Estrogen Rapidly Modulates 5-Hydroxytrytophan-Induced Visceral Hypersensitivity via GPR30 in Rats

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BACKGROUND & AIMS: Sex hormones have been reported to modulate visceral hypersensitivity (VH). Estrogen regulates neurons not only by binding to estrogen receptors (ERα and ERβ) to initiate transcription but also via the G-protein coupled receptor GPR30, which binds and rapidly mediates actions of estrogen. We examined the role of sex hormones in a VH model without colonic inflammation. **METHODS:** 5-Hydroxytryptophan (5HTP) was injected subcutaneously into awake female rats to induce VH; the 5HT3 antagonist (granisetron) or saline (control) were injected 30 minutes later. Immunohistochemistry was used to quantify calcitonin gene-related peptide-immunoreactive (CGRP-IR) neurons in the dorsal root ganglion (DRG). 5HTP-induced VH was evaluated in ovariectomized rats injected with 17β-estradiol, progesterone, or both. ER α/β agonist, GPR30 agonist, ER antagonist (ICI-182,780) or GPR30 antisense oligodeoxynucleotide were given to 5HTP-primed, estrogen-treated ovariectomized rats. **RESULTS:** Rats given 5HTP had increased VH that was inhibited by granisetron, accompanied by a decrease in CGRP-IR in the DRG. Ovariectomy eliminated 5HTP-induced VH, whereas estrogen and the combination of estrogen and progesterone, but not progesterone alone, restored the VH. The GPR30 agonist, but not the ERβ agonist, rapidly restored VH. VH was preserved by coadministration of ICI-182,780 and estrogen but was absent after administration of the GPR30 antisense oligodeoxynucleotide. GPR30 colocalized with 5HT3 in DRG neurons; no significant inflammation occurred in colonic mucosa. **CONCLUSIONS: In the absence of mucosal inflammation, estrogen can rapidly modulate 5HTP-induced VH. Loss of gonad hormones suppresses VH, whereas estrogen replacement restores it. Estrogen-mediated VH appears to act through GPR30.**

Sex hormones are reported to be important in animal models in the modulation of "visceral hypersensitivity" (VH), a defining factor in pathogenesis of irritable bowel syndrome (IBS). However, the VH models in these reports were mainly secondary to severe colonic mucosal inflammation,[1,2] which is not observed in IBS patients. Therefore, the role of estrogen in the VH model in the absence of mucosal inflammation should be examined. To achieve this, subcutaneous injection of 5-hydroxytryptophan (5HTP, a serotonin precursor) to awake rats is used to induce VH without mucosal inflammation.[3] Additionally, serotonin (5HT) is a critical molecule in mediating gut-to-brain signaling. Much of the pharmaceutic efforts over the past 2 decades has been focused on serotonin and 2 of its receptors: 5HT3 and 5HT4.[4] Therapeutics targeting 5HT3 and 5HT4 receptors is effective in the treatment of IBS, particularly in female patients.[5,6] Thus, it would be beneficial to understand the role of estrogen in 5HTP-induced VH.

Estrogen signals neurons by binding to estrogen receptors (ERs), which then interact with estrogen response elements to initiate transcription, ie, the classic "genomic" mechanism of steroid action. This effect is usually delayed at onset (within several hours to days) and prolonged in duration.[7] Two nuclear ERs, ERα and ERβ, have been previously cloned.[8] In addition to these classical ERs, emerging data suggest that other estrogen receptors can also function as a cytoplasmic signaling molecule. This rapid action of estrogen can occur within seconds to minutes and does not involve transcriptional regulation.[7] For example, we have shown that estrogen can restore mustard oil-induced VH in ovariectomized (OVX) rats in 90 minutes.[2] Others have demonstrated that a single injection of estrogen can rapidly phosphorylate CREB in gonadotropin-releasing neurons in OVX mice within only 15 minutes, and this effect can persist

Abbreviations used in this paper: 5HT, serotonin; 5HTP, 5-hydroxytryptophan CGRP, calcitonin gene related peptide; CRD, colorectal distension; DPN, diarylpropionitrile; DRG, dorsal root ganglion; EMG, electromyogram; ER, estrogen receptor; GPCR, G-protein-coupled receptor; IR, immunoreactive; ODN, oligodeoxynucleotide; OVX, ovariectomized; PPT, propylpyrazole-triol; VH, visceral hypersensitivity; VMR, visceral motor response.

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for 4 hours.[9] A G-protein-coupled receptor, GPR30 (an alternative to the classical ERs), has recently been identified to be involved in the rapid action of estrogen through its direct association with estrogen.[10] Therefore, we aimed to test the following hypotheses: (1) sex hormones can rapidly modulate 5HTP-induced VH in the absence of colonic inflammation, and (2) estrogen-dependent 5HTP-induced VH acts through the GPR30 receptor rather than through the traditional ER α and β pathway.

Materials and Methods

Animal Preparation, Electrode Implantation, and Intrathecal Catheterization

This study used female Sprague–Dawley rats (220–330 g), which were maintained on a 12-hour light-dark cycle with standard laboratory chow and tap water administered ad libitum. For rats undergoing visceral pain studies, electrodes were implanted using the following procedure. Under ether anesthesia, electromyogram (EMG) electrodes made from Teflon-coated stainless steel wire (7 strand) (A-M Systems, Inc, Carlsborg, WA) were implanted in the rat's abdominal external oblique muscle at least 7 days prior to experimentation. Electrodes were exteriorized onto the back of the neck. For the antisense oligonucleotide (ODN) study, rats were prepared with both electrode implantation and intrathecal catheterization. After sodium pentobarbital (65 mg/kg, intraperitoneal [IP]) anesthesia, the rat's occipital magnus from the dorsal side of the neck was opened, and the dura mater was incised. A catheter (PE-10 polyethylene tubing) was inserted intrathecally and advanced caudally to the lumbar enlargement. Catheter placements were verified by visual inspection after the animal was killed.

Colorectal Distension Procedure

Rats were placed in plastic tunnels (6-cm diameter, 25-cm length) for the described experiments. During the 3 days preceding the experiments, the rats were trained to the experimental conditions by placing them singly in the tunnel for 3 hours per day. The colorectal distension (CRD) balloon was composed of a latex glove finger (7 cm long) attached to a rectal catheter (Medtronic, Skovlunde, Denmark). The balloon was inflated and left overnight to help equilibrate the tension in its wall. The inflatable device was introduced through the anal canal completely into the rectum in conscious rats and secured to the tail base. The tube was then connected to a barostat (Medtronic). The colon was distended by inflating the balloon to the desired pressure (20, 40, or 60 mm Hg) for 10-second intervals with 30-second intervals between distensions. Distensions were repeated 4 times for each experimental protocol with 5-minute intervals between each series.[2]

Ovariectomy Procedure

An ovariectomy was performed on experimental animals as previously described.[2] Briefly, ovaries were excised utilizing forceps through a 1-cm incision over both flanks while the rat was sedated under light ether anesthesia. A ligature was placed below the ovary, and the ovary was removed. Ovaries in sham-OVX rats were externalized from the abdominal cavity and then replaced without being excised. Rats were allowed 1-week recovery prior to experimentation. Implantation of EMG electrodes was conducted during ovary surgery in several rat groups.

Experiment Protocol

All experiments were performed at the same time of day (between 9:00 AM and 12:00 PM [noon]) to minimize the influence of circadian rhythms. The first experiment was designed to verify the 5HTP-induced VH in awake female rats and to explore its potential mechanism. The rats underwent 2 rounds of CRD during the proestrous stage. These 2 sessions were separated by 4–5 days, which corresponds to the rat ovarian cycle. During session 1 in the experimental group (n = 7), distilled water (vehicle) was injected subcutaneously 30 minutes prior to CRD; whereas in session 2, 5HTP (10 mg/kg; Sigma–Aldrich, St Louis, MO) was injected subcutaneously prior to the CRD. In the control group (n = 5), a subcutaneous distilled water injection was administered during both sessions. The CRD procedure with simultaneous EMG recording was performed as described. The EMG was recorded using a CED 1401 instrument and analyzed using Spike 2 software for Windows (Cambridge Electronic Design, Cambridge, UK). The raw EMG signal was rectified off-line, and the area under the curve (AUC) for baseline activity in each session was subtracted from the AUC for the rectified response to CRD to obtain an AUC difference.[2] In each session, the EMG values from individual distensions were averaged. We further tried to confirm the role of 5HT3 receptors in 5HTP-induced VH[3] and to identify the potential site of sensitization. Thirty minutes after 5HTP priming, subcutaneous injection of 5-HT3 receptor antagonist (granisetron, 10 μg/kg, n = 6, Sigma–Aldrich) or saline (n = 6) were applied. CRD with simultaneous EMG recording as described was then performed. Thereafter, the rats were anesthetized with sodium pentobarbital (65 mg/kg, IP) and perfused transcardially with normal saline followed by 4% paraformaldehyde in ice-cold phosphate-buffered saline (PBS). For quantifying the calcitonin gene-related peptide immunoreactive (CGRP-IR) neurons, dorsal root ganglions (DRGs) at L6 level were excised and postfixed in the same fixative at 4°C for 4 hours. All specimens were cryoprotected in 30% (wt/vol) sucrose, and sections were collected in PBS. The immunohistochemical study was performed as described.[2] Briefly, after washing in PBS (pH 7.4), free-floating sections were treated with