Corticotropin-Releasing Hormone Receptor 1 Antagonist Blocks Brain–Gut Activation Induced by Colonic Distention in Rats

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Background & Aims: **The corticotropin-releasing hormone receptor 1 mediates stress-induced changes in colonic motor activity and emotion. We tested the hypothesis that pretreatment with JTC-017, a specific corticotropin-releasing hormone receptor 1 antagonist, blocks colorectal distention–induced hippocampal noradrenaline release and visceral perception in rats. We also investigated whether pretreatment with JTC-017 blocks acute or chronic colorectal distention–induced adrenocorticotropic hormone release, anxiety, and stress-induced changes in colonic motility.** ***Methods:*** **Rats were pretreated intrahippocampally with α-helical corticotropin-releasing hormone (1.25 μg/kg; vehicle), a nonspecific corticotropin-releasing hormone receptor antagonist, or intraperitoneally with JTC-017 (10 mg/kg). Hippocampal noradrenaline release after microdialysis and the frequency of abdominal contractions were measured in response to acute colorectal distention. Plasma adrenocorticotropic hormone levels, anxiety-related behavior, and stress-induced changes in colonic motility were evaluated after acute or chronic colorectal distention followed by exposure to an elevated plus maze.** ***Results:*** **Administration of α-helical corticotropin-releasing hormone or JTC-017 significantly attenuated hippocampal noradrenaline release and reduced the frequency of abdominal contractions induced by acute distention. In addition, JTC-017 significantly reduced plasma adrenocorticotropic hormone and anxiety after acute distention. After chronic distention, changes in plasma adrenocorticotropic hormone and anxiety were not significant because of habituation. In contrast, a significant increase in fecal pellet output during the elevated plus maze was observed after chronic distention. This increase in fecal pellet output was blocked by pretreatment with JTC-017.** ***Conclusions:*** **Our results suggest that JTC-017, a specific corticotropin-releasing hormone receptor 1 antagonist, attenuates hippocampal noradrenaline release, visceral perception, adrenocorticotropic hormone release, and anxiety after acute colorectal distention in rats. In addition, JTC-017 blocks stress-induced changes in colonic motility after chronic colorectal distention in rats.**

Two major G protein–coupled receptors for the corticotropin-releasing hormone (CRH) have been identified as CRH receptor (CRHR)1 and CRHR2.[1–3] CRHR1, which is highly expressed in the anterior pituitary, neocortex, hippocampus, amygdala, and cerebellum has been reported to mediate stress-induced physiological changes, including stimulation of the hypothalamo-pituitary-adrenal axis, elevation of plasma levels of catecholamines, increased colonic motility,[4–7] and exaggerated stress-related behavior, especially anxiety.[6,8] In addition, stimulation of this receptor is believed to activate adenylate cyclase, an enzyme that catalyzes the formation of adenosine 3′,5′-cyclic monophosphate (cAMP).[1–3]

We have previously reported increased colonic motility and visceral perception in response to the administration of CRH in patients with irritable bowel syndrome (IBS).[9] In addition, earlier studies have indicated that gastrointestinal dysmotility[9,10] and visceral hypersensitivity[11] are major events in the pathophysiology of IBS. Moreover, patients with IBS have been reported to suffer from a variety of chronic or acute psychiatric conditions, including depression, generalized anxiety, panic, social phobia, and somatization.[12,13] Various studies have suggested a relationship between stress-induced changes in colonic motility and CRH action in the paraventricular nucleus (PVN) of the hypothalamus.[14,15] Accordingly, it has been shown that intracerebroventricular injection of CRH stimulates gastrointestinal motility in a way similar to that induced by stress[7,16,17] and that intraperitoneal injection of CRH induces defecation and clustered spike bursts longer than the basal spike bursts in rats.[5] CRHR1 antagonists have been shown to

Abbreviations used in this paper: CRH, corticotropin-releasing hormone; CRHR, CRH receptor; EPM, elevated plus maze; HPLC, high-performance liquid chromatography; IBS, irritable bowel syndrome; IC_{50}, median inhibitory concentration; LC, locus ceruleus; PVN, paraventricular nucleus.

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prevent stress-like gastrointestinal motor responses after central or peripheral injection of CRH.[5,7] In addition, it has been reported that CRHR1-deficient mice show an impaired response to stress, as indicated by the absence of increased adrenocorticotropic hormone (ACTH) and corticosterone levels after exposure to stress and less pronounced anxiety-related behavior.[6,8] From these findings, it is reasonable to assume that CRH mediates gastrointestinal and behavioral responses to stress via CRHR1.

Colorectal distention is widely used to assess the response to gastrointestinal stimulation in human and animal experiments. Repetitive sigmoid distention has been shown to induce rectal hyperalgesia in patients with IBS,[18] whereas acute noxious colorectal distention under restraint conditions induces abdominal contractions and significant c-Fos expression in the brainstem, limbic areas, cortical areas, and lumbosacral spinal cord in rats.[19–21] Gastrointestinal stimulation especially has been shown to activate the locus ceruleus (LC), a nearly homogeneous nucleus containing approximately 50% of brain noradrenaline neurons.[22] Along this line, we have previously reported that colorectal distention induces hippocampal noradrenaline release and visceral perception in rats.[23] Other studies have suggested that brain noradrenergic response to colonic distention plays an important role in anxiety-related behavior and central symptoms of IBS.[24] Only a few reports have examined the effects of chronic colorectal distention. One study has shown that colon irritation for 2 weeks results in chronic visceral hypersensitivity.[25]

Although the involvement of CRH in stress-related behavior has been widely studied, no study has investigated the effects of CRHR1 antagonists on hippocampal noradrenaline release and visceral perception after gastrointestinal stimulation. In addition, the effects of acute and chronic gastrointestinal stimulation on emotional behavior, which is important to clarify the pathophysiology of IBS, have not been investigated. In this study, we tested the hypothesis that pretreatment with JTC-017, a specific CRHR1 antagonist, blocks colorectal distention–induced hippocampal noradrenaline release and somatic motor response to visceral distention in rats. We also used α-helical CRH (a nonspecific CRHR antagonist) to block CRHR1. If effects of the nonspecific CRHR antagonist and a specific CRHR1 antagonist are almost identical, then CRHR1 may play a salient role in the process described previously. We also investigated whether pretreatment with JTC-017 blocks acute or chronic colorectal distention–induced ACTH release, anxiety-related behavior, and stress-induced changes in colonic motility.

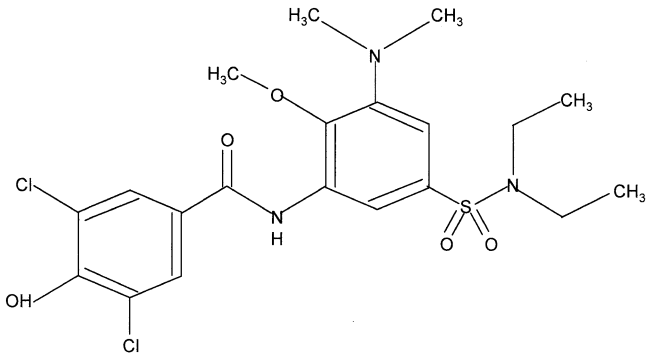

Figure 1. Structure of JTC-017.

Materials and Methods

Animals

Male Wistar rats (n = 73) weighing 180–210 g were provided by Charles River Japan Inc. (Yokohama, Japan). The rats were housed under controlled illumination (12:12-hour light/dark cycle starting at 8:00 AM) and temperature (23°C ± 1°C) with free access to food and water. This study was designed in accordance with the guidelines for animal experiments and was approved by the Ethics Committee of Laboratory Animals of Tohoku University.

Drugs

The drugs used in this study were α-helical CRH, a nonspecific CRHR antagonist, and JTC-017 (3,5-dichloro-*N*-[5-diethylsulfamoyl-3-dimethylamino-2-methoxphenyl]-4-hydroxybenzamide; Figure 1), a specific CRHR1 antagonist developed by Japan Tobacco Inc. α-Helical CRH was dissolved in hydrochloride acidic saline and kept at −40°C, and JTC-017 was dissolved in 10% hydroxypropyl-β-cyclodextrin and kept at 14°C.

Corticotropin-Releasing Hormone Receptor 1 Binding Assays and Cell Biology Assays

Competition binding experiments were performed according to the procedure described by Okuyama et al,[26] with some modifications. Membrane homogenates of rat pituitary glands (150 μg of protein) were incubated for 120 minutes at 22°C with 0.1 nmol/L [^{125}I]Tyr^{0}-CRH in the absence or presence of the competing test compound in 250 μL of assay buffer containing 50 mmol/L Tris-HCl (pH 7.4), 10 mmol/L $MgCl_2$, 2 mmol/L ethylenediaminetetraacetic acid, and 0.1% bovine serum albumin. After incubation, the samples were rapidly filtered under a vacuum through glass-fiber filters (GF/B; Packard) and rinsed several times with an ice-cold buffer containing 50 mmol/L Tris-HCl, 150 mmol/L NaCl, and 0.01% Triton X-100 by using a 96-sample cell harvester (Unifilter; Packard). The filters were then dried and counted for radioactivity in a scintillation counter (Topcount; Packard) by using a scintillation cocktail (Microscint 0; Packard). Nonspecific binding was determined in the presence of 1 μmol/L