

# Impact of corticotropin-releasing hormone on gastrointestinal motility and adrenocorticotrophic hormone in normal controls and patients with irritable bowel syndrome

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## Abstract

**Background**—Corticotropin-releasing hormone (CRH) plays a key role in modulating intestinal motility in stressed animals.

**Aims**—To evaluate the effect of CRH on intestinal motility in humans and to determine whether patients with irritable bowel syndrome (IBS) have an exaggerated response to CRH.

**Subjects**—Ten IBS patients diagnosed by Rome criteria and 10 healthy controls.

**Methods**—CRH (2 µg/kg) was intravenously administered during duodenal and colonic manometry and plasma adrenocorticotrophic hormone (ACTH) was measured by radioimmunoassay.

**Results**—CRH induced motility of the descending colon in both groups ( $p < 0.001$ ) and induced greater motility indexes in IBS patients than in controls ( $p < 0.05$ ). CRH produced duodenal phase III motor activity in 80% of the subjects and duodenal dysmotility in 40% of IBS patients. Abdominal symptoms evoked by CRH in IBS patients lasted significantly longer than those in controls ( $p < 0.05$ ). CRH induced significant increases in plasma ACTH levels in both groups ( $p < 0.001$ ) and produced significantly higher plasma ACTH levels in IBS patients than in controls ( $p < 0.001$ ).

**Conclusion**—Human intestinal motility is probably modulated by exogenous CRH. The brain-gut in IBS patients may have an exaggerated response to CRH.

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Keywords: irritable bowel syndrome; corticotropin releasing factor; adrenocorticotrophic hormone; colonic motility; duodenal motility

Stress can alter gastrointestinal function but the mechanism of the stress induced intestinal response is still obscure.<sup>1</sup> The detail of the brain-gut interaction is one of the most important factors of stress induced intestinal response and irritable bowel syndrome (IBS) is presumed to be a disorder of the brain-gut link.<sup>2</sup> We have reported that psychological stress induces colonic segmental contractions and irregular contractile activity (phase II) of the duodenal migrating motor complex (MMC) in humans and that these responses are exaggerated in IBS patients.<sup>3,4</sup> Wrap restraint stress in rats is also reported to facilitate colonic motility and to inhibit small intes-

tinal motility.<sup>5,6</sup> These phenomena in rats are mimicked by intracerebroventricular<sup>6-9</sup> or intravenous<sup>9</sup> administration of corticotropin releasing hormone (CRH) and are blocked by the CRH antagonist,  $\alpha$  helical CRH<sub>9-41</sub>.<sup>6-9</sup> Stress induces anxiogenic behaviour in rats and intracerebroventricular administration of CRH mimics the behavioural changes under stress.<sup>10</sup> Furthermore, intravenous administration of CRH decreases slow wave sleep in humans.<sup>11</sup>

These findings led us to hypothesise that CRH plays a major role in the stress response of humans, both normal subjects and IBS patients. The purpose of this study was to determine whether intravenous administration of CRH affects human gastrointestinal motility and whether CRH discriminates physiological responses in normal control subjects from those in IBS patients.

## Methods

### SUBJECTS

Ten normal healthy volunteers and 10 IBS patients were studied. Both groups consisted of five men and five women. Ages (controls: 20.7 (0.5) years versus IBS: 23.8 (3.6) years) and body mass indexes (controls: 21.8 (0.6) versus IBS: 21.0 (1.1)) were almost matched. Control subjects were paid volunteers who had no symptoms or history of major diseases. IBS patients were diagnosed by the Rome criteria<sup>12</sup>; they also had recurrent abdominal pain with alternating diarrhoea and constipation for more than two years, with temporal exacerbation of these symptoms by psychosocial stress. No patient had a history of abdominal surgery or evidence of organic disease by diagnostic studies including blood tests, urinalysis, stool analyses, plain x ray film of the abdomen, barium enema, colonoscopy, and the lactose tolerance test. Because of the unstable bowel habit, our patients were not classified into two subgroups of diarrhoea or constipation. This notion is supported by an earlier study in which these symptoms of IBS fell into the same principle component in multivariate analysis.<sup>13</sup> Informed consent was obtained from all subjects and this study was approved by the Tohoku University Ethics Committee.

### CANNULATION AND RECORDING ASSEMBLIES

Intestinal motility was recorded by previously reported methods.<sup>4</sup> In brief, an assembly consisting of three transducers (Sentron, Amsterdam, Netherlands) was inserted into the descending colon using colonoscopy at 08.30. On the evening before colonoscopy, the

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Table 1 Influence of CRH on patterns of gastrointestinal motility, abdominal symptoms, and duodenal pH

	Controls (n=10)	Patients with IBS (n=10)	p Value
Latency of evoked motility (min)			
Colon	3.0 (0.6)	2.8 (0.8)	NS
Duodenum	3.1 (0.5)	3.0 (0.9)	
Occurrence of phase III during first 15 min of			
Baseline (n)	2	1	NS
CRH	8*	8*	NS
Clustered contractions or other dysmotility after CRH (n)	0	4§	0.05†
Abdominal pain or discomfort during			
Baseline (n)	0	0	NS
CRH (n)	2	6	NS
Duration of abdominal pain or discomfort (min)	1.3 (1.0)	20.8 (11.5)	0.05‡
Area below pH 7.0			
Baseline (n)	-0.84 (0.17)	-0.85 (0.13)	NS
CRH (n)	-0.95 (0.24)	-0.68 (0.12)	NS

Data are presented as mean (SE) or number of cases (n).

\*p<0.05 significant variation by McNemar's test; †p<0.05 significant difference by Fisher's exact test; ‡p<0.05 significant difference by the Mann-Whitney U test.

§Three cases with clustered contractions and a case with retrograde phase III-like contractions.

subjects ingested a solution composed of 125 ml of magnesium citrate (13.6%) and 2.5 mg of sodium picosulphate to lessen the faecal effluent. Based on an earlier report,<sup>14</sup> this bowel preparation was unlikely to affect the colonic motility. Another assembly with three transducers and a pH sensor (Monocrystal Antimony, Synectics, Stockholm, Sweden) was inserted transnasally into the third portion of the duodenum. The position of catheters without major drift were certified by fluoroscopy with x ray before and after the study. These catheters consisted of three sensors 5 cm apart. The pressure at the following points (distance from the mouth or anus) was measured: duodenal bulb (60 cm), proximal second portion of the duodenum (65 cm), distal second portion of the duodenum (70 cm), descending colon (60 cm), proximal sigmoid colon (55 cm), and mid sigmoid colon (50 cm). The sensors were tip transducers made from a semiconductor. The measuring range of the sensors was from -50 mm Hg to 1000 mm Hg, with -3 dB (0-180 Hz) of the frequency property. The pH sensor was located in the third portion of the duodenum (75 cm). A pneumogram was taken from a pick up belt around the

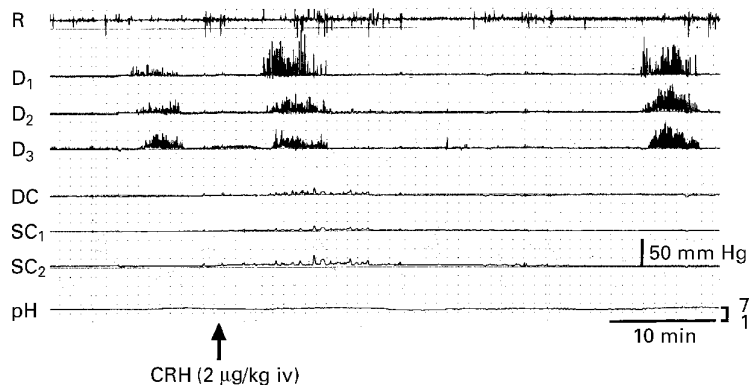


Figure 1 Manometric recordings of a normal control subject. R, respiration; D1, duodenal bulb/antrum; D2, proximal second portion of the duodenum; D3, distal second portion of the duodenum; DC, descending colon; SC1, proximal sigmoid colon; SC2, mid sigmoid colon; pH, duodenal pH. Note the fine colonic contractions after intravenous CRH. Even though CRH was injected during duodenal phase I immediately after phase III at the end of baseline, administration of CRH evoked duodenal phase III.

chest. A Teflon cannula was inserted into an arm vein for blood sampling and saline was infused at a speed of 0.5 ml/min. Two pressure catheters, the pH sensor, and pick up, were connected to an analogue to digital converter (PC-Polygram, Synectics). The analogue signals were sampled at 8 Hz, digitised, entered into a computer (PC-9801 ES, NEC, Tokyo, Japan) via fiberoptic cable, and stored on magnetic hard disk for later analysis.

#### EXPERIMENTAL DESIGN

The subjects lay on a bed at 11.00 after the above procedures were completed. Respiration, duodenal pressure, colonic pressure, and duodenal pH were monitored for 240 minutes. The subjects were instructed to inform the investigators of the beginning and the end of abdominal symptoms. These symptoms were notable on the motility data record. The experiment consisted of the three periods: the initial 60 minutes for adaptation; the following 60 minutes for baseline; and the last 120 minutes for effect of CRH. Saline (20 ml) was injected at 12.00. Human CRH (Peptide Institute, Osaka, Japan) was dissolved in 20 ml of saline immediately before the experiment and 2 µg/kg was injected intravenously within one minute at 13.00. This is the dose which alters gastrointestinal function in animals<sup>9</sup> and increases plasma adrenocorticotrophic hormone (ACTH) secretion to stress levels with detectable plasma CRH in humans.<sup>15</sup> Furthermore, blood pressure is known to be unchanged at this dose.<sup>16</sup> Blood (5 ml) was drawn from the cannula placed in the vein at 0, 15, 30, 60, 90, and 120 minutes after CRH injection. Blood was collected into two tubes: one with EDTA (1 mg/ml) as the first reagent for ACTH assay, and the other without reagent for cortisol assay. Plasma and serum were obtained by centrifugation of the samples at 3000 rpm for five minutes, frozen, and stored at -45°C for later analysis.

#### DATA ANALYSIS

A colonic motility index was calculated by measuring the area under the pressure records for each 15 minute period using a computerised planimeter (Gastrosoft).<sup>17</sup> Motility index was calculated as follows: motility index = 100 (%) × area under the curve (mm Hg/sec)/(15 × 60 sec). Duodenal motility was analysed by duration of three phases: phase I, period of quiescence; phase II, period of irregular contractile activity; and phase III, period of regular contractile activity with at least three minutes of uninterrupted phasic pressures at the maximum frequency of 11-13 per minute of MMC.<sup>18</sup> The timing of CRH injection was in phase I or II at the end of the baseline.

Patterns of duodenal dysmotility were defined and analysed as follows. Clustered contractions were groups of phasic waves, occurring at a rate of 10-12 per minute and lasting overall five minutes or longer; individual waves had amplitudes of at least 15 mm Hg, often with some tonic elevation of the basal pressure.<sup>19</sup> Individual clusters were preceded and followed by at least 30 seconds of quiescence.<sup>19</sup> Retrograde phase III-like contractions were regular contractile

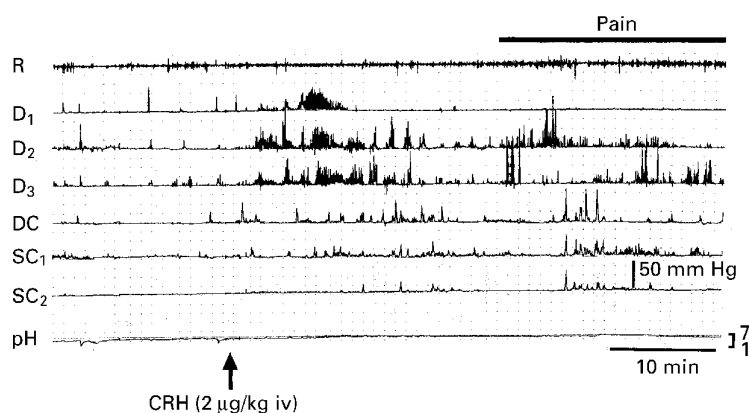


Figure 2 Manometric findings of a patient with IBS. Abbreviations as for fig 1. Administration of CRH stimulated the colon, particularly the descending colon, and evoked duodenal phase III which was followed by phase II contractions. This patient complained of abdominal pain during the period expressed by a solid bar.

activity at a frequency of 11–13 per minute with the retrograde propagation.<sup>20</sup> Duodenal pH was estimated by area under pH 7.0 (pH · sec) divided by duration of periods (15 × 60 sec). Frequency and duration of abdominal pain or discomfort were calculated from the event marks. The stored samples were defrosted and levels of plasma ACTH and serum cortisol were measured by radioimmunoassay. The minimal detectable value and interassay variability were as follows: ACTH, minimum 4 pg/ml, variability 3.89%; cortisol, minimum 0.64 mg/dl, variability 2.64%.

#### STATISTICAL ANALYSIS

Data were expressed as mean (SE) unless indicated otherwise. Means of two groups were compared by one way or two way analysis of variance (ANOVA). Post hoc analysis was carried out using Scheffé's F test, *t* tests, and non-parametric tests. Cross table analysis was used for qualitative data. A *p* value less than 0.05 was regarded as significant.

### Results

#### PATTERNS OF GASTROINTESTINAL MOTILITY AND DUODENAL pH

At baseline, there was no prominent difference in patterns in colonic or duodenal motility between controls and IBS patients. CRH induced segmental contractions in the descending and sigmoid colon within the first 15 minutes in controls (fig 1) and these responses

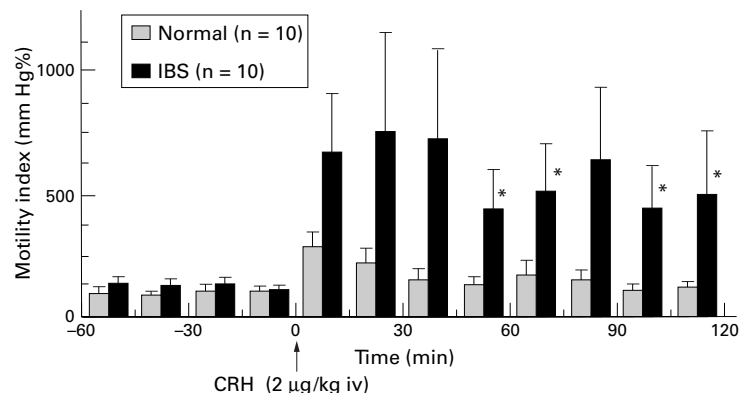


Figure 3 Changes in motility indexes of the descending colon induced by CRH. \**p* < 0.05 versus controls by Mann-Whitney *U* test.

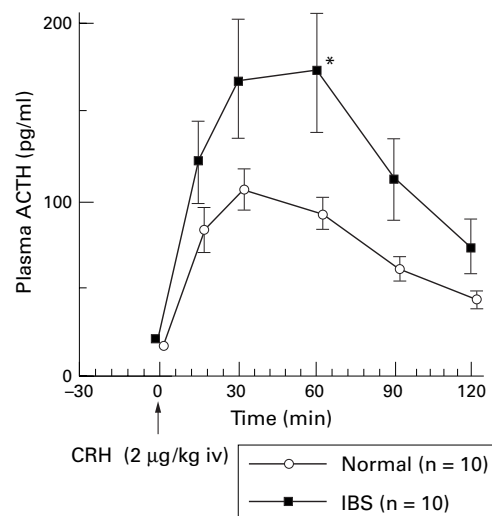


Figure 4 Effects of CRH on plasma ACTH. \**p* < 0.05 versus controls by Scheffé F test and Mann-Whitney *U* test.

were prominent in IBS patients (fig 2). Phase III motor activity of the duodenum was significantly evoked by the CRH injection during the first 15 minutes in both groups (*p* < 0.05; table 1). After CRH administration, IBS patients had a significantly higher incidence of duodenal dysmotility and longer duration of abdominal symptoms than controls (*p* < 0.05; table 1). Duodenal pH was not changed by CRH.

#### CHANGES IN MOTILITY INDEXES OF THE COLON

At baseline, there was no significant difference in motility indexes of the descending colon between controls (87.2 (29.3)) and IBS patients (101.3 (22.2)). CRH induced a significant increase in motility indexes of both groups (controls: 274.0 (72.6), IBS patients: 663.5 (251.6), *p* < 0.001) and produced significantly higher motility indexes in IBS patients in controls (*p* < 0.05), especially in the later time periods (60–120 minutes) after administration of CRH (for example, during 45–60 minutes, controls: 118.1 (38.4) versus IBS patients: 430.4 (174.3), *p* < 0.05, fig 3). Motility indexes of two sites in the sigmoid colon showed the same pattern but the values were smaller than those of descending colon (data not shown). There was no significant correlation between duration of abdominal pain and colonic motility indexes.

#### NEUROENDOCRINE DATA

Basal levels of plasma ACTH were almost identical in both groups (controls: 20.4 (2.5) pg/ml versus IBS patients: 23.3 (2.7) pg/ml). CRH induced a significant increase in plasma ACTH in both groups (*p* < 0.001) and produced significantly higher plasma ACTH in IBS patients than in controls (*p* < 0.01), especially at 60 minutes after administration (controls: 93.1 (9.2) pg/ml versus IBS patients: 174.5 (35.1) pg/ml, *p* < 0.05; fig 4).

Serum cortisol showed a significant increase after CRH and responses were identical in both groups (from baseline to peak value at 60 minutes, controls: 13.6 (1.2) mg/dl to 28.9 (1.3) mg/dl; IBS patients: 13.6 (1.6) mg/dl to 30.2 (1.8) mg/dl; *p* < 0.001).

## Discussion

This is the first study confirming that exogenous CRH can produce considerable changes in phasic contractions in human colon and small intestine. CRH is a peptide containing 41 amino acids,<sup>21</sup> distributed in the whole brain with dense localisation in the paraventricular nucleus of the hypothalamus,<sup>22</sup> and now considered to be a major mediator of the stress response.<sup>10</sup> Stress releases CRH from the paraventricular nucleus and CRH stimulates pituitary ACTH secretion.<sup>23</sup> Growing evidence from animal experiments indicates that endogenous CRH plays a role in mediating stress induced alteration of gastrointestinal motor function.<sup>6-9</sup> Intracerebroventricular administration of CRH mimics the effects of various stressors in inhibiting small intestinal transit and stimulating colonic motor function through autonomic pathways in rats.<sup>6-9</sup> Stress induced alterations in gastrointestinal motility in animals are abolished by intracerebroventricular administration of the CRH antagonist,  $\alpha$  helical CRH<sub>9-41</sub>.<sup>6-9</sup> Our data provide strong support for the hypothesis of a role of CRH in alterations of human gastrointestinal motility.

The precise site of action of intravenous CRH on human gastrointestinal motility is unknown. Because intravenous administration of ACTH or  $\beta$  endorphin does not mimic CRH effects on gut motility,<sup>5</sup> increased plasma ACTH is not likely to be involved in intestinal responses to CRH. There are three forms of CRH receptors: CRH<sub>1</sub>, CRH<sub>2 $\alpha$</sub> , and CRH<sub>2 $\beta$</sub> .<sup>24</sup> The mRNA for CRH<sub>1</sub> and CRH<sub>2 $\alpha$</sub>  is predominantly expressed in the brain, whereas the mRNA for CRH<sub>2 $\beta$</sub>  is expressed in both the brain and the periphery.<sup>24</sup> There are functional CRH receptors in the smooth muscle of the colon but the precise subtype of the receptors is unknown.<sup>25</sup> As there is a specific unidirectional brain to blood transport system for CRH,<sup>26</sup> non-specific penetration of intravenous CRH into the brain is unlikely to occur.<sup>23</sup> A more plausible possibility is that intravenous CRH affects gut motility through brain CRH receptors at circumventricular organs that are relatively unprotected by the blood-brain barrier.<sup>27</sup> This hypothesis is supported by the report that CRH given intracerebroventricularly and intravenously was essentially equipotent in modulating intestinal motility.<sup>9</sup> In vitro effects of CRH on contractions of colonic smooth muscle cells are not excitatory but inhibitory.<sup>25</sup> In contrast, in vivo effects of intracerebroventricular CRH on colonic motility is always excitatory.<sup>5-9</sup> Therefore, altered gastrointestinal motility in our results is probably not mediated by peripheral receptors, but by central CRH receptors in the circumventricular organs. Administration of a specific antagonist would determine the precise sites and effects of intravenous CRH on gut motility.

Colonic motor function, of the descending colon in particular, was stimulated by CRH in our study. This finding is compatible with the results of animal experiments.<sup>7-9</sup> The mechanism by which intracerebroventricular CRH influences colonic motility involves peripheral cholinergic neurotransmission.<sup>6-8</sup> This neuro-

transmission is probably mediated through the sacral parasympathetic pathways.<sup>8</sup> Replicating previous reports,<sup>3,4</sup> there is no significant difference in basal colonic motility between IBS patients and healthy control subjects in this study. In contrast, provocation tests such as loading psychological stress or injection of neostigmine are reported to induce colonic dysmotility in IBS patients.<sup>3,4</sup> These observations and our current data suggest that the colon of IBS patients is hypersensitive to acetylcholine and CRH which are presumably released by stress. Duodenal contraction was also induced by intravenous CRH in humans for a short duration. A significantly greater incidence of phase III within 15 minutes after CRH injection than that within 15 minutes of the start of baseline suggested that this is not incidental. Exogenous CRH induces a faster rhythm of the MMC period in the proximal jejunum in dogs<sup>28</sup> and increases post-prandial motor activities in humans,<sup>29</sup> suggesting its stimulatory action on motility of the small intestine in certain species. Prolonged ambulatory recording of duodenal motility showed that most IBS patients show increased incidence of clustered contractions under alert conditions.<sup>18</sup> In our study, administration of CRH in IBS patients induced duodenal dysmotility with abdominal pain. These observations suggest that not only the colon but also the small intestine is sensitive to the centrally derived stimuli in IBS patients.

We found an increased ACTH response to CRH in IBS patients. Psychosocial stress induces onset and/or exaggeration of gastrointestinal symptoms in the majority of IBS patients.<sup>2-4</sup> The responses of the hypothalamo-pituitary-adrenal axis during chronic stress in rats are characterised by increased hypothalamic CRH mRNA and immunoreactive CRH, decreased pituitary CRH receptors, higher pituitary content of ACTH, normal or slightly elevated plasma ACTH, and hypersecretion of the ACTH responses to a novel stress.<sup>30,31</sup> Another study showed that pretreatment with short inescapable stress induced exaggerated ACTH secretion to a novel stress, whereas cortisol secretion did not differ between previously stressed and control rats.<sup>32</sup> These results from stressed animals resemble our human data. Stress experience may account for a sensitised ACTH response in the pituitary gland and a desensitised adrenocortex of IBS patients. Furthermore,  $\alpha_2$  adrenergic antagonists potentiate exogenous CRH induced ACTH secretion in rats.<sup>33</sup> A blunted growth hormone response to desipramine in IBS patients, which suggests impaired  $\alpha_2$  adrenergic function, was also reported.<sup>34</sup> Therefore, exogenous CRH induced ACTH hypersecretion in IBS patients may be due to  $\alpha_2$  blockade in the brain. It is also possible that decreased levels of CRH binding protein, which inhibits the ACTH releasing properties of CRH, may play a role in ACTH hypersecretion in IBS patients.<sup>35</sup> This possibility should be explored in the future.

Anxiety and depression are common psychological features in IBS patients.<sup>2</sup> Intracerebroventricular administration of CRH or an  $\alpha_2$

antagonist induces noradrenaline release in the locus caeruleus with concomitant production of anxiogenic behaviour in rats.<sup>22</sup> Patients with depression have CRH hypersecretion in the brain, especially the paraventricular nucleus.<sup>36</sup> Neuronal circuits relay visceral information to these nuclei. Distension of the distal colon increases the firing rate of the locus caeruleus.<sup>37</sup> As the majority of IBS patients have a decreased visceral threshold to colonic distension,<sup>38</sup> increased visceral information to the locus caeruleus may cause more activation of CRH neurones in the paraventricular nucleus.<sup>39</sup> Exogenous CRH decreases the visceral threshold to rectal distension in humans<sup>40</sup> and this mechanism probably relates to CRH induced abdominal symptoms in IBS patients as well as motility change. Furthermore, intravenous administration of CRH decreases slow wave sleep in humans<sup>11</sup> and the proportion of rapid eye movement (REM) sleep is notably increased in IBS patients.<sup>41</sup> We previously reported the stress induced increase in electroencephalographic beta power in IBS patients.<sup>4</sup> These findings support our hypothesis that CRH is increased in the brain of IBS patients.

In conclusion, intravenous administration of CRH partially mimicked the stress response of the gastrointestinal motility and neuroendocrine response in humans. These responses were exaggerated in IBS patients. Our present study suggests that CRH plays an important role in modulating brain-gut functions under stress in humans, and that this neuropeptide relates to the pathophysiology of IBS.

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