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RESEARCH ARTICLE | *Neurogastroenterology and Motility*

The effect of intravenous corticotropin-releasing hormone administration on esophageal sensitivity and motility in health

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Broers C, Melchior C, Van Oudenhove L, Vanuytsel T, Van Houtte B, Scheerens C, Rommel N, Tack J, Pauwels A. The effect of intravenous corticotropin-releasing hormone administration on esophageal sensitivity and motility in health. *Am J Physiol Gastrointest Liver Physiol* 312: G526–G534, 2017. First published March 23, 2017; doi:10.1152/ajpgi.00437.2016.—Esophageal hypersensitivity is important in gastroesophageal reflux disease (GERD) patients who are refractory to acid-suppressive therapy. Stress affects visceral sensitivity and exacerbates heartburn in GERD. Peripheral CRH is a key mediator of the gut stress response. We hypothesize that CRH increases esophageal sensitivity and alters esophageal motility in health. Esophageal sensitivity to thermal, mechanical, electrical, and chemical stimuli was assessed in 14 healthy subjects after administration of placebo or CRH (100 μg iv). Perception scores were assessed for first perception, pain perception threshold (PPT), and pain tolerance threshold (PTT). Esophageal motility was investigated by high-resolution impedance manometry, before and after CRH and evaluated by distal contractile integral (DCI) and intrabolus pressure (IBP). Pressure flow analysis assessed bolus clearance (impedance ratio), degree of pressurization needed to propel bolus onward (IBP slope), and pressure flow (pressure flow index, PFI). Stress and mood were assessed during the study. Sensitivity to mechanical distention was increased after CRH compared with placebo (PPT: $P = 0.0023$; PTT: $P = 0.0253$). CRH had no influence on the other stimulations. DCI was increased for all boluses (liquid, $P = 0.0012$; semisolid, $P = 0.0017$; solid, $P = 0.0107$). Impedance ratio for liquid ($P < 0.0001$) and semisolid swallows ($P = 0.0327$) decreased after CRH. IBP slope increased after CRH for semisolid ($P = 0.0041$) and solid ($P = 0.0003$) swallows. PFI increased for semisolid ($P = 0.0017$) and solid swallows ($P = 0.0031$). CRH increased esophageal sensitivity to mechanical distention, not to the other stimulation modalities. CRH increased esophageal contractility and tone, decreased LES relaxation, increased esophageal bolus pressurization, improved esophageal bolus clearance, and increased pressure flow.

NEW & NOTEWORTHY This is the first study to address the effect of corticotropin-releasing hormone (CRH) on esophageal sensitivity and alterations in motility in health. CRH administration increased esophageal sensitivity to mechanical distention. This effect is accompanied by an increase in esophageal contractility and tone and a decrease in lower esophageal sphincter relaxation. CRH increased esophageal bolus pressurization, improved esophageal bolus clearance, and increased pressure flow. The changes in esophageal contractile properties may underlie the increased sensitivity to mechanical distention after CRH.

corticotropin-releasing hormone; gastroesophageal reflux disease; esophageal sensitivity; esophageal motility; stress

GASTRO-ESOPHAGEAL REFLUX DISEASE (GERD) is defined by Vakil et al. (50) as a condition that develops when reflux of gastric contents causes troublesome symptoms such as heartburn, regurgitation, and/or complications, such as esophagitis, peptic stricture, Barrett's esophagus, and adenocarcinoma. GERD is increasingly prevalent in Western societies (50) with ~10–30% of the general population in Europe and the United States suffering from reflux symptoms at least once a week (9, 14, 27, 39). Proton pump inhibitors (PPIs) are very effective in healing esophagitis (13); however, it is estimated that 10–40% of patients with GERD continue to experience reflux symptoms despite adequate acid-suppressive treatment (5, 16, 49). The cause of these refractory reflux symptoms remains largely unclear. Numerous factors, including absence of underlying GERD, inadequate intake of PPIs, ongoing weakly acid reflux, and esophageal hypersensitivity, have been implicated (6–9). Among these, a key role has been attributed to esophageal hypersensitivity, which is demonstrable in a large subset of patients with PPI-refractory GERD symptoms (8–10).

The mechanisms underlying esophageal hypersensitivity have not been fully elucidated, but stress is considered a potentially important underlying factor (11, 12). Up to 64% of individuals with heartburn report that psychological factors, including life stress, aggravate their GERD-related symptoms (32). Fass et al. (15) showed that auditory stress exacerbated symptom perception during esophageal acid perfusion in patients with GERD. Although most studies focused on esophageal sensitivity to acid reflux or acid perfusion, there is increasing evidence that esophageal hypersensitivity in patients with PPI-refractory GERD symptoms is of a multisensory nature. Indeed, a number of studies have shown that these patients are also hypersensitive to mechanical or thermal-esophageal stimulation, and this may also be relevant to symptom generation (13–15). Furthermore, stress is not only able to alter esophageal sensitivity but may also affect esophageal

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motility. As early as 1962, Rubin et al. (40) showed a significant increase in nonperistaltic contractions during a stressful condition in healthy volunteers. More recent studies have also documented esophageal motility changes in response to stressors in healthy subjects and patients with preexisting esophageal dysmotility abnormalities (2, 21, 42).

Stress induces the release of peripheral CRH, which is a pivotal player in the stress response of the GI tract. CRH plays a key role in the acute regulation of stress- and anxiety-related behaviors and in the regulation of endocrine responses during chronic stress via activation of the hypothalamic-pituitary-adrenal axis (HPA axis) (46, 48). During acute and chronic stress, CRH drives secretion of adrenocorticotropic hormone from the pituitary, ultimately leading to the release of cortisol from the adrenal glands (48). The effect of stress on the GI tract is mediated, at least in part, via a direct effect of CRH on the CRH_1 receptor identified on human intestinal mucosal mast cells and to a lesser extent via CRH_2 receptors (44).

In this study, CRH is administered to mediate one of the key molecules involved in the GI stress response. We hypothesize that CRH will increase multimodal esophageal sensitivity and alter esophageal motility. Hence, we investigated whether administration of CRH affects esophageal sensitivity to thermal, mechanical, electrical, and chemical stimulation in healthy volunteers (HV) and whether the CRH is involved in alterations in esophageal motility.

MATERIALS AND METHODS

Subjects. Sensitivity and motility studies were performed in HV. Before the initiation of the study, all participants provided informed consent. Inclusion criteria included age between 18 and 60 yr old. Exclusion criteria included a history of GI symptoms, a history of allergic reaction to CRH, atopy (e.g., eczema, asthma, food allergies, and allergic rhinoconjunctivitis), or multiple allergies to several drugs, pregnancy or lactation, concomitant administration of monoamine oxidase inhibitors, verapamil or diltiazem, or medication affecting esophageal motility, significant comorbidities (neuromuscular, psychiatric, cardiovascular, pulmonary, endocrine, autoimmune, renal, and hepatic), prior history of ear, nose and throat surgery or endoscopic antireflux procedure, and first-degree relatives with Crohn's or celiac disease. Both study protocols have been registered to ClinicalTrials.gov (NCT02736734, NCT02674256) and the European Union Drug Regulating Authorities Clinical Trials (EudraCT) registry under the numbers 2014-000602-36 and 2014-002239-33. The protocols were in accordance with the Declaration of Helsinki and were approved by the Ethics Committee of the University of Leuven (approval numbers S56177 and S57111).

Test conditions. After an overnight fast, HV came to the endoscopy unit of the university hospital. All study visits started between 1 PM and 3 PM, to reduce diurnal variation. CRH administration was executed as follows: a solution of 100 μg CRH powder for injection (CRH Ferring, Ferring, Aalst, Belgium) in 1 ml of NaCl 0.9% was injected intravenously over the course of 1 min (52). This dose of CRH is known to alter gastrointestinal function and increases in plasma ACTH secretion to stress levels with detectable plasma CRH in humans (18, 43). Furthermore, this dose has been previously shown to reproduce the gastrointestinal effects of stress in a mast cell-dependent fashion (52). In this way, side effects are limited to transient facial flushing lasting from 5 to 45 min in ~75% of subjects. Intravenous CRH administration is clinically used as a diagnostic tool in locating the source of hypercortisolemia in Cushing's disease. Following intravenous administration of 100 μg CRH, maximal plasma concentrations of CRH are achieved after 5 min. The elimination half-life of one dose 100 μg CRH is ~9 min. Cortisol levels reach a maximal concentration ~30 min after CRH administration, and cortisol normalizes 120 min after CRH administration (52).

In the first protocol, we investigated the effect of CRH on esophageal sensitivity using a multimodal stimulation protocol in which all participants underwent two conditions: *1*) placebo (0.9% NaCl) and *2*) CRH administration. Over time, each participant received placebo or CRH (crossover, counterbalanced) with an interval at least of 1 wk, in a single-blind fashion.

In the second study, esophageal motility was assessed before and after the administration of CRH on the same day by a standard high-resolution impedance manometry.

Esophageal sensitivity testing by multimodal stimulation. Esophageal sensitivity was evaluated by a multimodal esophageal stimulation probe (Ditens, Aalborg, Denmark), which allows thermal, mechanical, electrical, and chemical stimulation of the esophagus in one single protocol (Fig. 1) (12).

The multimodal stimulation probe was positioned through the mouth in the esophagus with the top of the inflatable balloon positioned 10 cm above the lower esophageal sphincter (LES). To locate the LES, the balloon was inserted into the stomach and filled with 20 ml of saline with subsequent retraction of the probe to identify the LES. Subsequently, after deflating the balloon, the probe was further retracted 10 cm proximal to the LES. The subjects remained in a semirecumbent position for the entire study period. After a 15-min adaptation period, HV received an intravenous injection of placebo (0.9% NaCl) or CRH. Immediately after the injection, four types of stimulations were performed in the following order: thermal, mechanical, electrical, and chemical stimulation, according to our experimental design (Fig. 2).

Before the start of the sensitivity study, all subjects were instructed how to use a pain-scoring scale, which has been shown to be reliable in discriminating esophageal sensations (10). Thresholds for first

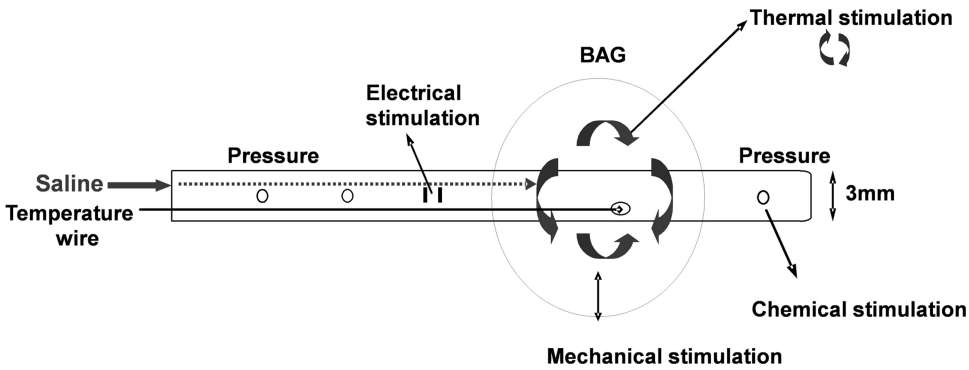

Fig. 1. Schematic representation of the multimodal esophageal stimulation probe.

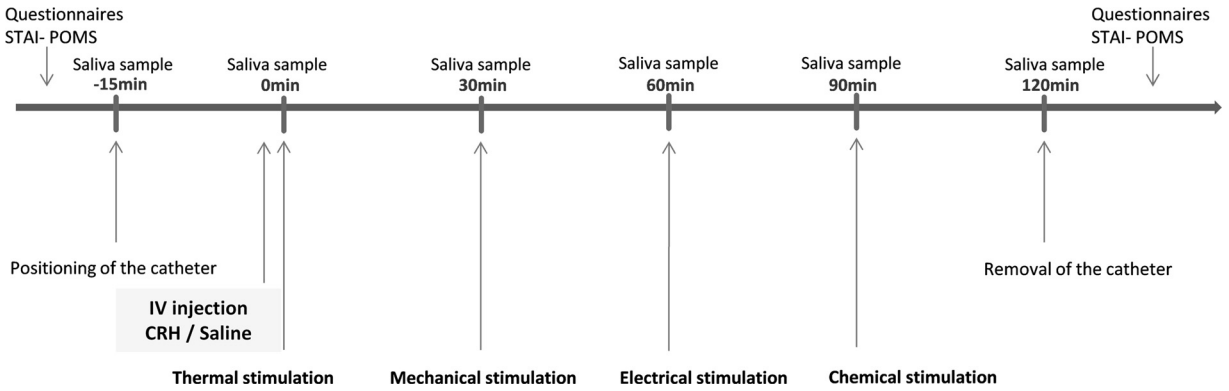

Fig. 2. Study outline of corticotropin-releasing hormone (CRH) sensitivity. STAI, state-trait anxiety inventory; POMS, profile of mood states; CRH, corticotropin-releasing hormone; IV, intravenous.

perception, pain perception threshold (PPT), and pain tolerance threshold (PTT) were recorded.

Thermal stimulation was performed by recirculating a saline solution (0.09% NaCl) through the balloon mounted on the probe, and infusion water was heated by a water bath with a maximal temperature of 62°C. The stimulation temperature was steadily increased by increasing the flow rate from the water bath to the balloon; flow rate was controlled by a computer-operated pump (Harvard PHD 2000). The volume in the balloon was kept constant at 5 ml to avoid mechanical stimulation of the esophagus. A temperature sensor present in the balloon continuously monitored the stimulation temperature. Thermal stimulation started immediately after the intravenous bolus injection of CRH or placebo and was terminated when the subject reached PTT.

Mechanical stimulation of the esophagus was executed by distention of the balloon. The flow of a saline solution (0.09% NaCl) into the balloon, inducing the distention, was regulated by a computer-controlled pump (25 ml/min, ramp distention). Mechanical stimulations were performed using a solution of 37°C, to avoid thermal stimulation of the esophagus. The stimulation started 30 min after the injection of CRH or placebo and was terminated when the subject reached PTT.

Two electrodes mounted on the probe proximal to the inflatable balloon were used to administer short electrical pulses. Electrical block pulses with a duration of 1 ms at 200 Hz were given using a standard electrical stimulator (12). The amplitude of the pulses steadily increased, with steps of 0.5 mA at an interval of 15 s. The maximum intensity was limited to 50 mA, as previous studies have shown atrial capturing with higher intensities (12, 17). ECG monitoring was performed as a safety measure during the electrical stimulations. Electrical stimulation started 60 min after the injection of CRH or placebo and was terminated when the subject reached PPT.

Finally, after pulling back the probe 3 cm, chemical stimulation was performed in the distal esophagus by infusing an acidic solution (0.1 N HCl), an adaptation of the Bernstein test, used in clinical practice to diagnose reflux disease since the early sixties (3). Chemical stimulation was controlled by a peristaltic infusion pump with a flow rate of 2 ml/min. The stimulation lasted for a maximum period of 30 min or was terminated when subjects reached PTT. The stimulation was initiated 90 min after the injection of CRH or placebo.

Esophageal motility testing by standard high-resolution impedance manometry. A solid-state, high-resolution, impedance manometric (HRiM) catheter consisting of 36 manometry channels spaced at 1-cm intervals and 16 impedance channels (Unisensor, Attikon, Switzerland) was placed transnasally under topical anesthesia (lignocaine gel) and positioned along the esophagus with the distal two sensors in the stomach. Pressure and impedance data were acquired at 20 Hz (Solar GI, Medical Measurement Systems, Enschede, The Netherlands). After the catheter was positioned, subjects remained in a semirecumbent position for the entire study period, and pressure and impedance measurements were recorded.

Test boluses of 5 ml of liquid (water), 5 ml of semisolid (apple sauce), and 2 cm^2 solid (white bread) were administered orally. All bolus stock contained 1% NaCl to enhance conductivity. Ten swallows of each consistency were executed. After being measured under baseline conditions, CRH was administered intravenously, and after 30 min, the same procedure was repeated (Fig. 3).

For each type of bolus consistency, data gathered from multiple swallows were averaged for each HV. These mean values were used for further analysis (Solar GIHRM; Medical Measurement Systems). Contraction patterns during the different swallows were compared between baseline and CRH recordings, according to Chicago Classification v3.0 (22). Although the evaluation scheme of the Chicago Classification is based on the analysis of ten 5-ml liquid swallows performed in a supine position, we used the Chicago Classification for the analysis of liquid, semisolid, and solid swallows in a semirecumbent position. Esophageal contractile function was evaluated before and after administration of CRH by assessing the distal contractile integral (DCI) and measuring contractile vigor and the intrabolus pressure (IBP). Furthermore, the integrated relaxation pressure of the LES, mean of the 4 s of maximal deglutitive relaxation in the 10-s window beginning at the upper esophageal sphincter relaxation (integrated relaxation pressure, IRP) was calculated. IRP was used as a marker of resistance at the level of the esophago-gastric junction (EGJ)/LES.

Combined esophageal manometry and multichannel intraluminal impedance recordings allow one to describe the complex interplay between bolus transport and pressure generation. Therefore, pressure flow analysis was performed using esophageal automated impedance manometry software (AIMPlot_OES_V4.2, copyright T. Omari, 2014), a purpose-designed analysis program written in MATLAB (version 7.9.0.529 R2009b, The MathWorks, Natick, MA). Five space-time landmarks were defined on a standard pressure isocontour plot of the esophageal swallow: *1)* time of onset of swallow, *2)* time of proximal peak pressure, *3)* proximal margin of the esophageal pressure wave sequence, *4)* position of the transition zone, and *5)* distal margin of the esophageal pressure wave sequence. The following parameters were evaluated: *1)* the ratio of nadir impedance to impedance at the time of peak pressure (NI/IIPP ratio or the impedance ratio, IR), which is used as a marker of bolus clearance, *2)* the intrabolus pressure slope (IBP slope), the rate of change in IBP recorded during the phase of transition from a full lumen to an occluded lumen. IBP slope is a marker of the pressurization needed to propel a bolus forward, and *3)* pressure flow index (PFI), which reflects the relationship between intrabolus pressure and bolus flow timing in the esophagus. The PFI is calculated using the formula: (IBP $\times$ IBP slope)/(time from nadir impedance to peak pressure) and

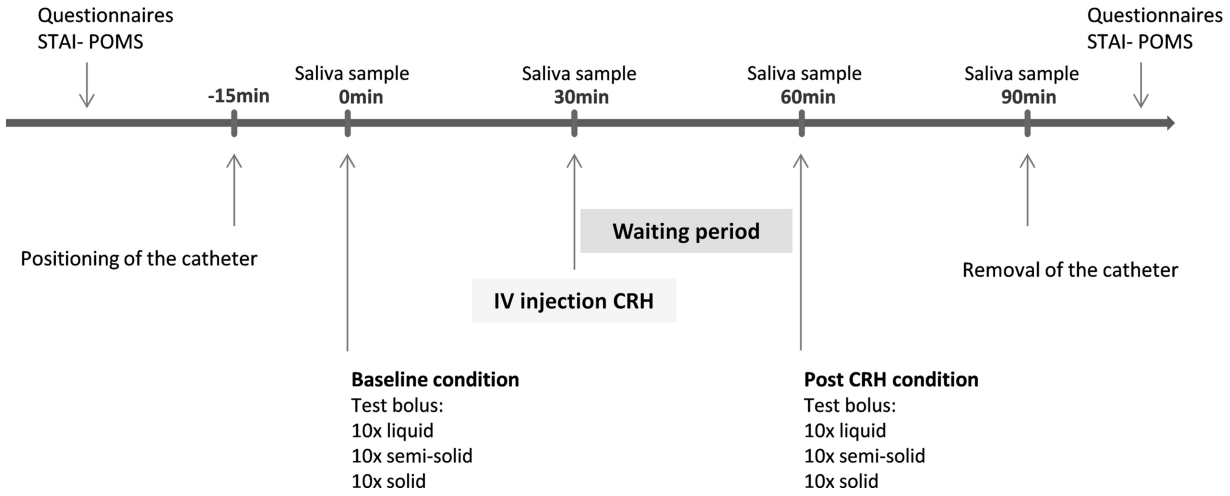

Fig. 3. CRH-motility study outline.

serves as a global measure of pressure flow or EGJ resistance to bolus flow (35, 38).

Evaluation of stress symptoms and hormones, emotion, and general mood. In both protocols, an assessment of momentary anxiety levels and mood state was performed by using the State-Trait Anxiety Inventory (STAI, state scale) and the Profile of Mood States (POMS) questionnaires before and after the study procedures. The STAI scale is a validated and widely used questionnaire measuring levels of transitory anxiety (51). The scale consists of 20 items, which are answered on a four-point scale. A total score was calculated according to the instructions of the questionnaire. The POMS questionnaire, validated for the measurement of different emotional/mood states, contains 32 questions and is designed to measure feelings of tension-anxiety, depression-dejection, anger-hostility, fatigue, vigor, and confusion (29).

Salivary samples were collected (Salivette, Sarstedt, Nümbrecht, Germany) to determine the concentration of salivary cortisol. In the sensitivity study, samples were collected before the positioning of the probe, immediately before the placebo or CRH administration, and every 30 min for 2 h after administration of placebo or CRH (Fig. 2). In the motility study, saliva samples were collected before the positioning of the probe, immediately before the CRH administration, and at 30 and 60 min after administration of CRH (Fig. 3). The samples were stored at −20°C after centrifugation (4°C, 3,000 rpm, 10 min). Salivary cortisol was determined by ELISA (DRG Diagnostics, Marburg, Germany), according to the manufacturer's instructions.

Statistical analysis. Statistical analysis was performed using Prism 5.01 (GraphPad Software, La Jolla, CA). Threshold comparisons were performed as well as a comparison of differences in change in questionnaire data after and before the stimulations between CRH and placebo conditions within subjects. Comparisons were done using a paired Student's *t*-test or the nonparametric paired Wilcoxon signed rank test with Bonferroni correction for multiple testing. Deviations from Gaussian distribution were tested using the Shapiro-Wilk normality test. Cohen's *d* index, a measure for the size of observed effects, was performed for tests within groups using the mean and standard deviation. Cohen's *d* can be calculated as the difference between the means of two conditions divided by the pooled standard deviation (0.2 = small effect, 0.5 = medium effect, >0.8 large effect) (7). Results are expressed as median (25–75th percentile), unless indicated otherwise. A P value < 0.05 was considered statistically significant.

RESULTS

Esophageal sensitivity. Fourteen HVs (8 male/6 female, 30.7 ± 10.6 yr, and body mass index of 23.7 ± 2.0 kg/m^2) were recruited to investigate the effect of CRH administration on esophageal sensitivity assessed by multimodal stimulation. Esophageal sensitivity to mechanical distention was significantly increased after CRH administration compared with that in placebo condition. After CRH administration, PPT levels during mechanical stimulation were reached at significantly lower distending balloon volumes compared with those in placebo administration (24.10 vs. 28.48 ml, $P = 0.0023$, survives Bonferroni correction), with a large size effect (Cohen's $d = 0.89$). Similarly, PTT levels were reached earlier after CRH than placebo administration (30.24 vs. 32.30 ml, $P = 0.1953$), with a small size effect (Cohen's $d = 0.42$) (Table 1). However, this did not reach statistical significance since we only evaluated subjects reaching the PTT at the maximal inflation volume of 50 ml. In addition, we observed that 6 (43%) HV did not reach PTT in the placebo condition at the maximal inflation volume, whereas this was only the case in two (14%) HV in the CRH condition (Fisher's exact test, $P = 0.2087$). Administration of CRH had no influence on esophageal sensitivity to thermal, electrical, or chemical stimulation in HV compared with the placebo condition (Table 1).

Esophageal motility. Fourteen HV (8 males/6 females, mean age 26.6 ± 5.8 yr, and body mass index of 23.1 ± 1.2 kg/m^2) were included in the study. After CRH administration, DCI values significantly increased for all three types of bolus (liquid $P = 0.0012$, semisolid $P = 0.0017$, solid $P = 0.011$, all survive Bonferroni correction), whereas no differences in IBP were seen. Finally, IRP values for all three bolus consistencies significantly increased after administration of CRH (liquid $P = 0.039$, semisolid $P = 0.0085$, solid $P = 0.0039$; except for liquid, all survive Bonferroni correction) (Table 2). Differences in Chicago Classification v3.0 outcome before and after administration of CRH were assessed for all three bolus consistencies (data not shown) although Chicago Classification is currently only validated for liquid bolus swallows. No significant changes were seen when the Chicago Classification was applied to liquid or solid boluses. When the Classification was applied to semisolid boluses, a significant decrease in the prevalence of ineffective esophageal motility was found (pre-CRH 6 out of 14 subjects, 42.86% compared with 0 out of 14, 0% after CRH, $P = 0.015$).

Table 1. *Results of esophageal sensitivity tests*

	CRH	Placebo (Saline)	*P* Value Uncorrected	Cohen's *d* †
Temperature stimulation, °C				
PPT	43.99 [41.03–47.06]	45.13 [42.14–48.91]	0.27	0.22
PTT	46.48 [45.00–49.09]	49.07 [44.81–50.66]	0.35	0.19
Mechanical Stimulation, ml				
PPT	24.10 [18.71–26.15]	28.48 [23.39–43.88]	0.0023*	0.89
PTT ($n = 8$)	30.24 [23.98–35.08]	32.30 [28.43–45.20]	0.20	0.42
Electrical stimulation, mA				
1st perception	5.42 [4.45–9.58]	7.58 [5.00–10.00]	0.88	0.04
PPT	11.08 [8.0–16.38]	12.92 [9.38–15.38]	0.95	0.03
Chemical stimulation, ml				
1st perception	12.00 [4.00–24.00]	12.00 [4.00–26.00]	0.55	0.08

Results are presented as median [25–75th percentile]; $n = 14$. For mechanical stimulation, only volunteers reaching PTT at the maximal inflation volume (50 ml) are included in the analysis. CRH, corticotropin-releasing hormone; PPT, pain perception threshold; PTT, pain tolerance threshold. Correction for multiple testing was performed. *Survives Bonferroni correction. †Effect size expressed as Cohen's *d* (0.2 = small effect, 0.5 = medium effect, and >0.8 = large effect).

Pressure flow analysis. The impedance ratio for liquid and semisolid swallows decreased significantly after CRH administration (liquid $P < 0.0001$, survives Bonferroni correction; semisolid $P = 0.0327$). No significant effect was reached for the difference in impedance ratio with solid boluses ($P = 0.059$). Mean IBP slope (mmHg/s) increased after CRH administration for semisolid and solid swallows (semisolid: $P = 0.0041$, solid: $P = 0.0003$; all survive Bonferroni correction), and no statistically significant increase was reached for liquid swallows ($P = 0.058$). PFI increased for semisolid ($P = 0.0017$, survives Bonferroni correction) and solid swallows ($P = 0.0031$, survives Bonferroni correction), but no changes were seen for liquid swallows ($P = 0.1937$) (Table 3).

Salivary cortisol, stress, and mood. In the sensitivity study, salivary cortisol levels were compared at each time point between placebo and CRH conditions. CRH administration resulted in elevated salivary cortisol levels between 30 and 120 min compared with placebo (Fig. 4*A*). Cortisol levels at 30 min after CRH injection were significantly higher compared with cortisol levels after placebo injection [8.68 ng/ml (6.36–12.34) vs. 3.43 ng/ml (2.55–4.21), $P < 0.0001$, survives Bonferroni correction] (Fig. 4*A*). No correlation was found between cortisol levels at 30 min and the balloon volume reached at PPT ($P = 0.81$) and PTT ($P = 0.95$).

Similar results were found in the motility study where cortisol levels were measured up to 60 min after CRH administration (Fig. 4*B*). When compared with baseline (−15 min), an increase in salivary cortisol was seen at 30 min after the intravenous CRH injection [4.40 (2.35–5.40) vs. 5.87 ng/ml (5.79–6.79), $P = 0.0002$, survives Bonferroni correction] (Fig. 4*B*). No correlation was found between changes in cortisol and HRiM parameters.

In the sensitivity study, CRH administration exerted effects at a behavioral level. Anxiety scores were compared between CRH and placebo at the end of the procedure. No differences were found in state anxiety scores on the STAI at the end of the CRH session compared with placebo [50.00 (49.00–52.00) vs. 49.50 (48.75–50.00), $P = 0.058$]. This difference could not be assessed in the motility study since baseline and CRH measurements were performed during one single procedure. However, we did not see a difference in state anxiety scores before and after CRH administration [50.00 (49.00–51.00) vs. 50.00 (50.00–51.25), $P = 0.4346$]. The POMS anxiety scores did not differ before and after the motility procedure [30.85 (26.53–41.98) vs. 31.55 (28.63–42.00), $P = 0.0960$]. When POMS anxiety scores at baseline and at the end of the sensitivity study were compared, no differences could be found between CRH or placebo conditions [6.40 (3.10–8.90) vs. 3.60 (0.50–6.20), $P = 0.3368$].

DISCUSSION

In the current study, our aim was to elucidate the effect of exogenous CRH on esophageal sensitivity and motility. We

Table 2. *High-resolution manometry results of esophageal motility tests*

	Pre-CRH	Post-CRH	*P* Value Uncorrected	Cohen's *d* +
Liquid				
DCI, $mmHg \cdot s^{-1} \cdot cm^{-1}$	686 [541.30–1149.00]	1391 [926.00–2035.00]	0.0012*	0.94
IBP, mmHg	7.00 [5.00–8.25]	6.00 [4.50–8.00]	0.075	0.26
mIRP, mmHg	8 [7–9]	12 [9–14]	0.039	0.62
Semisolid				
DCI, $mmHg \cdot s^{-1} \cdot cm^{-1}$	620.50 [381.50–915.30]	1180.00 [639.80–1811.00]	0.0017*	0.92
IBP, mmHg	5.00 [3.75–9.25]	5.00 [4.00–7.25]	0.79	0.02
mIRP, mmHg	8 [7–9]	10 [7–14]	0.0085*	0.64
Solid				
DCI, $mmHg \cdot s^{-1} \cdot cm^{-1}$	1261.00 [832.80–2596.00]	1947.00 [1405.00–3329.00]	0.0107*	0.63
IBP, mmHg	4.50 [2.75–8.50]	5.00 [2.75–8.50]	1.00	0.06
mIRP, mmHg	8 [6–12]	12 [10–16]	0.0039*	0.85

Changes in esophageal motility before and after intravenous CRH administration. Values for distal contractile integral (DCI), intrabolus pressure (IBP) and median integrated relaxation pressure (mIRP4) are shown for liquid, semisolid, and solid boluses. Results are presented as median [25–75th percentile]; $n = 14$. Correction for multiple testing was performed for each bolus type. *Survives Bonferroni correction.

Table 3. *Pressure flow analysis metrics based on HRiM before and after intravenous CRH administration*

	Pre-CRH	Post-CRH	*P* Value Uncorrected	Cohen's *d* +
Liquid				
Impedance ratio	0.29 [0.22–0.34]	0.25 [0.20–0.28]	<0.0001*	0.73
IBP slope, mmHg/s	2.12 [1.35–2.58]	2.57 [1.85–3.35]	0.06	0.46
PFI	5.47 [3.12–7.64]	6.20 [3.57–10.60]	0.19	0.31
Semisolid				
Impedance ratio	0.36 [0.25–0.48]	0.29 [0.25–0.35]	0.03	0.63
IBP slope, mmHg/s	5.67 [3.72–7.65]	7.02 [5.57–8.86]	0.0041*	0.65
PFI	32.25 [25.82–65.03]	51.50 [36.03–79.54]	0.0017*	0.49
Solid				
Impedance ratio	0.47 [0.39–0.58]	0.43 [0.33–0.55]	0.06	0.25
IBP slope, mmHg/s	10.87 [5.10–15.42]	16.08 [12.09–21.81]	0.0003*	0.96
PFI	140.80 [53.85–276.60]	223.00 [109.80–455.00]	0.0031*	0.52

The ratio of mean nadir impedance and impedance at peak pressure (or impedance ratio), intrabolus pressure slope (IBP slope), and pressure flow index (PFI) are shown for liquid, semisolid, and solid boluses. Results are presented as median [25–75th percentile]; $n = 14$. Correction for multiple testing was performed for each bolus type. *Survives Bonferroni correction.

demonstrated that intravenous CRH administration *1*) increased salivary cortisol levels; *2*) enhanced esophageal sensitivity to mechanical distention; *3*) did not alter esophageal sensitivity to thermal, electrical, and chemical stimulation; *4*) increased esophageal contractile amplitude and decreased LES relaxation; and *5*) improved esophageal bolus clearance (reflected by decreased impedance ratio), increased esophageal bolus pressurization (reflected by increased IBP slope), and increased EGJ resistance to bolus flow (reflected by increased PFI).

Noxious stimuli in the esophagus are sensed by nociceptive receptors located on esophageal nerves and transmitted via spinal or vagal nerves to the central nervous system (31). Esophageal sensitivity is modulated at both peripheral and central levels. However, the details of interaction of peripheral and central factors in modulating esophageal pain perception and sensitivity have not been elucidated yet. Stressful conditions are known to increase esophageal nonperistaltic contractions (1, 2). CRH is a key mediator of responses of the body to stress and is well known to be involved in stress-related hyperalgesia. Both central and peripheral CRH signaling has been implicated in the pathogenesis of visceral hypersensitivity (26, 33, 41).

The available literature has already established a role for stress in the generation of acid-related symptoms. Fass et al. (15) showed that acute auditory stress can exacerbate heartburn symptoms in GERD patients, through an enhanced perceptual response to intra-esophageal acid exposure. Similarly, it has been shown that stress tasks can increase subjective ratings of reflux symptoms in patients with GERD, without increasing objective parameters of acid reflux. Moreover, in patients who are chronically anxious and exposed to prolonged stress, there was no habituation of reflux symptom perception upon repeated exposure to stress tasks (6). At a central level, an upregulation of central stress and arousal circuits has been postulated (28).

CRH has been implicated in the acute regulation of stress and anxiety-related behaviors and in the regulation of behavior and endocrine responses during chronic stress. Furthermore, it is well known to mediate stress and anxiety via activation of the HPA axis. When a stressor is perceived, the hypothalamus will be activated to release CRH, a hypothalamic peptide which, in its turn, will activate the release of cortisol (48). Besides its actions on the central nervous system, peripheral CRH signaling pathways are also known to be involved in stress-related changes in GI physiology (25, 46). Larauche et al. (25) stated an equally important role of the peripheral CRH signaling in visceral hypersensitivity. CRH is able to cross the blood-brain barrier via a well-characterized saturating efflux system (24). Also peripheral sources of CRH have been identified. Zheng et al. (55) demonstrated that eosinophils are able to express CRH in the jejunum in response to psychological stress in mice. Furthermore, mast cells have been shown to express CRH receptors (25, 47, 53). CRH exerts its biological actions by interacting with CRH_1 and CRH_2 receptors (25, 26, 33, 41, 45). Genetic alterations of the CRH system have been

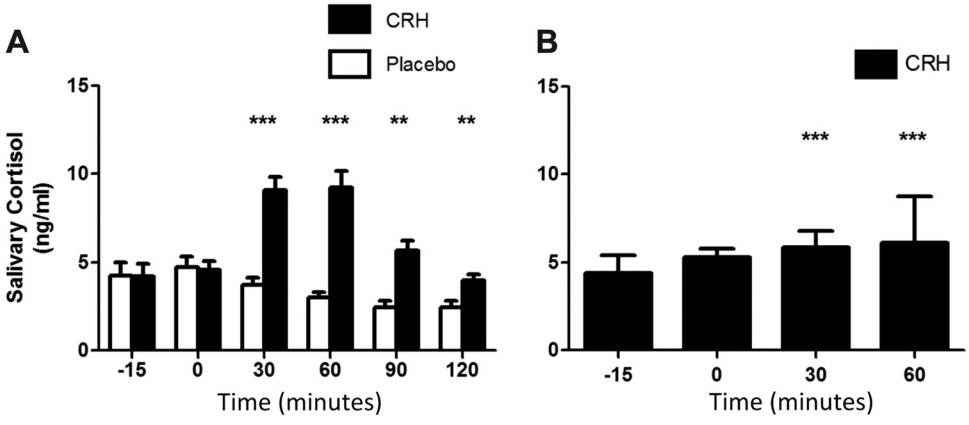

Fig. 4. Hormonal effect of CRH administration on salivary cortisol levels. *A*: in the sensitivity study, salivary cortisol was increased at 30, 60, 90 and 120 min after CRH administration compared with placebo. *B*: in the motility study, cortisol levels were increased 30 and 60 min after administration of CRH. Median (interquartile ranges) are indicated on the graph. ***$P < 0.0001$ and **$P < 0.01$, all significant *P* values survive Bonferroni correction.

implicated in pathophysiology of anxiety and depression (4). Preclinical and clinical data support an important role for the CRH_1 receptor in mediating acute and chronic stress-induced colonic hyperalgesia. In IBS patients, CRH may modulate visceral hypersensitivity (23). Stress induces the release of peripheral CRH, which mediates the stress response of the GI tract. Hence, we used an intravenous CRH administration to mimic this effect on esophageal sensorimotor function. CRH is able to exert physiological effects rapidly after administration (52), and the timing of procedures in the study design was based on that knowledge.

Esophageal sensitivity has been investigated in previous studies: thermal, mechanical, electrical, and chemical stimuli can all be perceived in the esophagus. Because pain is a multidimensional experience, the optimal way to evaluate this sensation is to use a multimodal stimulation approach, as previously published (11, 12). We demonstrated that CRH lowered the threshold for pain perception to mechanical distention. However, we were unable to find an effect of CRH on sensitivity to thermal, electrical, and chemical stimulation. These findings suggest a sensory modality-dependent effect of exogenous CRH.

Although the data in the current study show that CRH mainly has an impact on sensitivity to mechanical distention, it is conceivable that other sensory modalities are implicated in hypersensitive GERD patients. In a previous study, it was shown that nonerosive reflux disease patients are hypersensitive to chemical, thermal, and mechanical stimulation, and they react with a higher number of esophageal contractions to balloon distention compared with controls (37).

Previous studies, focusing on the colon, have shown that administration of CRH induces hypersensitivity to colorectal distention in rodents and humans (26, 33). These reports are in agreement with our findings in the esophageal sensitivity study. Nevertheless, visceral mechanosensitivity is strongly influenced by contractile activity. Hence, we used HRiM to also evaluate the impact of CRH on esophageal contractility and bolus flow (20, 36). CRH administration resulted in higher DCI values, indicating increased amplitude of esophageal contractions in response to liquid, semisolid, and solid bolus swallows. We also found an increase in IRP values for all three types of bolus consistencies, indicating reduced swallow-induced LES relaxation. These findings indicate an increase outflow resistance, which could be the main effect of intravenous CRH administration on esophageal motility. On the other hand, the median IRP values remained within the normal range and did not exceed the cut-off values for EGJ outflow obstruction [>28.28 mmHg for 36 solid-state unidirectional sensors (Unisensor AG)] (19, 22). In agreement with an increased resistance at the EGJ, we could show an increased resistance to bolus flow reflected by an increase in pressure flow index. This was accompanied by higher values of IBP slope for semisolid and solid bolus swallows, indicative of an increased degree of pressurization needed to propel the bolus onward (34). The impedance ratio for liquid and semisolid bolus swallows, a marker for incomplete bolus transit, was decreased after administration of CRH, showing more effective bolus clearance. The findings on manometry and impedance, suggesting increased contractile tone, are in line with older studies evaluating the effects of stress on esophageal function in healthy volunteers (8, 30). Many GERD patients attribute a worsening of their symptoms to stress (15, 21, 32), by increasing contractile tone, CRH could decrease esophageal distensibility and provoke higher symptom perception in response to reflux of gastric contents into the esophagus (54). However, because we studied healthy subjects, these statements should be verified in a separate study in which we investigate the effect of CRH on esophageal sensitivity and motility in GERD patients.

We acknowledge that the current study has some limitations. We did not perform dose-response studies in our experiments; the study is, therefore, vulnerable given the choice of dose was based on available literature. Furthermore, technical limitations of the stimulation probe available at our institution prevent us from measuring the cross-sectional area of the distending balloon used for mechanical stimulation. Therefore, we are unable to separate an effect on esophageal sensitivity from an effect on motor function, particularly esophageal compliance. Salivary cortisol levels were not maximally elevated at the time of temperature stimulation, and this precludes us from fully evaluating actions of CRH through the HPA axis on thermosensitivity.

In conclusion, we demonstrated that intravenous CRH administration increased esophageal sensitivity to mechanical distention in health. However, no changes were seen in sensitivity to the other stimulation modalities. Furthermore, we observed an increase in esophageal contractility and tone and a decrease in LES relaxation. As expected peripheral CRH administration increased cortisol levels. The changes in esophageal contractile properties may underlie the increased sensitivity to balloon distention after CRH.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

C.B., T.V., J.F.T., and A.P. conceived and designed research; C.B., C.M., B.V.H., and A.P. performed experiments; C.B., C.M., C.S., and A.P. analyzed data; C.B., C.M., L.V.O., T.V., C.S., N.R., J.F.T., and A.P. interpreted results of experiments; C.B. and C.M. prepared figures; C.B. and C.M. drafted manuscript; C.B., C.M., L.V.O., T.V., B.V.H., C.S., N.R., J.F.T., and A.P. edited and revised manuscript; C.B., C.M., L.V.O., T.V., B.V.H., C.S., N.R., J.F.T., and A.P. approved final version of manuscript.

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