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Estrogen suppresses the stress response of prolactin-releasing peptide-producing cells

Sachika Adachi[a], Akikazu Mochiduki[a], Haruki Nemoto[a], Binggui Sun[a], Ken Fujiwara[a,b], Hirokazu Matsumoto[c], Kinji Inoue[a,*]

[a] *Department of Regulation Biology, Faculty of Science, Saitama University, 255 Shimo-ohkubo, Sakura-ku, Saitama 338-0825, Japan*
[b] *Department of Physiology, Jichi Medical School, Minamikawachi, Kawachi, Tochigi 329-0498, Japan*
[c] *Discovery Research Laboratories I, Pharmaceutical Discovery Research Division, Takeda Chemical Industries Ltd, 10 Wadai, Tsukuba, Ibaraki 300-4293, Japan*

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Abstract

Prolactin-releasing peptide (PrRP) is known to be produced in A1/A2 noradrenergic neurons and to mediate the stress response. Our preliminary experiment showed that PrRP neurons in the A2 region differed between males and females in terms of c-Fos expression. In addition it has been reported that estrogen receptor α is detectable in A2 PrRP neurons. Therefore, we speculated that the stress response of PrRP neurons is modified by estrogen. We, therefore, examined c-Fos expression in A2 PrRP neurons during the estrous cycle and found that c-Fos accumulation in PrRP neurons was significantly decreased in estrus compared with in proestrus, metestrus and diestrus. This suggests that estrogen suppresses the activation of PrRP neurons. We thus administered diethylstilbestrol (DES) to ovariectomized rats and then added restraint stress. The data clearly showed that PrRP cells in DES-administered rats significantly suppressed c-Fos accumulation induced by stress.

Keywords: Prolactin-releasing peptide; Stress; Estrogen; c-Fos protein; Immunocytochemistry; Rat

Prolactin (PRL)-releasing peptide (PrRP) was first isolated as a ligand of an orphan G protein-coupled receptor (hGR3), which is highly expressed in the human anterior pituitary [5]. PrRP is known to stimulate PRL release from the anterior pituitary both in vitro [5] and in vivo [14,20]. It has been demonstrated that PrRP neurons exist in the dorsomedial hypothalamic nucleus and the A1/A2 noradrenergic neurons of the medulla oblongata [3,6,7,10]. It is known that PrRP neurons project to the paraventricular hypothalamic nucleus (PVN) and make contact with corticotropin-releasing hormone (CRH) cell bodies in the PVN [10,13,15], in which PrRP receptors exist [18]. Intracerebroventricular (i.c.v.) administration of PrRP stimulates oxytocin and vasopressin secretion from magnocellular neurons [12,17], and CRH secretion from parvocellular neurons in the PVN [13,19]. In addition, i.c.v. administration of PrRP stimulates adrenocorticotropic hormone (ACTH) and β-endorphin secretion through hypothalamic CRH [13,19]. It has been revealed that PrRP evokes the hypothalamo–pituitary–adrenal axis, and water immersion-restraint stress stimulates PrRP neurons [11]. Therefore, we have demonstrated that PrRP plays an important role as a stress mediator in the central nervous system [8,11,16].

We have also observed that c-Fos expression in A2 PrRP neurons differs between male and female rats. Our previous study demonstrated that activated A2 PrRP neurons were few in the male under normal conditions [11], but that the neurons varied in the normal female. A2 PrRP neurons have been reported to have estrogen receptor α (ERα) [9]. We, therefore, considered that activation of PrRP neurons might be modified by gonadal steroid hormone. To elucidate the effect of estrogen on PrRP neurons, in this study, we performed immunocytochemical studies, i.e., we examined the change

* Corresponding author. Tel.: +81 48 858 3422; fax: +81 48 858 3422.
E-mail address: kininoue@seitai.saitama-u.ac.jp (K. Inoue).

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in c-Fos expression in A2 PrRP neurons during the estrous cycle. We also examined the effect of estrogen on the stress response of A2 PrRP neurons.

Adult Wistar female rats were housed in group cages illuminated from 08:00 to 20:00 h (12 h cycle). The room temperature was controlled at between 21 and 24 °C. The animals had free access to food and water. All procedures were performed in accordance with institutional guidelines for animal care of NIH. The estrous cycle stage was monitored by collection of daily vaginal smears for 1 week. For the ovariectomized group (OVX group), bilateral ovariectomy was performed 1 week before sampling. In addition, the group of diethylstilbestrol (DES; Sigma Chemical Co., St. Louis, MO) -treated ovariectomized rats (OVX + DES group) had a subcutaneously inserted silastic tube (inner diameter 1.58 mm, outer diameter 2.41 mm, length 20 mm; Dow Corning, MI), which was filled with DES at the time of ovariectomy. The rats were immobilized in stainless restrainers (Natsume, Tokyo, Japan). Restraint stress was performed for 30 min for ovariectomized (OVX + Stress group) and DES-treated ovariectomized rats (OVX + DES + Stress group), and the rats were fixed 2 h after the end of the immobilization stress. For fixation, animals were deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.; Dainippon Pharmaceutical, Osaka, Japan), and perfused first with saline and then with 5% acrolein (Tokyo Kasei Kogyo, Tokyo, Japan) in 0.07 M phosphate buffer (pH 7.4). Their brains were removed and immersed in the same fixative for 24 h, and then in PBS containing 30% sucrose for more than 24 h at 4 °C. Frontal sections (40 μm thick) of the brains were cut with a cryomicrotome. Immunocytochemistry was performed using the free floating technique, which is a sensitive method. Briefly, the sections were washed with PBS and then immersed sequentially in the following solutions: (1) 0.5% sodium metaperiodate in PBS for 20 min; (2) 1% sodium borohydride in PBS for 20 min; (3) 1% normal fetal bovine serum and 0.4% Triton X-100 in PBS (TNBS) for 1 h; (4) mouse anti-PrRP monoclonal antibody (P2L-1T, 2 μg/ml) and rabbit polyclonal anti-c-Fos (1:2000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) in TNBS for 24 h; (5) biotinylated anti-rabbit IgG (1:300; Vector Laboratories, Inc., Temecula, CA) in TNBS for 2 h; (6) avidin-biotinylated HRP complex (ABC Elite; Vector Laboratories, Inc.) for 30 min; (7) 0.05% 3,3-diaminobenzidine-tetrachloride (DAB) mixed with 1%

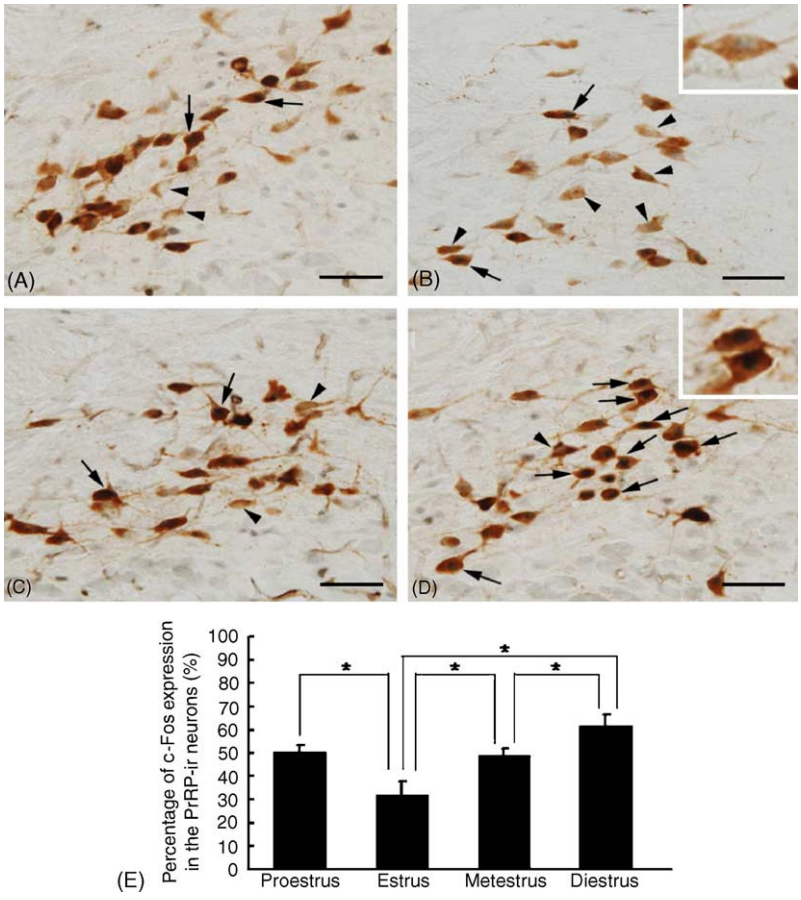

Fig. 1. Change in c-Fos expression in PrRP-ir neurons during the estrous cycle. The photomicrographs show double immunocytochemistry for PrRP and c-Fos in the A2 region. PrRP and c-Fos were stained brown and black, respectively. Enlargements of the neurons are shown as insets. (A) proestrus, (B) estrus, (C) metestrus, and (D) diestrus. *Arrows* indicate the c-Fos positive nuclei in PrRP-ir neurons, and *arrowheads* the c-Fos negative nuclei in PrRP-ir neurons. The histogram shows the percentages of c-Fos expression in PrRP-ir neurons (E). Scale bars: 100 μm. The values are mean ± S.E.M. ($n = 4$–5, $^*P < 0.05$).