornical organ (SFO), and area postrema (AP) (Arima et al., 1998). The circumventricular organs (CVOs) have been suggested to be involved in monitoring alterations of plasma osmolality after both hyperosmotic challenge and feeding (Starbuck and Fitts, 2002; Hiyama et al., 2004). Conformational changes in osmoreceptor cells stimulate neurons in the supraoptic (SON) and paraventricular (PVN) nuclei, which secrete vasopressin (AVP) and oxytocin (OT). These hormones act on water, sodium, and chloride excretion mechanisms (Sladek and Knigge, 1977; Oliet and Bourque, 1993).

Plasma osmolality may increase after feeding or sodium intake (Bloom et al., 1975; Burlet et al., 1992). During feeding, the action of AVP contributes to controlling the balance of water and changes in blood pressure as well as ceasing food intake (Pittman et al., 1982; Palkovits, 1984; Langhans et al., 1991).

Estrogen may influence fluid balance by modulating the vasopressinergic and oxytocinergic systems (Caligioni and Franci, 2002; Somponpun, 2007). However, this action is not fully understood. Some studies have shown that estrogen has no significant effect on OT and AVP levels in the PVN (Van Tol et al., 1988; Crowley et al., 1993). Other researchers have demonstrated that estrogen treatment can reduce OT-mRNA levels in the PVN of previously ovariectomized rats (Shughrue et al., 2002). Studies involving double labeling *in vivo*, autoradiography, and immunohistochemistry have also shown that radiolabeled estrogen concentrates in OT and AVP neurons (Rhodes et al., 1981).

The expression of estrogen receptor subtype β (ERβ) in OT and AVP neurons has been well described in the literature (Hrabovszky et al., 1998; Laflame et al., 1998; Sladek and Somponpun, 2008). Estrogen may directly

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Abbreviations: ANOVA, analysis of variance; AVP, vasopressin; BSA, bovine serum albumin; CVOs, circumventricular organs; dMnPO, dorsal median preoptic nucleus; ERα, estrogen receptor-subtype α; ERβ, estrogen receptor-subtype β; FOS, fos-coded protein; IgG, immunoglobulin G; IR, immunoreactivity; MnPO, median preoptic nucleus; mRNA, messenger ribonucleic acid; OT, oxytocin; OVLT, organum vasculosum of the lamina terminalis; OVX, bilateral ovariectomy; PaML, lateral subdivision of the PVN; PaMM, medial subdivision of the PVN; PBS, phosphate-buffered saline; PFA, paraformaldehyde; PVN, paraventricular nucleus; RIA, radioimmunoassay; SEM, standard error of the mean; SON, supraoptic nucleus; vMnPO, ventral median preoptic nucleus.

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regulate AVP and OT secretion via ERβ; these receptors are expressed in both parvocellular and magnocellular neurons (Shughrue et al., 2002; Sladek and Somponpun, 2008). Indirect action of estrogen on OT and AVP neurons in rats via estrogen receptor subtype α (ERα) cannot be ruled out (Somponpun et al., 2004; Grassi et al., 2010) because the neurons of the MnPO and OVLT that express these receptors project to the SON (Voisin et al., 1997). In castrated rats, estrogen treatment either significantly reduces (Suzuki and Handa, 2004) or induces no change in (Greco et al., 2001) ERβ expression in the PVN. The disparity of these results may be due to differences in the doses of estrogen administered, the duration of treatment, or the amount of time between ovariectomy and treatment initiation. Changes in ER expression may alter the impact of gonadal steroids by amplifying or reducing the effects of increased ligand levels (Greco et al., 2001; Patisaul et al., 2001; Somponpun and Sladek, 2003; Suzuki and Handa, 2004).

Little is known regarding the mechanisms and pathways by which estrogen interferes with the activity of AVP neurons and with the liquid and electrolyte balance associated with the food state. Our aim was to analyze the interactions between food state-related changes in osmolality and the action of estrogen through ERα on the activity of AVP neurons.

EXPERIMENTAL PROCEDURES

Animals

The experimental protocols and animal manipulations followed the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Ethics Committee of the Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo. Female Wistar rats weighing 160–180 g were housed in individual cages under controlled temperature conditions (22 ± 1 °C) with a 12-h light–dark cycle and given free access to water and chow unless otherwise specified. Bilateral ovariectomy (OVX) was performed under 2.5% tribomoethanol anesthesia (1 ml/100 g body weight, i.p., Sigma–Aldrich, Steinheim, Germany). After surgery, all rats were treated with a prophylactic dose of pentabiotic (0.1 ml/rat, Fort Dodge, Campinas, Brazil) and analgesic flunixin meglumine (2.5 mg/kg i.m., Banamine®, Schering-Plough, Rio de Janeiro, Brazil).

Experimental protocol

Fourteen days after surgery, the OVX animals were treated with either estradiol cypionate (10 μg/0.1 ml/animal, 10 μg/0.1 ml, s.c., Pfizer, Paulinia, SP, Brazil) or vehicle (oil) during the 3 days prior to the experiment and divided into four groups subjected to the following treatment conditions: *ad libitum* standard chow; 48 h of fasting (started at 1 p.m. on the second day of treatment); refeeding for 2 h with standard chow after 48 h of fasting; and refeeding for 2 h with sodium-free food after 48 h of fasting. At 3 p.m. on the 4th day. Each group of animals was subdivided in two subgroups. One subgroup was anesthetized with 2.5% tribromoethanol (1 ml/100 g of body weight, i.p.) followed by the injection of paraformaldehyde (PFA)-4%. Transcardiac perfusion was conducted via the ascending aorta with approximately 80 ml of 0.01 M phosphate-buffered saline (PBS pH 7.4), followed by 320 ml of cold PFA-4% to remove the brain. Other group was decapitated to withdraw trunk blood. The plasma obtained by centrifugation was used to measure osmolality and AVP concentration.

Osmolality

Plasma osmolality was determined by assessing the freezing point with a Fisk Mark-3 Osmometer (Fiske Associates, Norwood, MA, USA).

Radioimmunoassay (RIA)

Plasma AVP was measured via RIA following extraction with petroleum ether and acetone. RIA was performed using specific standard antibodies from Bachem-Peninsula Labs (Torrance, CA, USA) and a secondary antibody produced by Dr. Franci's Laboratory (Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Brazil). Results are reported as mean ± standard error of the mean (SEM). The minimum detectable dose was 0.2 pg/ml, with intra- and inter-assay errors of 5.8% and 12%, respectively.

Immunofluorescence

Tissue preparation. Rat brains were removed immediately after perfusion and post-fixed in PFA-4% for 2 h, then immersed in 30% sucrose in PBS for cryoprotection. The brains were frozen in cooled isopentane for storage at −70 °C. Serial coronal sections with a thickness of 14 μm for the PVN and SON or 20 μm for the OVLT and MnPO were sliced using a cryostat according to the brain atlas (Swanson, 1992). The medial (PaMM) and lateral (PaML) subdivisions of the PVN were defined at −1.53 and −1.78 mm from the bregma, respectively, and the SON was defined at −1.08 mm from the bregma. The OVLT, dorsal MnPO (dMnPO), and ventral MnPO (vMnPO) were defined at −0.00, −0.46, and −0.26 mm from the bregma, respectively.

Sections of different brain areas were rinsed several times in PBS prior to immersion in 0.1 M glycine in PBS to remove excess aldehydes. After washing in PBS, the sections were incubated in PBS containing 0.1% Triton X-100 and 1% bovine serum albumin (BSA), followed by incubation with primary antibodies and then secondary antibodies. All antibodies were diluted in PBS containing 0.1% Triton X-100 and 1% BSA.

Double-labeling for AVP/FOS or ERα/FOS

AVP and fos-coded protein (FOS) labeling was performed in the PVN and SON sections via overnight incubation with a mouse monoclonal anti-AVP antibody diluted 1:1000 (a kind gift from Dr. Harold Gainer, National Institutes of Health, Bethesda, MD, USA) and rabbit polyclonal anti-FOS (SC-52, Santa Cruz Biotechnology,