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Vagal Afferents Mediate Antinociception of Estrogen in a Rat Model of Visceral Pain: The Involvement of Intestinal Mucosal Mast Cells and 5-Hydroxytryptamine 3 Signaling

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Abstract: **Estrogen reportedly facilitates visceral nociception at the spinal or supraspinal level. The present study was aimed to investigate whether estrogen modulates visceral pain through the vagal pathway. Ovariectomized rats received estradiol, which was administered subcutaneously (to act through both the vagal and spinal pathways) or intraduodenally (to preferentially act through the vagal pathway). Luminally applied estradiol induced a rapid and significant decrease in the visceromotor response to colorectal distension, with increased c-Fos expression in nodose ganglion neurons. Systemically injected estradiol increased visceromotor response and c-Fos expression in both nodose and dorsal root ganglion (T6-12) neurons. The antinociceptive effect of estrogen was abolished by surgical vagotomy or chemical denervation of vagal afferents. Both luminally and systemically administered estradiol elicited selective 5-hydroxytryptamine secretion from the duodenum. Granisetron, a 5-hydroxytryptamine 3 receptor antagonist, reversed the antinociceptive effect of estrogen. Intestinal mucosal mast cell stabilizers prevented estradiol-induced antinociception and 5-hydroxytryptamine secretion. Ultrastructural analysis revealed that estradiol caused piecemeal degranulation of intestinal mucosal mast cells. The actions of estradiol were inhibited by an estrogen receptor β antagonist and mimicked by an estrogen receptor β agonist. These results suggest that estrogen can trigger vagus-mediated antinociception, which is masked by its spinally mediated pronociception.**

Perspective: ***This study is the first to show a vagus-mediated estrogenic antinociception, in which the nongenomic estrogen receptor β–mediated, intestinal mucosal mast cell–derived 5-hydroxytryptamine/5-hydroxytryptamine 3 receptor pathway is involved. This work may provide new insights into the sex hormone modulation of visceral sensitivity related to irritable bowel syndrome and indicate potential therapeutic targets to manage this disease.***

Key words: *Visceral pain, sex hormone, intestinal mucosal mast cells, serotonin, vagal afferents.*

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Many chronic pain syndromes, including irritable bowel syndrome (IBS), temporomandibular disorder, and fibromyalgia, are more prevalent in women.[1,5] Gonadal hormone modulation of nociceptive sensitivity is likely a major factor underlying the female prevalence of these disorders. Females suffering from IBS outnumber males by a ratio of 2:1.[15,67] Animal studies have revealed that estrogen facilitates pain signaling via acting at the spinal or supraspinal level.[29,30,40,44] These results

suggest that estrogen is pronociceptive. However, the antinociceptive effects of estrogen have also been reported in animal models.[28,37,38,49] Clinically, the severity of abdominal pain in a subgroup of female patients with IBS is exacerbated during menses; this pain is not associated with psychological traits such as anxiety and depression.[1,27,64] Several possibilities might account for these conflicting findings, including the various functions mediated by different estrogen receptors (ERs), ER expression fluctuation with the estrous cycle, or reduced functioning of the endogenous opioid system.[13,43,53]

Vagal afferents, which are extensively distributed throughout the upper gastrointestinal (GI) tract, are involved in the reflex control of GI secretion and motility. In addition, they are capable of conveying primary sensory information (both innoxious and nociceptive) from the gut to brain. Vagal afferents can project to the brainstem, where they make synaptic connections with second-order neurons involved in the descending control of spinal nociceptive transmission.[3,22] Converging lines of evidence suggest that in addition to modulating somatic pain,[20,34,51] they may engage visceral perception via driving a descending inhibition of spinal pain signaling, which may involve the 5-hydroxytryptamine (5-HT)/5-HT 3 receptor ($5\text{-HT}_3\text{R}$) pathway.[7,12,24,71] Of note, clinical data have shown reduced vagal tone in a subset of premenopausal female patients with IBS, which correlates with IBS symptoms, such as abdominal pain or discomfort.[17,55] It has been demonstrated that ERα and β are expressed in the upper GI tract and may be involved in regulation of intestinal functions,[2,8,9,59,60] indicating that the GI tract may be a target of steroid hormone action. Whether activating these ERs affects intestinal vagal afferent-mediated antinociception is unknown.

Intestinal mucosal mast cells (IMMCs), a mast cell population residing in the GI mucosa, play critical roles in relaying information between the gut and brain[56] and may be implicated in the pathophysiology of disorders with a high prevalence in women, such as IBS.[26] Evidence has shown that IMMCs are in close contact with vagal afferent terminals and may functionally communicate with each other.[56,66,68] In addition to mucosal enterochromaffin (EC) cells, IMMCs are another source of 5-HT released in the gut.[23] It has been reported that the activational state and secretory activity of mast cells from bone marrow, the peritoneal cavity, or the brain are under the modulation of sex hormones.[63,65,70] Whether estrogen can regulate IMMC secretory activity and, in this manner, participate in vagal antinociception is unclear.

Therefore, we aimed to test the following hypotheses in a rat model of visceral pain: 1) estrogen can prevent visceral pain via the vagal pathway and 2) IMMCs participate in estrogen's actions via 5-HT secretion. In this study, estradiol was subcutaneously injected to act through both vagal and spinal pathways, or intraduodenally infused to preferentially act through the vagal pathway, as demonstrated in our previous studies.[71]

Methods

Animals

Adult female Sprague Dawley rats (200–250 g) were purchased from Shanghai Laboratory Animal Center, Chinese Academy of Science (Shanghai, China), and were maintained on a normal light-dark cycle. Rats were provided with food and water ad libitum and were housed singly after surgery. The experimental procedures met the guidelines for experimental pain in animals published by the International Association for the Study of Pain and approved by School of Medicine, Shanghai Jiao Tong University. All experiments were performed at the same period of the day (between 10:00 AM and 3:00 PM) to minimize the influence of circadian rhythms.

Drugs and Chemicals

17β-Estradiol, granisetron, diarylpropionitrile (DPN), methyl-piperidino-pyrazole, 1,3-bis(4-hydroxyphenyl)-4-methyl-5-[4-(2-piperidinylethoxy)phenol]-1*H*-pyrazole (MPP), cyclofenil, G15, and pyrilamine were obtained from Sigma-Aldrich (St. Louis, MO). Estradiol, DPN, MPP, cyclofenil, and G15 were first dissolved in dimethyl sulfoxide (DMSO) to obtain a stock solution and then diluted with saline. The final concentration of DMSO in the working solutions was .5%. Granisetron and pyrilamine were dissolved and diluted with saline.

Ovariectomy Procedure

An ovariectomy was performed on experimental animals to minimize the potential influence of endogenous estrogen. Keeping the animals under anesthesia with a combination of ketamine (150 mg/kg, intraperitoneally [i.p.]; Sigma-Aldrich) and light ether, the ovaries were excised with forceps through a 1-cm incision over both flanks. The rats were allowed a 2-week recovery period prior to experimentation. This time was sufficient to reduce the plasma estradiol concentration below 5 pg/mL[29] but was too short to result in ovariectomy-induced hyperalgesia.[49]

Implantation of Electrodes and Intraluminal Catheter

Nine days after ovariectomy surgery, the rats were anesthetized with 45 mg/kg sodium pentobarbital (i.p.; Sigma-Aldrich), and electromyogram (EMG) electrodes made from Teflon-coated, 32-gauge stainless steel wires were inserted into the abdominal external oblique muscles. The lead wires were then tunneled subcutaneously and exteriorized at the back of the neck. Then, a polyethylene tube (outer diameter, .9 mm; Shanghai Industrial Ceramics Electrical Co, Ltd, Shanghai, China) was gently inserted into the duodenum (positioned at 2 cm away from the pylorus) from the fundus of the stomach for drug administration. The cannula was fixed with sutures, and its remaining free end was exteriorized at the back of the neck. The rats that were subjected to surgery were allowed to recover for 5 days.