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GPR30 mediates anorectic estrogen-induced STAT3 signaling in the hypothalamus

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ABSTRACT

Objective. Estrogen plays an important role in the control of energy balance in the hypothalamus. Leptin-independent STAT3 activation (i.e., tyrosine[705]-phosphorylation of STAT3, pSTAT3) in the hypothalamus is hypothesized as the primary mechanism of the estrogen-induced anorexic response. However, the type of estrogen receptor that mediates this regulation is unknown. We investigated the role of the G protein-coupled receptor 30 (GPR30) in estradiol (E2)-induced STAT3 activation in the hypothalamus.

Materials/methods. Regulation of STAT3 activation by E2, G-1, a specific agonist of GPR30 and G-15, a specific antagonist of GPR30 was analyzed *in vitro* and *in vivo*. Effect of GPR30 activation on eating behavior was analyzed *in vivo*.

Results. E2 stimulated pSTAT3 in cells expressing GPR30, but not expressing estrogen receptor ERα and ERβ. G-1 induced pSTAT3, and G-15 inhibited E2-induced pSTAT3 in primary cultures of hypothalamic neurons. A cerebroventricular injection of G-1 increased pSTAT3 in the arcuate nucleus of mice, which was associated with a decrease in food intake and body weight gain.

Conclusions. These results suggest that GPR30 is the estrogen receptor that mediates the anorectic effect of estrogen through the STAT3 pathway in the hypothalamus.

1. Introduction

Estrogen reduces food intake and body weight and promotes an increase in the ratio of subcutaneous to visceral fat in both animals and humans [1]. The main targets of this CNS-mediated metabolic effect of estrogen are the hypothalamic nuclei. A lack of hypophagia and weight loss has been observed in STAT3-knockout mice following estradiol

Abbreviations: CNS, central nervous system; DAB, 3,3′-diaminobenzidine; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; EGFR, epidermal growth factor receptor; ER, estrogen receptor; E2, estradiol; GPR30, G protein-coupled receptor 30; ICV, intracerebroventricular; JAK, Janus kinase; PLC, phospholipase C; POMC, proopiomelanocortin; pSTAT3, tyrosine[705]-phosphorylation of signal transducer and activator of transcription 3; RT-PCR, reverse transcription polymerase chain reaction; SDS, sodium dodecyl sulfate; SEM, standard error of the mean; STAT3, signal transducer and activator of transcription 3.

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(E2) treatment [2], which suggests that the anorectic action of estrogen is mediated by STAT3 activation in the arcuate nucleus of the hypothalamus. This anorectic action of estrogen also appears to be independent of leptin, a hormone that exerts its effects on energy homeostasis via the JAK-STAT pathway in the hypothalamus [2,3]. However, the estrogen receptor that mediates the STAT3-dependent anorexigenic effect of estrogen has not been identified.

The estrogen receptors (ERs), ERα and ERβ, are ligand-activated nuclear transcription factors that are members of the nuclear receptor superfamily. G protein-coupled receptor 30 (GPR30), which was originally identified as an orphan G protein-coupled receptor, also interacts with estrogen and is involved in the rapid effects of estrogen [4]. ERα, ERβ, and GPR30 are all reportedly expressed in several nuclei of the hypothalamus, which suggests that all three receptors play important roles in hypothalamic regulation [5]. ERα and ERβ can form homodimers as well as heterodimers [6], and GPR30 has been shown to interact and crosstalk with ERα [7]. Thus, it is plausible that the anorectic effect of estrogen is mediated by the activation of either a single type of receptor or a composite of GPR30-mediated rapid signaling and ER-mediated nuclear events. Before the discovery of GPR30-estrogen binding, the action of estrogen in the hypothalamus was assumed to be mediated by ERα and the role of ERβ in the regulation of energy balance is negligible [1,8]. For example, ERα-knockout mice did not show E2-induced reduction of food intake and body weight compared with wild-type mice [9]. Increased body weight has also been observed in mice that lack GPR30, which indicates a potential role of GPR30 in the estrogen-mediated control of energy balance [10]. Despite these findings, it is difficult to address how ERs and GPR30 act alone or in combination to regulate feeding behavior and weight due to a lack of experimental evidence that links specific receptors to the anorectic signaling pathway.

The rapid action of the membrane-bound estrogen receptor, GPR30, has attracted considerable attention as a potential new regulatory mechanism of the estrogen metabolic network. GPR30 couples with trimeric G proteins to initiate diverse rapid signaling events. GPR30 activation has been linked to protein kinase A, protein kinase C, and the pertussis-sensitive transactivation of epidermal growth factor receptor (EGFR) [11,12]. GPR30 plays an important role in the regulation of calcium oscillations in luteinizing hormone-releasing neurons in the hypothalamus and in the control of serotonin receptor signaling in the paraventricular nucleus [13,14].

The development of the GPR30-specific agonist, G-1, and the GPR30-specific antagonist, G-15, has facilitated studies exploring the role of GPR30 in mediating estrogen's actions *in vivo* independent from the role of ERs [15,16]. The blockade of estrogen effects by GPR30-specific antagonists demonstrates that some of estrogen's reproductive and non-reproductive functions are mediated through GPR30 rather than the ERs [17]. However, the contribution of GPR30 activation to the anorectic STAT3 pathway in the hypothalamus remains underexplored. In this study, we demonstrate that the activation of GPR30 by estrogen can initiate STAT3 phosphorylation, which leads to the activation of the anorectic pathway in the arcuate nucleus of hypothalamus.

2. Materials and methods

2.1. Materials

All cell culture reagents, media, and sera were purchased from Invitrogen (Carlsbad, CA, USA) except for Dulbecco's modified Eagle's medium (DMEM), which was purchased from Welgene (Daegu, Korea). G-1 and G-15 were purchased from Tocris (Bristol, UK), and water-soluble β-estradiol (E2) was purchased from Sigma (St. Louis, MO, USA). All primary antibodies were purchased from Cell Signaling (Danvers, MA, USA) except the GPR30 antibody, which was purchased from Novus Biologicals (Littleton, CO, USA).

2.2. Cell line and primary hypothalamic neuron culture

HeLa cells were grown in DMEM supplemented with 10% fetal bovine serum in a 5% CO_2 atmosphere at 37 °C. Primary hypothalamus cultures were extracted from Sprague Dawley rat embryos on the 18th day of gestation using a technique reported in Ref. [18] with modifications. Briefly, medial portions of the hypothalamic tissue were dissected and incubated at 37 °C for 10 min in a solution containing 0.05% trypsin in Hank's balanced salt solution (HBSS). The tissue was washed and triturated in HBSS by pipetting. Cells were centrifuged and the resulting pellet was re-suspended in Neurobasal medium supplemented with B-27 and 2 mmol/L L-glutamine. Cells were plated onto 6-well plates that were pre-coated with poly-D-lysine, and cultured in a 5% CO_2 atmosphere at 37 °C. Primary cultures were used for drug treatment 2 weeks after plating.

2.3. Transfection and Western blot

HeLa cells were transfected with either human GPR30 cDNA or a mock vector by Lipofectamine (Invitrogen, Grand Island, NY, USA). Cells were starved of serum and treated with E2 or G-1 48 h after transfection. For Western blot, HeLa cells or primary hypothalamic neurons were lysed with 1% Triton X-100 plus 0.1% SDS solution containing protease/phosphatase inhibitors. Lysates were separated by SDS-polyacrylamide gel electrophoresis and then subjected to immunoblotting analysis using antibodies for STAT3 (1:5000), phospho-STAT3 (1:1000), and GPR30 (1:1000).

2.4. Reverse transcription polymerase chain reaction (RT-PCR)

Total RNA from MCF-7 and HeLa cells were isolated and extracted, and the cDNA was synthesized as templates. The following primer sequences for human ERα and ERβ were used: ERα forward 5′-CTACTGCATCAGATCCAAGG-3′ and reverse 5′-GTCATTGGTACTGGCCAATCT-3′, ERβ forward 5′-CGATGCTTTGGTTTGGGTGAT-3′ and reverse 5′-GCCCTCTTTGCTTTTACTGTC-3′. The following PCR reaction conditions were used: 95 °C (5 min), followed by 34 cycles at 95 °C (30 sec)/53 °C (60 sec) for ERα or 57 °C (60 sec) for ERβ/72 °C (60 sec), followed by 72 °C (5 min).