Impaired Emotional Learning and Involvement of the Corticotropin-Releasing Factor Signaling System in Patients With Irritable Bowel Syndrome

JENNIFER S. LABUS,[1,2,4] CATHERINE S. HUBBARD,[1,2] JOSHUA BUELLER,[1,2] BAHAR EBRAT,[1,2] KIRSTEN TILLISCH,[1,2] MICHELLE CHEN,[1,2] JEAN STAINS,[1,2] GEORGE E. DUKES,[8] DENNIS L. KELLEHER,[8] BRUCE D. NALIBOFF,[1,2,7] MICHAEL FANSELOW,[4,5] and EMERAN A. MAYER[1,2,3,4,5,6]

[1]Gail and Gerald Oppenheimer Family Center for the Neurobiology of Stress, Departments of [2]Medicine, [3]Physiology, [4]Psychiatry, [5]Psychology, and [6]Ahmanson-Lovelace Brain Mapping Center, University of California, Los Angeles, Los Angeles, California; [7]VA Greater Los Angeles Healthcare System, Los Angeles, California; and [8]GlaxoSmithKline, Research Triangle Park, North Carolina

See Covering the Cover synopsis on page 1172.

BACKGROUND & AIMS: Alterations in central corticotropin-releasing factor signaling pathways have been implicated in the pathophysiology of anxiety disorders and irritable bowel syndrome (IBS). We aimed to characterize the effects of the corticotropin-releasing factor receptor 1 (CRF-R1) antagonist, GW876008, on brain and skin conductance responses during acquisition and extinction of conditioned fear to the threat of abdominal pain in subjects with IBS and healthy individuals (controls). **METHODS:** We performed a single-center, randomized, double-blind, 3-period crossover study of 11 women with IBS (35.50 ± 12.48 years old) and 15 healthy women (controls) given a single oral dose (20 mg or 200 mg) of the CRF-R1 antagonist or placebo. Blood-oxygen level–dependent responses were analyzed using functional magnetic resonance imaging in a tertiary care setting. **RESULTS:** Controls had greater skin conductance responses during acquisition than extinction, validating the fear-conditioning paradigm. In contrast, during extinction, women with IBS had greater skin conductance responses than controls—an effect normalized by administration of a CRF-R1 antagonist. Although the antagonist significantly reduced activity in the thalamus in patients with IBS and controls during acquisition, the drug produced greater suppression of blood-oxygen level–dependent activity in a wide range of brain regions in IBS patients during extinction, including the medial prefrontal cortex, pons, hippocampus, and anterior insula. **CONCLUSIONS: Although CRF signaling via CRF-R1 is involved in fear acquisition and extinction learning related to expected abdominal pain in patients with IBS and controls, this system appears to be up-regulated in patients with IBS. This up-regulation might contribute to the previously reported abnormal brain responses to expected abdominal pain.**

Keywords: Corticotropin-Releasing Factor Receptor 1 (CRF-R1) Antagonist; Fear Conditioning; Extinction.

Irritable bowel syndrome (IBS) is a common gastrointestinal disorder characterized by chronically recurring abdominal pain and discomfort, altered bowel habits, and increased anxiety and hypervigilance to symptom-related stimuli.[1] We have previously demonstrated that group differences in brain responses to aversive rectal distension are almost completely accounted for by differences in brain responses to the expectation of such a stimulus, suggesting an important role of conditioned responses to chronic abdominal pain.[2] Consistent with this concept, hypervigilance to and arousal by symptom-related stressors (interoceptive and contextual cues) previously associated with distressing gastrointestinal sensations/symptoms can be viewed in the context of Pavlovian fear conditioning, where neutral stimuli (eg, a light signal) are paired with aversive sensations (eg, abdominal pain).[3] Further evidence to support an important role for aversive visceral learning and memory processes in visceral pain comes from a recent report demonstrating fear conditioning (learned anticipatory fear response) to rectal pain stimuli in healthy control subjects (HCs).[4] From this perspective, persistent hypervigilance to and arousal by symptom-related stressors in IBS might result from deficits in the ability to extinguish conditioned fear responses, resulting in symptom persistence, even in the absence of abnormal visceral input.[5] Similar impairments in fear conditioning and extinction learning have been implicated in several other stress-related disorders, including post-traumatic stress disorder.[6–8] Extinction is not a process of "unlearning," but rather a process by which new learning of fear inhibition occurs and is superimposed over the initially acquired learned response.[9,10] Such

Abbreviations used in this paper: aINS, anterior insula; aMCC, anterior midcingulate cortex; AMYG, amygdala; BOLD, blood-oxygen level–dependent; CRF-R1, corticotropin-releasing factor receptor 1; dlPFC, dorsolateral prefrontal cortex; HCs, healthy controls; HIPP, hippocampus; HYPO, hypothalamus; IBS, irritable bowel syndrome; LCC, locus coeruleus complex; mPFC, medial prefrontal cortex; pACC, pregenual anterior cingulate cortex; PLA, placebo; SCR, skin conductance responses; SNS, sympathetic nervous system; THAL, thalamus; vlPFC, ventrolateral prefrontal cortex.

0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2013.08.016

impaired learning processes could play a role in post-infectious IBS, where failure to extinguish associations between gastrointestinal activity and abdominal pain acquired during the acute infection results in persistent hypervigilance toward gut-related signals.

Corticotropin-releasing factor is considered the principal coordinator of the vertebrate stress response via widespread actions on peripheral and central targets, all of which serve to orchestrate a host of autonomic, neurochemical, and behavioral responses to stress.[11,12] Perturbations to this system in humans have been linked to a variety of psychiatric disorders and stress-sensitive syndromes, including anxiety disorders[13] and IBS.[14–16] For example, we have recently demonstrated that exaggerated brain responses to a pain threat in IBS patients were attenuated by acute administration of a corticotropin-releasing factor receptor 1 (CRF-R1) antagonist.[17]

It remains unknown if IBS patients show similar alterations in the acquisition and/or extinction of fear conditioning, as has been reported for patients with certain anxiety disorders, and which brain regions and neuromodulatory systems might be involved. Indirect evidence from clinical trials with cognitive behavioral therapies in IBS suggests that a significant component of the success of these therapies is related to the effort to extinguish persistent conditioned fear responses toward gastrointestinal-related signals and sensations. Given the high comorbidity of stress-related anxiety disorders with IBS,[18,19] coupled with the link between disturbances in fear conditioning and extinction and dysregulation of the CRF/CRF-R1 signaling system,[20] we hypothesized that a similar emotional learning process might underlie the chronicity of IBS symptoms. We used functional magnetic resonance imaging and skin conductance response (SCR) measurement in female IBS patients and HCs to characterize the effects of a selective CRF-R1 antagonist, GW876008, on brain responses during acquisition and extinction of conditioned fear to an abdominal pain stimulus. By using a pain stimulus to the body site most often reported by IBS patients as the site of their abdominal pain (eg, left lower quadrant) as the unconditioned stimulus, and a visual cue as the conditioned stimulus, we aimed to test the following hypotheses: (1) IBS patients compared with HCs show impaired fear conditioning and extinction learning associated with increased sympathetic nervous system (SNS) responses (as indexed by SCR) and altered activity in fear-related brain circuits. (2) Acute administration of GW876008 modulates activity in these networks in IBS patients compared with HCs during acquisition and extinction, normalizing the exaggerated response in IBS, and this drug effect might be dose dependent.

Materials and Methods

Characterization of the Sample

An age-matched sample of right-handed females recruited from the greater Los Angeles community, 14 of whom were diagnosed with IBS (mean age, 35.50 ± 12.48 years) and 17 non-IBS HCs (mean age, 33.65 ± 15.87 years) participated in this study. All study participants were recruited by advertisement from the greater Los Angeles population or a database review from participation in one of our studies previously. IBS was diagnosed based on ROME II criteria and assessment by a gastroenterologist or a nurse practitioner trained in the diagnosis of functional bowel disease. Patients included all bowel habits, 43% constipation-predominant, 21% diarrhea predominant, and 36% alternating symptoms of constipation and diarrhea. Participants had a negative urine test for drugs of abuse, lacked any significant medical problems other than IBS, were free of past or present psychiatric illness, as determined by the Mini International Neuropsychiatric Interview,[21] and were not currently taking any centrally acting medications. All participants were tested in the follicular phase of their menstrual cycle, defined as day 3–14 post menses. The University of California, Los Angeles Medical Institutional Review Board approved all procedures, and each subject provided informed consent.

Of the 31 subjects in our sample, 5 individuals (3 IBS, 2 HC) were excluded from the analysis as a result of blood-oxygen level–dependent (BOLD) signal loss in brain regions of interest across all or at least 1 of the 3 functional magnetic resonance imaging study treatment visits.

Study Design

This was a single-center, randomized, double-blind, placebo (PLA)-controlled, 3-period crossover study of 2 single oral doses (20 mg or 200 mg) of the CRF-R1 antagonist, GW876008, vs PLA. The study consisted of an initial screening and familiarization visit, followed by 3 study treatment visits, each separated by approximately 1 month. Details about study design have been published previously in Hubbard et al.[17]

Drug, Dosage, and Administration

GW876008 (GlaxoSmithKline, Research Triangle Park, NC) is a highly selective and potent antagonist for the G-protein–coupled CRF-R1 subtype.[22] Based on phase II clinical trials in patients with IBS, 20-mg and 200-mg doses of GW876008 were chosen in an attempt to provide a sufficient therapeutic range.[23,24] PLA tablets were identical to the active GW876008 tablets in all respects, with the exception of omission of the active ingredient. Subjects were assigned to study treatment in accordance with the randomization schedule provided by GlaxoSmithKline.

Experimental Design

Conditioned fear learning and extinction were examined using a simple fear conditioning paradigm composed of 3 phases, using a pain stimulus applied to the left lower abdomen as an unconditioned stimulus and a visual cue (red light) as the conditioned stimulus: (1) acquisition: 5 trials of the visual cue presentation always followed by an aversive abdominal stimulus (750 milliseconds); (2) test phase: 10 trials in which the cue was followed by the aversive stimulus on only 50% of the trials; and (3) extinction: 5 trials in which none of the cues were followed by an aversive stimulus. Each cue presentation lasted for 9 seconds in each phase and the inter-trial interval was 20.75 seconds. Supplementary Figure 1*A* illustrates the experimental design.

To deliver the unconditioned stimulus, 2 electrode stimulation pads were placed 6 cm apart over each subject's lower left abdomen in the region overlaying the sigmoid colon. The threat of a pain experience in this region would be expected to generate anticipatory anxiety and hypervigilance because many IBS patients