Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 1417–1420, February 1989
Physiological Sciences

Regulation of duodenal bicarbonate secretion during stress by corticotropin-releasing factor and β-endorphin

(neuropeptides/mucosal defense/autonomic nervous system/brain–gut axis)

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Communicated by Roger Guillemin, November 7, 1988 (received for review September 22, 1988)

ABSTRACT **Proximal duodenal mucosal bicarbonate secretion is an important factor in the pathogenesis of duodenal ulcer disease. To examine the central nervous system regulation of duodenal bicarbonate secretion, an animal model was developed that allowed cerebroventricular and intravenous injections as well as collection of duodenal perfusates in awake, freely moving rats. The hypothalamic peptide corticotropin-releasing factor (CRF) and stress (physical restraint) significantly stimulated duodenal bicarbonate secretion. These responses were abolished by pretreatment of the animals with the CRF receptor antagonist α-helical CRF-(9–41), hypophysectomy, and naloxone. In contrast, blockade of autonomic efferents by surgical and pharmacological means did not prevent the stimulatory effects of stress and CRF. Intravenous, but not cerebroventricular, administration of β-endorphin that produced plasma concentrations of β-endorphin that were similar to those produced by exogenous CRF and stress significantly stimulated duodenal bicarbonate secretion. These results indicate that endogenous CRF released during stress and exogenously administered CRF stimulate duodenal bicarbonate secretion by release of β-endorphin from the pituitary, thus, demonstrating a functional hypothalamus–pituitary–gut axis.**

Development of ulcers in the gastroduodenal mucosa results from an imbalance between aggressive and defensive factors (1). The role of aggressive factors (such as gastric acid and pepsin) in ulcer pathogenesis has been studied extensively. The duodenum secretes bicarbonate protecting the mucosa from acid-peptic damage (2). Furthermore, patients with duodenal ulcer disease have decreased proximal duodenal mucosal bicarbonate production at rest and in response to hydrochloric acid (3). Diminished proximal duodenal bicarbonate secretion appears to be an important factor in the development and natural history of duodenal ulcer disease (3). To date, only local mediators of duodenal bicarbonate secretion (acid, hormones, and prostaglandins) have been studied (2). The role of the central nervous system or stress in regulating duodenal bicarbonate secretion, to the best of my knowledge, has not been characterized. The hypothalamic peptide corticotropin-releasing factor (CRF) (4, 5) initiates various stress responses in experimental animals (6–12). The central nervous system effects of CRF and stress on gastrointestinal function are characterized by decreases in gastric acid secretion, emptying, and small bowel transit but by marked increases in large bowel transit (13–19). These responses are mediated by autonomic efferents and not by the pituitary (13, 17–19). The present study shows that endogenous CRF released during stress and exogenously administered CRF stimulate duodenal bicarbonate secretion in rats by the release of β-endorphin from the pituitary, thus, demonstrating a functional hypothalamus–pituitary–gut axis.

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MATERIALS AND METHODS

Animal Preparation. Male Sprague–Dawley rats (250–300 g) were obtained from Wiga (Sülzfeld, F.R.G.). Animals were fed a standard rat diet before surgery and a liquid diet (Altromin Sonderdiät, Hamburg, F.R.G.) thereafter. Four to 7 days before the experiment and under general anesthesia [xylazine at 6 mg/kg (Rompun, Bayer AG, Leverkusen, F.R.G.) and ketamine hydrochloride at 50 mg/kg (Ketanest, Parke–Davis)], the proximal duodenum was isolated. After a midline abdominal incision, the proximal 2 cm of the duodenum (from the pylorus to a few millimeters proximal to entry of the pancreatic duct) were carefully prepared to avoid damage to blood supply and pancreatic tissue. The pylorus was anastomosed to the distal duodenum to reestablish gastrointestinal continuity. The proximal and the distal ends of the isolated duodenal segment were connected with polyethylene catheters (PE-90) that were subcutaneously routed to exit at the interscapular region of the animal's neck where they were secured by adhesive tape. To avoid mucus plugging, the duodenal segment was flushed every 8 hr with 0.15 M NaCl. Two to 4 days before the experiment, in a second operation and under stereotaxic guidance, a stainless steel cannula was implanted so that its tip was inside the right lateral cerebral ventricle (18). Jugular and/or femoral venous catheters were implanted and routed subcutaneously to exit at the interscapular region of the animal's neck (17). This animal model allowed simultaneously cerebroventricular and i.v. injections as well as perfusion of the duodenal segment and venous aspirations in awake, freely moving rats. Adrenalectomy and subdiaphragmatic truncal vagotomy were performed 4–7 days before the experiment as described (17), and hypophysectomized animals were obtained commercially. Adrenalectomized and hypophysectomized animals had free access to water and 0.15 M NaCl. The average weight loss after surgery was 20 g. During the initial phase of this work, the duodenum was histologically examined after the experiment. No ulcerations, erosions, necrosis, capillary leakage, or venous congestion were observed, and the mucosa of the duodenal test segment appeared intact compared with the duodenal mucosa of nonoperated animals.

Experimental Design. After a 24-hr fast, the animals were placed in 15-liter buckets and the cerebroventricular cannula, i.v. catheters, and duodenal catheters were connected with polyethylene tubing. The duodenal test segment was perfused with 0.15 M NaCl (30 ml/hr, 39°C), and the effluent was collected in cone-shaped test tubes by gravity drainage at intervals of 15 min and subsequently subjected to determination of bicarbonate concentration. After a 45-min wash period, basal (resting) duodenal bicarbonate secretion was

Abbreviations: CRF, corticotropin-releasing factor; ACTH, adrenocorticotropic hormone; i.c.v., intracerebroventricular(ly).

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measured. Peptides were obtained from Jean E. Rivier (Salk Institute, La Jolla, CA). They were injected cerebroventricularly (5 μl) or i.v. (0.5 ml as a bolus or 1 ml/hr as a constant infusion) as described (17). The bicarbonate response was recorded for the subsequent 3 hr.

To determine the responsiveness of the duodenal mucosa in this model, various concentrations of the prostaglandin E_1 analogue (±)-methyl-(11α,13*E*)-11,16-dihydroxy-16-methyl-9-oxoprost-13-en-1-oate (misoprostol, Searle) were administered as a constant intraluminal duodenal perfusion (1 μM, 10 μM, and 0.1 mM; 30 ml/hr for 1 hr each at 39°C). The prostaglandin E_1 analogue was stored at −20°C and freshly dissolved in sterile water before the experiment. In stress experiments, the animals were subjected to partial body restraint for 3 hr that was produced by use of three arch-shaped clamps around their trunk. This restraint results in characteristic endocrine and autonomic stress responses (10). In some experiments, the ganglionic response was blocked with chlorisondamine (Ecolid, CIBA–Geigy), the noradrenergic response was blocked with bretylium tosylate (Bretylol, American Critical Care, American Hospital Supply, McGaw Park, IL), and opiate receptors were blocked with naloxone (Sigma) (17, 18).

Bicarbonate Measurements. Bicarbonate concentrations in the effluent from the duodenal test segment were determined in triplicate immediately after collection. A 2-ml aliquot of the effluent was added to 5 ml of CO_2-free H_2O and acidified with 125 μl of 100 mM H_2SO_4. To remove any residual CO_2, the solution was gassed for 5 min with N_2 and washed with $Ba(OH)_2$. The sample then was titrated by the addition of 15 mM NaOH under a continuous stream of N_2 to pH 8.4 by using an automated titration system (Autoburette ABU SO, Titrator TTT 80, pH meter PHM 82, Radiometer, Copenhagen). To validate these measurements, bicarbonate solutions of known concentrations (0.65 mM, 1.3 mM, and 2.6 mM) were determined on 6 days. There was good correlation between actual and measured bicarbonate concentrations: $r = 0.979$, $y = 0.965x - 0.031$. The coefficient of variation at these three concentrations was 14.1%, 7.9%, and 6.8%, respectively. On each test day, the perfusate as a blank and bicarbonate standards (0.65 mM, 1.3 mM, and 2.6 mM) were measured to assure reproducibility.

Radioimmunoassays. Two milliliters of venous blood was removed, collected in chilled tubes containing 100 μl of 0.5 M EDTA, centrifuged at 3000 × *g* at 4°C for 10 min, and plasma was stored at −20°C. Plasma concentrations of β-endorphin and adrenocorticotropic hormone (ACTH) were determined in duplicate with radioimmunoassay kits (DRG-Instruments GmbH, Marburg, F.R.G.) (10).

Statistical Analysis. The data were subjected to analysis of variance, and differences between treatment groups were determined by the Newman–Keuls multiple range test (20). The data obtained from six to eight animals are expressed as the mean ± SEM and results are considered significant if $P < 0.05$.

RESULTS

Basal (resting) duodenal bicarbonate secretion was 19.7 ± 0.7 μmol/hr. Intraluminal perfusion of the duodenal test segment with increasing concentrations (1 μM, 10 μM, and 0.1 mM) of the synthetic prostaglandin E_1 analogue misoprostol significantly ($P < 0.01$) increased the bicarbonate responses to 45.4 ± 4.4, 77.6 ± 6.9, and 98.8 ± 8.4 μmol/hr, respectively.

Intracerebroventricular (i.c.v.) and i.v. administration of increasing doses of rat CRF (5) resulted in significant and dose-dependent stimulation of duodenal bicarbonate secretion in freely moving rats (Fig. 1*A*). The responses after i.c.v. and i.v. administration of CRF were similar (Fig. 1*A*). In contrast, i.c.v. or i.v. administration of 1 nmol of other hypothalamic peptides (somatostatin-14, growth hormone-releasing factor, or gonadotropin-releasing hormone) did not significantly alter bicarbonate secretion (data not shown).

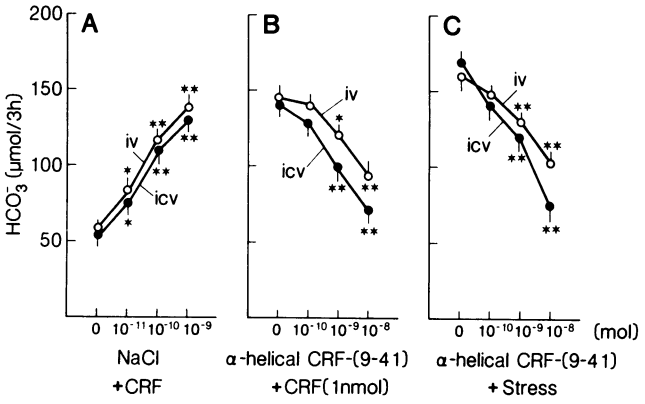

FIG. 1. Effect of CRF and stress on proximal duodenal bicarbonate secretion in conscious rats. (*A*) NaCl (0.15 M) was injected i.c.v. or i.v. 15 min before various doses of i.c.v. or i.v. rat CRF. (*B*) The CRF receptor antagonist α-helical CRF-(9–41) was injected in increasing doses 15 min before CRF. (*C*) α-Helical CRF-(9–41) was given 15 min before stress exposure. Peptide doses were given on separate days. *, $P < 0.05$; **, $P < 0.01$ compared with control. ○, i.v.; ●, i.c.v.

Pretreatment of the animals with various doses of the CRF receptor antagonist, α-helical CRF-(9–41) (6) given i.c.v. or i.v. reversed the increase in bicarbonate secretion produced by i.c.v. or i.v. administration of 1 nmol of CRF (Fig. 1*B*). The antagonist was significantly ($P < 0.05$) more effective in preventing the bicarbonate response after i.c.v. than after i.v. administration.

Partial body restraint increased the bicarbonate response that was slightly, yet significantly ($P < 0.05$), higher than the bicarbonate response induced by 1 nmol of exogenous CRF (Fig. 1*C*). Pretreatment of the animals with increasing doses of α-helical CRF-(9–41) given i.c.v. or i.v. resulted in a dose-related inhibition of stress-induced bicarbonate secretion (Fig. 1*C*). The antagonist was significantly ($P < 0.01$ at 10 nmol) more effective after i.c.v. than after i.v. administration (Fig. 1*C*).

CRF given i.c.v. and stress significantly stimulated bicarbonate secretion for at least 2.5 hr (Fig. 2*A*). These responses were abolished by pretreatment of the animals with α-helical CRF-(9–41) (Fig. 2*B*). Stress resulted in sustained stimulation of bicarbonate secretion while the effect of a single dose of CRF (1 nmol) steadily declined after 1 hr (Fig. 2*A*). Hypophysectomy (Fig. 2*C*) and pretreatment of the animals with the opiate receptor antagonist naloxone (Fig. 2*D*) abolished CRF- and stress-induced stimulation of duodenal bicarbonate.

Infusion of β-endorphin or ACTH i.v. (100 pmol per kg per hr) produced plasma concentrations that were similar to those observed after 60 min of stress or cerebroventricular administration of CRF (1 nmol) (Table 1). At the dose of 100 pmol per kg per hr, β-endorphin, but not ACTH, produced a bicarbonate response that was similar to the bicarbonate response observed after 60 min of stress or cerebroventricular administration of CRF (1 nmol) (Table 2). In hypophysectomized animals, β-endorphin significantly ($P < 0.01$) stimulated duodenal bicarbonate secretion from 19.2 ± 0.9 μmol/hr (at basal) to 29.5 ± 1.9 μmol/hr (at 10 pmol per kg per hr) and 43.4 ± 3.4 μmol/hr (at 100 pmol per kg per hr). In contrast, 1 nmol of β-endorphin given i.c.v. did not significantly alter bicarbonate secretion (data not shown).

Blockade of the autonomic nervous system with chlorisondamine, blockade of noradrenergic and adrenergic systems with bretylium and adrenalectomy, respectively, and block-

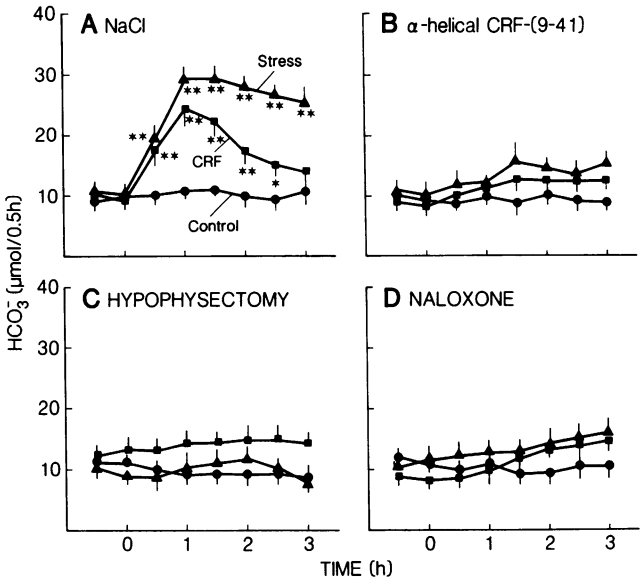

FIG. 2. Effect of α-helical CRF-(9–41), hypophysectomy, and opiate blockade on duodenal bicarbonate secretion stimulated by exogenous CRF and stress. CRF (1 nmol) or the control solution (0.15 M NaCl) was given i.c.v. at time 0 and restraint stress began at time 0. (*A*) Effect of i.v. 0.15 M NaCl administered at 15 min prior to time 0. (*B*) Effect of i.v. α-helical CRF-(9–41) (10 nmol bolus 15 min prior to time 0 followed by infusion at 10 nmol/hr). (*C*) Effect of hypophysectomy. (*D*) Effect of i.v. naloxone (1 mg/kg bolus at 15 min prior to time 0 followed by infusion at 1 mg per kg per hr). *, $P < 0.05$; **, $P < 0.01$ compared with control. α-Helical CRF-(9–41), hypophysectomy, and naloxone abolished CRF- and stress-induced stimulation of duodenal bicarbonate secretion. ▲, Stress; ■, CRF; ●, control.

ade of parasympathetic efferents to the intestine by truncal vagotomy did not prevent the CRF- or stress-induced increases in duodenal bicarbonate secretion (Table 3).

DISCUSSION

Duodenal bicarbonate secretion is an important gut secretory process in animals and in humans that protects the mucosa from acid-peptic damage (2, 21). Diminished bicarbonate secretion within the proximal duodenum, the duodenal bulb, is likely to facilitate the development and influence the natural history of duodenal ulcer disease (3). The factor(s) responsible for diminished bicarbonate secretion as well as the role of the central nervous system and stress in regulating the bicarbonate response are unknown. Therefore, a model was developed that permitted cerebroventricular injections and measurement of proximal duodenal bicarbonate secretion in freely moving, as well as in physically restrained, rats. Resting bicarbonate secretion was ≈20 μmol/hr. Intraluminal perfusion of the duodenal test segment with increasing concentrations of a synthetic prostaglandin E_1 analogue resulted in dose-dependent stimulation of the bicarbonate response, suggesting a viable and functioning mucosa (22).

Table 1. Plasma concentrations of ACTH and β-endorphin

	ACTH, pM		β-Endorphin, pM	
	0 min	60 min	0 min	60 min
Stress	18 ± 4	202 ± 29*	25 ± 3	280 ± 22*
CRF	19 ± 3	185 ± 24*	21 ± 3	245 ± 31*
β-Endorphin	—	—	26 ± 2	309 ± 27*
ACTH	21 ± 4	235 ± 31*	—	—

Stress was initiated and 1 nmol of CRF was given i.c.v. at 0 min. Intravenous infusions of β-endorphin or ACTH (at 100 pmol per kg per hr) began at 0 min. Plasma concentrations of ACTH and β-endorphin at 60 min were similar between the treatment groups.
*$P < 0.01$ compared with 0 min.

Table 2. Effects of stress, CRF, β-endorphin, and ACTH on bicarbonate secretion

	Bicarbonate, μmol/hr	
	0 min	60 min
Stress	19.4 ± 0.5	50.4 ± 2.4*
CRF	19.9 ± 0.6	47.3 ± 2.7*
β-Endorphin	19.1 ± 0.6	45.9 ± 3.3*
ACTH	18.9 ± 0.7	19.7 ± 0.6

Stress, CRF, β-endorphin, and ACTH were administered as described in Table 1.
*$P < 0.01$ compared with 0 min.

Administration of CRF and physical restraint, a nonulcerogenic stress-producing stimulus (10), increased duodenal bicarbonate secretion by as much as 3-fold. This response was quantitatively similar to that produced by intraluminal hydrochloric acid, a physiologic stimulus of duodenal bicarbonate secretion (22). Pretreatment of the animals with a specific CRF receptor antagonist, α-helical CRF-(9–41) (6), attenuated the bicarbonate responses produced by exogenous CRF and stress in a dose-dependent fashion. These findings suggest that exogenously administered CRF and endogenously released CRF stimulate duodenal bicarbonate secretion by a receptor-mediated event. This biological action of CRF appeared to be unique since other hypothalamic peptides did not alter bicarbonate secretion. Furthermore, α-helical CRF-(9–41) was more effective after i.c.v. than after i.v. administration. This is likely due to the more direct delivery and greater availability of the CRF antagonist at its receptor or secondary to enhanced inactivation in the peripheral circulation.

The bicarbonate responses after i.c.v. and i.v. administration of CRF were similar suggesting a single site rather than multiple sites of action. Cerebroventricularly administered CRF does not enter the peripheral circulation and i.v. administered CRF does not cross the blood-brain barrier (23). However, CRF administered i.c.v. and i.v. reach the anterior pituitary through the portal hypophyseal vessels and the peripheral circulation, respectively. Both hypophysectomy and naloxone abolished the stimulatory effects of exogenous CRF and stress on bicarbonate secretion. These results indicate that duodenal bicarbonate secretion in response to exogenously administered CRF and endogenously released CRF during stress is mediated by pituitary- and opiate-dependent pathways.

Table 3. CRF- and stress-induced stimulation of duodenal bicarbonate secretion: Role of the autonomic nervous system

	Bicarbonate, μmol/3 hr		
	Control	CRF*	Stress*
NaCl	58 ± 5	142 ± 17	161 ± 16
Chlorisondamine	30 ± 4	100 ± 15	119 ± 10
Bretylium tosylate	60 ± 7	141 ± 12	160 ± 9
Sham-adrenalectomy	55 ± 5	139 ± 13	149 ± 13
Adrenalectomy	59 ± 7	122 ± 13	148 ± 14
Sham-vagotomy	62 ± 6	137 ± 9	155 ± 17
Vagotomy	41 ± 5	103 ± 10	118 ± 11

Ganglionic blockade with chlorisondamine (3 mg/kg, i.v. bolus, followed by 3 mg per kg per hr, i.v. infusion) and noradrenergic blockade with bretylium tosylate (25 mg/kg, i.v. bolus, followed by 25 mg per kg per hr, i.v. infusion) were performed 0.5 hr before i.c.v. injection of 1 nmol of CRF or before stress. NaCl (0.15 M) served as control for chlorisondamine and bretylium tosylate. Bicarbonate secretion was measured for 3 hr after injection of CRF and during a 3-hr period of stress.
*$P < 0.01$ compared with control.

To further examine if ACTH and β-endorphin, the principal pituitary peptides released by stress and CRF (4, 24), are involved in mediating the bicarbonate responses, studies were carried out in which exogenous administration of ACTH and β-endorphin produced plasma concentrations that were similar to those observed after stress and exogenous CRF. β-Endorphin but not ACTH significantly increased the bicarbonate response, which was similar to the response observed after stress and CRF. Furthermore, exogenous β-endorphin stimulated bicarbonate secretion in hypophysectomized animals. These results indicate that exogenously administered CRF and endogenously released CRF during stress stimulate duodenal bicarbonate secretion by the release of β-endorphin from the pituitary, thus, demonstrating a functional hypothalamus–pituitary–gut axis. In contrast, inhibition of gastric acid secretion by CRF appears to be mediated in part by the release of central nonpituitary opiates (25).

In addition to its action on the anterior pituitary, CRF activates the sympathetic nervous system producing increased plasma concentrations of catecholamines and glucose, arterial hypertension, and gastric hyposecretion (13, 14, 26, 27). In this study, ganglionic and noradrenergic blockade, vagotomy, and adrenalectomy did not abolish the stimulatory effects of stress and CRF on duodenal bicarbonate secretion. This suggests that CRF does not stimulate duodenal bicarbonate by altering autonomic nervous system activity. In contrast, alterations in gastric acid secretion and gastrointestinal motility in response to CRF are mediated by activation of the autonomic nervous system and not by pituitary-dependent pathways (13, 17–19).

Gastrointestinal functions are thought to be regulated predominantly by the autonomic nervous system and gastrointestinal hormones. The present investigation indicates that an important gut secretory process is controlled by the hypothalamus and pituitary. Increased gastric acid secretion and decreased duodenal bicarbonate secretion are important factors in the pathogenesis of duodenal ulcer disease (1, 3). CRF likely serves as an endogenous, physiologic regulator of gastroduodenal secretions during stress by decreasing gastric acid (10) and by β-endorphin release increasing duodenal bicarbonate production, thereby protecting the mucosa from an increased acid-peptic load.

I thank I. Forquignon, E. Friemel, and K. Feutlinske for expert technical assistance, J. E. Rivier for the generous supply of the peptides, and J. I. Isenberg and M. R. Brown for critical reading of the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft Grant Le 470/2-1, Dr. Martini Stiftung, and Hamburgische Wissenschaftliche Stiftung.

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