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Research Report

Prolactin-sensitive neurons express estrogen receptor-α and depend on sex hormones for normal responsiveness to prolactin

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ABSTRACT

Estrogens and prolactin share important target tissues, including the gonads, brain, liver, kidneys and some types of cancer cells. Herein, we sought anatomical and functional evidence of possible crosstalk between prolactin and estrogens in the mouse brain. First, we determined the distribution of prolactin-responsive neurons that express the estrogen receptor α (ERα). A large number of prolactin-induced pSTAT5-immunoreactive neurons expressing ERα mRNA were observed in several brain areas, including the anteroventral periventricular nucleus, medial preoptic nucleus, arcuate nucleus of the hypothalamus, ventrolateral subdivision of the ventromedial nucleus of the hypothalamus (VMH), medial nucleus of the amygdala and nucleus of the solitary tract. However, although the medial preoptic area, periventricular nucleus of the hypothalamus, paraventricular nucleus of the hypothalamus, retrochiasmatic area, dorsomedial subdivision of the VMH, lateral hypothalamic area, dorsomedial nucleus of the hypothalamus and ventral premammillary nucleus contained significant numbers of prolactin-responsive neurons, these areas showed very few pSTAT5-immunoreactive cells

Abbreviations: 3v, third ventricle; ARH, arcuate nucleus of the hypothalamus; ARH tub, tuberal ARH; ARH cau, caudal ARH; AVPV, anteroventral periventricular nucleus; BST, bed nucleus of the stria terminalis; DAB, 3,3′-diaminobenzidine; DEPC, diethylpyrocarbonate; DMH, dorsomedial nucleus of the hypothalamus; ERα, estrogen receptor α; ERE, estrogen response element; f, fornix; KPBS, 0.02 M potassium PBS; LH, luteinizing hormone; LHA, lateral hypothalamic area; MCH, melanin-concentrating hormone; MeA, medial nucleus of the amygdala; MeApd, posterodorsal subdivision of the MeA; MPA, medial preoptic area; MPN, medial preoptic nucleus; NTS, nucleus of the solitary tract; PBS, phosphate-buffered saline; PeN, periventricular nucleus of the hypothalamus; PMV, ventral premammillary nucleus; PrlR, prolactin receptor; PVH, paraventricular nucleus of the hypothalamus; pSTAT5, phosphorylated form of signal transducer and activator of transcription-5; RCA, retrochiasmatic area; SOCS, suppressor of cytokine signaling; SSC, sodium chloride/sodium citrate; STAT5, signal transducer and activator of transcription-5; pSTAT5-ir, pSTAT5 immunoreactivity; oc, optic chiasm; OVLT, organum vasculosum of lamina terminalis; OVX, ovariectomized; VMH, ventromedial nucleus of the hypothalamus; VMHdm, dorsomedial subdivision of the VMH; VMHvl, ventrolateral subdivision of the VMH

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expressing ERα mRNA. Second, we evaluated prolactin sensitivity in ovariectomized mice and observed that sex hormones are required for a normal responsiveness to prolactin as ovariectomized mice showed a lower number of prolactin-induced pSTAT5 immunoreactive neurons in all analyzed brain nuclei compared to gonad-intact females. In addition, we performed hypothalamic gene expression analyses to determine possible post-ovariectomy changes in components of prolactin signaling. We observed no significant changes in the mRNA expression of prolactin receptor, STAT5a or STAT5b. In summary, sex hormones exert a permissive role in maintaining the brain's prolactin sensitivity, most likely through post-transcriptional mechanisms.

1. Introduction

Estrogens and prolactin share important target tissues. For example, a critical crosstalk occurs between prolactin and estrogens in the murine corpus luteum, where prolactin-induced signal transducer and activator of transcription 5 (STAT5) signaling is required for the appropriate expression of estrogen and luteinizing hormone (LH) receptors (Frasor and Gibori, 2003). Consequently, the *null* mutation of the prolactin receptor (PrlR) results in infertility (Ormandy et al., 1997a). The interaction between estrogens and prolactin is likely widespread in several physiological systems because knockout mice for *Esr1*, *Prlr* and

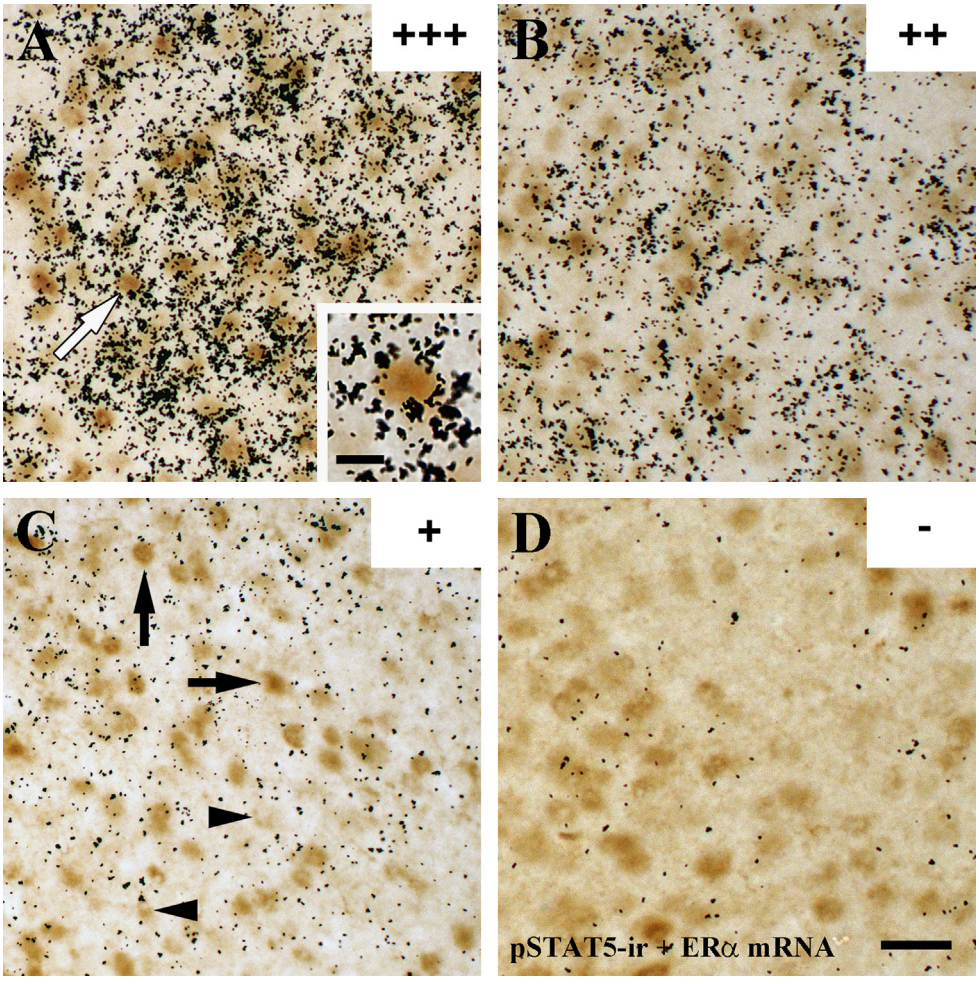

Fig. 1 – Classification of the proportion of prolactin-responsive cells expressing ERα mRNA. (A)–(D). Brightfield photomicrographs of coronal sections of the mouse brain showing representative areas that exhibited high (+++), moderate (++), low (+) and very low to virtually absent (−) proportion of prolactin-responsive cells expressing ERα mRNA. The inset shows an example of a double-labeled neuron exhibiting nuclear staining indicative of pSTAT5-ir, surrounded by a cluster of silver grains representing the cytoplasmic expression of ERα mRNA. The white arrow in (A) indicates the position of the neuron shown enlarged in the inset and the black arrows in (C) examples of pSTAT5-positive neurons. Arrowheads point to examples of neurons considered pSTAT5-negative. Scale bar: (A)–(D)=20 μm; Inset=5 μm.