Central CGRP inhibits pancreatic enzyme secretion by modulation of vagal parasympathetic outflow

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Li, Ying, Yi Cheng Jiang, and Chung Owyang. Central CGRP inhibits pancreatic enzyme secretion by modulation of vagal parasympathetic outflow. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G957–G963, 1998.—Calcitonin gene-related peptide (CGRP) is a potent inhibitor of pancreatic enzyme secretion in vivo. Recent studies have shown that CGRP exerts its inhibitory action at a central vagal site. The present study investigates the mechanism responsible for the central action of CGRP. Rats were fitted with lateral cerebroventricular cannulas, using stereotaxic instruments, 4 days before pancreatic secretion studies. In anesthetized rats, administration of 2-deoxy-D-glucose (2-DG) (75 mg/kg iv) or CGRP (40 pmol·kg−1·h−1) produced a 100 and 75% increase in protein secretion, respectively, which was completely blocked by atropine. Intracerebroventricular (ICV) administration of CGRP (0.03–0.6 nmol/h) resulted in a dose-related inhibition of pancreatic protein secretion evoked by 2-DG or CCK-8. CGRP administered by the ICV route was 10–40 times more potent than CGRP given by the intravenous route. In contrast, ICV administration of CGRP had no significant effect on pancreatic protein secretion evoked by electrical vagal stimulation or bethanecol, which directly activates the pancreatic muscarinic receptor. Chemical sympathectomy induced by pretreatment with guanethedine (20 mg/kg ip, 2 days) or α-adrenergic receptor blockade with phentolamine did not alter the inhibitory effects of CGRP. We recently demonstrated that CCK stimulated the enteropancreatic neural pathways to mediate pancreatic secretion in rats with a chronic vagotomy. ICV-administered CGRP did not affect CCK-stimulated pancreatic secretion in rats with a chronic vagotomy. In conclusion, CGRP in the central nervous system inhibits pancreatic enzyme secretion stimulated by 2-DG and CCK-8, which act through vagal pathways. The inhibitory action of CGRP is not mediated by the sympathetic nervous system but appears to depend on intact vagus nerves.

METHODS

Materials

Atropine sulfate, bethanecol chloride, 2-DG, phentolamine, guanethedine, and maltose were purchased from Sigma Chemical (St. Louis, MO). Rat α-CGRP and CCK-8 were purchased from Peninsula Laboratories (Belmont, CA).

Animal Preparations

Male Sprague-Dawley rats were used (body wt, 250–300 g). After an overnight fast, the rats were anesthetized with a mixture of xylazine and ketamine (13 and 87 mg/kg body wt im, respectively) and maintained with small doses, as required (one-third of the initial dose every 2 h). Rats were positioned stereotaxically, and a small hole was drilled through the skull. A 27-gauge stainless steel cannula attached to PE-20 polyethylene tubing was placed so that the tip of the cannula protruded into the middle of the left cerebral ventricle. The cannula was secured by two screws inserted into the surface of the parietal bone, using craniplastic powder (Plastics One). Coordinates from the bregma were as follows: anteroposterior, 0.6 mm; lateral, 2 mm; and ventral, 4 mm.
(34). Immediately before rats were killed, we administered 2 µl of methylene blue by ICV injection to verify the injection site. Data from animals that did not display the dye throughout the ventricular system were excluded from the analyses. After stereotaxic surgery, one or more polyethylene catheters were placed in the external jugular vein for intravenous infusion with a syringe-driven pump. A polyethylene cannula (Clay-Adams PE-100) was inserted through a midline incision into the common bile-pancreatic duct at the sphincter of Oddi. To permit intraduodenal infusion of pancreaticobiliary juice, a second cannula was placed in the duodenum, slightly above the sphincter of Oddi. Because CGRP is a potent central inhibitor of gastric secretion (15, 32), it is necessary to avoid any potential contribution of gastric acid on pancreatic secretion. A cannula was placed in the stomach to drain gastric juice, thus preventing gastric secretion from reaching the duodenum. The abdominal wound was covered with saline-moistened gauze. The rats were maintained at 37°C with a heating pad.

Pancreatic Secretion Study

After a 30-min stabilization period, combined bile-pancreatic secretions were collected for 15-min periods. The volume was measured, and aliquots were taken and diluted with distilled water for protein determination. The remainder of the undiluted bile-pancreatic juice was pumped back into the rat through the duodenal cannula during the next collection period after the secretion rate of the preceding collection period.

Assays

Pancreatic biliary juice protein was measured spectrophotometrically, using the assay method of Bradford (4). The total protein output was calculated as multiplied by volume and protein concentration and expressed as milligrams per collection period. We confirmed in a previous study (23) that the increase in protein output in the bile-pancreatic juice after CCK infusion largely reflected protein from the pancreatic source; biliary protein did not increase with CCK stimulation.

Experimental Design

2-DG studies. 2-DG, a central vagal stimulant, was dissolved in saline and administered as a bolus intravenous injection (75 mg/kg) at the end of a 30-min basal collection period. Pancreatic secretion was collected for 120 min. To investigate the CNS effects of CGRP on 2-DG-stimulated pancreatic secretion, graded doses of CGRP (0.03, 0.3, or 0.6 nmol every 2 h) were injected cerebroventriculally (0.7 µl), beginning 15 min before basal collection periods. Each rat received two doses of CGRP or saline given cerebroventriculally with a 1-h resting period between experiments.

CCK studies. CCK-8 was dissolved in 0.9% NaCl containing 1% BSA and stored in 20-µl aliquots at −20°C until the time of study. CCK-8 or 0.9% NaCl alone was infused at a rate of 1.08 ml/h. After two 15-min basal periods, rats were given an intravenous infusion of CCK-8 for 60 min at a dose of 40 pmol·kg⁻¹·h⁻¹, which produced plasma CCK levels similar to peak postprandial levels (22). An intracerebral microinjection of CCK-8 (0.3 nmol) or saline was administered 15 min before the basal period.

Non-CCK-dependent luminal stimuli. Recently, we have shown (18, 20) that similar to CCK, non-CCK-dependent luminal stimuli evoked pancreatic enzyme secretion through stimulation of vagal afferent nerve pathways originating in the duodenal mucosa. In the present study, we evaluated the CNS effect of CGRP on pancreatic enzyme secretion stimulated by intraduodenal administration of maltose and hypertonic saline. A 20-cm segment of the proximal small intestine (the entire duodenum and proximal jejunum) was isolated between two cannulas positioned at 4 and 24 cm from the pylorus. After a 30-min basal period, NaCl (500 mosM, pH 6.0) or maltose (300 mM, pH 6.0) was perfused at a rate of 3 ml/h for 60 min, using a peristaltic pump. Free intestinal drainage was established to avoid an increase in intra-luminal pressure. Each test solution was administered separately with a 1-h resting period in between experiments to allow pancreatic secretion to return to basal levels. In a separate study, an acute vagotomy was performed as described previously (22), and pancreatic secretion studies in response to maltose and hyperosmolar NaCl solution were performed 30 min after vagotomy. Similar pancreatic secretion studies were performed in a separate groups of rats that received an ICV injection of CGRP (0.3 nmol/7 µl) or saline 15 min before basal collection.

Nerve stimulation studies. Through a midline incision made on the anterior surface of the neck, the left vagus nerve was dissected free and severed. The distal end was placed on a nerve electrode and stimulated with a Grass stimulator for a period of 1 h (5 V, 4 Hz, 2-ms pulses), beginning at the end of the basal period. Infusion of atropine (50 µg·kg⁻¹·h⁻¹) or ICV injection of CCK-8 (0.3 or 0.6 nmol) or saline was begun 30 min after stimulation of the vagus nerve.

Bethanechol studies. Bethanechol (0.3 mg/kg) was dissolved in saline and injected subcutaneously as a bolus dose, after the 30-min basal collection period. ICV injection of saline or CGRP (0.6 nmol) was performed 15 min before the basal collection.

Chemical sympathectomy and α-adrenergic receptor blockade studies. To determine if the inhibitory action of central administration of CGRP on pancreatic secretion stimulated by 2-DG, CCK, or non-CCK luminal stimuli is mediated by the sympathetic nervous system, we examined the effect of the administration of guanethidine and phentolamine. 2-DG, CCK, and non-CCK luminal stimuli were given after a 30-min basal period. Phentolamine (1 µmol·kg⁻¹·h⁻¹), an α-adrenergic antagonist, was dissolved in 0.9% NaCl and given intravenously 30 min before basal collection. The effectiveness of this dose of phentolamine has been demonstrated previously (5). To evaluate the effect of chemical sympathectomy on the inhibitory action of central CCK, rats were injected with guanethidine (20 mg/kg ip) once daily for 2 consecutive days. This dose of guanethidine has been shown to deplete cardiac norepinephrine by >90% (35). ICV administration of CGRP (0.3 nmol) was begun 15 min before basal collection. Pancreatic secretion studies in response to 2-DG, CCK, and non-CCK dependent luminal stimuli were performed.

Chronic vagotomy. To determine the role of vagus nerves in mediating the inhibitory effects of CGRP, we used the chronic vagotomized rat model. Previously, we have shown that the pancreatic response to CCK returns to normal within 2 wk after chronic vagotomy. The response was not blocked by atropine but was abolished by TTX or surgical interruption of the enteropancreatic neural pathways (19). This suggests that adaptation occurs after chronic vagotomy and that CCK acts through enteropancreatic neural pathways to mediate pancreatic enzyme secretion in these animals. In the present study, vagotomy was performed as described earlier (22). Secretion studies were performed 2 wk after vagotomy in a similar manner as described for intact rats. Complete vagal section was confirmed by an absence of the pancreatic secretory response after administration of 2-DG (16). To show that CCK acts through enteropancreatic neural pathways after chronic vagotomy, the duodenal part of the pancreas was
meticulously dissected away from its vascular and neural connections to the duodenal wall and pyloric region. CCK-8 infusion studies were performed after a 30-min basal period. In a separate group of rats with chronic vagotomies, ICV injections of CGRP or saline were administered 15 min before basal period.

Statistical analysis. Results were expressed as means ± SE. The multivariate ANOVA method was used to evaluate the effects of repeated measurements over time and treatment effect and the interactions between them. Significance was determined using Student’s t-test and was accepted at the 5% level.

RESULTS

Effect of ICV Administration of CGRP on Pancreatic Protein Secretion Stimulated by 2-DG and CCK-8

In the ketamine-xylazine anesthetized rat model, basal pancreatic protein secretion remained stable and averaged 64.2 ± 5 mg/30 min (Fig. 1). ICV administration of CGRP did not significantly alter basal pancreatic secretion in our anesthetized rat. Intravenous administration of 2-DG (75 mg/kg iv) increased protein secretion to 112 ± 4 mg/30 min, similar to the increase produced by a physiological concentration of CCK-8. Central administration of CGRP inhibited 2-DG-stimulated protein secretion in a dose-related manner (Fig. 1). The lowest dose of CGRP to produce significant inhibition was 0.03 nmol (7 µl); this dose caused a reduction of pancreatic secretion from the control volume of 108 ± 6 to 85 ± 9 mg/30 min. Complete inhibition was produced with 0.6 nmol of CGRP (Fig. 1).

Intravenous infusion of CCK-8 (40 pmol·kg⁻¹·h⁻¹) produced a significant increase in pancreatic protein output, averaging 246 ± 18 mg/h, an 80% increase over basal. This increase was completely blocked by ICV administration of CGRP (0.3 nmol) (Fig. 2).

Effect of ICV Administration of CGRP on Pancreatic Secretion Stimulated by Non-CCK Luminal Stimuli

Infusion of hyperosmolar NaCl (500 mosM) and maltose (300 mM) at 3 ml/h increased protein secretion to 227 ± 12 and 230 ± 16 mg/h, respectively (Fig. 3). This represents a 70% increase in protein secretion over basal. Previously, we have shown that administration of hyperosmolar NaCl or maltose did not elevate plasma CCK levels (18, 20). Acute vagotomy did not affect basal secretion but abolished the response to hyperosmolar NaCl or maltose solution (Fig. 3A). This indicates that non-CCK-dependent luminal stimuli evoked pancreatic secretion by way of vagal pathways. ICV administration of CGRP (0.3 nmol) completely abolished the pancreatic protein response to hyperosmolar NaCl or maltose solution (Fig. 3B).

Effect of ICV Administration of CGRP on Electrical Vagal Stimulation of Pancreatic Secretion

Direct electrical stimulation of the left cervical vagus nerve with pulses of 5 V, 4 Hz, and 2-ms duration resulted in a twofold increase in pancreatic protein secretion over basal, similar to the increase produced by CCK-8 and 2-DG in this study. Atropine markedly inhibited this response (Fig. 4). ICV administration of CGRP at a dose as high as 0.6 nmol did not affect nerve-stimulated pancreatic protein secretion (Fig. 4).
Effect of ICV Administration of CGRP on Pancreatic Secretion Stimulated by Bethanechol

Bethanechol, a muscarinic receptor antagonist without significant nicotinic receptor effect, stimulated pancreatic protein secretion approximately twofold above basal when injected subcutaneously at a dose of 0.3 mg/kg (Fig. 5). ICV microinjection of CGRP at doses up to 0.6 nmol did not alter the protein output in response to bethanechol (Fig. 5).

Effect of Guanethidine and Phentolamine on Inhibitory Effect of Central CGRP

Intravenous administration of phentolamine, an α-adrenergic receptor antagonist, or chemical sympathectomy by guanethidine significantly increased basal pancreatic protein secretion by 15 ± 2 and 15 ± 3% (P < 0.01), respectively, but did not alter the inhibitory effect of ICV injection of CGRP (0.3 nmol) on 2-DG-, CCK-, and maltose-induced pancreatic protein secretion (Fig. 6). This indicates that the inhibitory effect of CGRP on stimulated pancreatic secretion is not mediated by the sympathetic nervous system.

Effect of ICV Administration of CGRP on CCK-Stimulated Pancreatic Secretion in Rats With Chronic Vagotomy

Acute vagotomy completely abolished the pancreatic response to the infusion of CCK-8 (40 pmol·kg⁻¹·h⁻¹), which produced a plasma CCK level similar to the peak postprandial plasma level (22). In rats with chronic vagal denervation, the pancreatic protein secretion in response to CCK was fully restored at 20 days postvagotomy (Fig. 7). This normalization of pancreatic function was not due to regeneration of the vagus nerve, because administration of 2-DG (75 mg/kg) failed to increase protein secretion in rats with a chronic vagotomy (19). In contrast to rats with intact vagus nerves, ICV microinjections of CGRP (0.3 and 0.6 nmol) failed to inhibit CCK-stimulated pancreatic protein secretion in rats with a chronic vagotomy (Fig. 7B). Conversely, local denervation by dissecting part of the pancreas away from its connection with the duodenal wall (interruption of enteropancreatic neural pathways) completely abolished pancreatic responses to CCK-8 after chronic vagotomy (Fig. 7B). We have
previously shown that the local surgical denervation of the pancreas did not alter CCK-stimulated pancreatic protein secretion in rats with intact vagus nerves (19).

**DISCUSSION**

We have shown that vagal afferent pathways represent the primary targets on which postprandial mediators (e.g., CCK and luminal stimuli) act to stimulate pancreatic secretion (17, 18, 20–22). In addition, we have shown that central vagal pathways are the key sites on which peptides, such as somatostatin (21) and CGRP (16), act to inhibit pancreatic secretion. Messmer et al. (25) have shown that ICV administration of CGRP inhibits basal pancreatic secretion in the conscious rat and the effects are mediated by the sympathetic nervous system (25). The objective of the present study was to provide direct evidence that CGRP in the CNS inhibits stimulated pancreatic enzyme secretion and to investigate the mechanism.

In the present study, we found that ICV administration of CGRP was highly effective in inhibiting pancreatic protein secretion evoked by 2-DG and CCK-8. 2-DG, a well-known central vagal stimulant, acts by competing with glucose for cell membrane transfer, causing intracellular glucopenia, especially at the hypothalamus. The resultant hypothalamic glucopenia excites the dorsal nucleus of vagus nerve and stimulates vagus nerve transmission (28). We confirmed that 2-DG acts through the vagal cholinergic pathway by demonstrating that pancreatic secretion induced by 2-DG could be completely abolished by administration of atropine or by truncal vagotomy (21). In the present study, ICV administration of CGRP at doses of 0.3 and 0.6 nmol produced 88 and 100% inhibition of pancreatic protein secretion stimulated by 2-DG, respectively. Our recent studies (22) have shown that CCK at physiological levels acts through stimulation of vagal afferent pathways, whereas CCK at supraphysiological levels acts directly on pancreatic acini to induce pancreatic enzyme secretion. In this study, CCK-8, at a dose of 40 pmol·kg⁻¹·h⁻¹, produced an 80% increase in protein secretion over basal and a plasma level similar to the peak postprandial level observed in rats (22). Central administration of CGRP at 0.3 nmol produced a 94% inhibition of pancreatic protein secretion induced by CCK-8 at physiological doses. This inhibition appears to be directed at a pre-acinar site, because direct...
stimulation of the muscarinic receptor by bethanechol was not affected by ICV administration of CGRP at a dose as high as 0.6 nmol.

Previously, we reported (16) that peripheral administration of CGRP at a dose of 25 µg (6.5 nmol) or 50 µg·kg⁻¹·h⁻¹ completely inhibited 2-DG- and CCK-8-stimulated pancreatic enzyme secretion. In our current study, pancreatic protein secretion was inhibited with ICV-administered CGRP, at smaller doses compared with the doses administered peripherally. Complete inhibition of 2-DG-stimulated pancreatic enzyme secretion was obtained with an ICV injection of CGRP at 0.6 nmol, a dose 10 times less than the dose administered peripherally. An ICV-administered dose of CGRP at 0.3 nmol completely blocked pancreatic protein secretion induced by CCK-8 (40 pmol·kg⁻¹·h⁻¹), a dose 40 times less than the peripherally administered dose. Therefore, although there is evidence that ICV-administered neuropeptides can rapidly diffuse into the circulation (26), the inhibitory effect of ICV injection of CGRP represents a CNS-mediated action. This effect is not due to the leakage of the peptide from the central to peripheral circulation, as only ~8% of the centrally administered CGRP leaked from the lateral ventricle into the circulatory system of the rat (3).

Apart from CCK, we used non-CCK-dependent luminal factors to activate the vagal afferent pathway. Luminal factors such as osmolarity, volume distension, and nutrients also play a major role in the intestinal phase of pancreatic secretion. Recent studies in rats (18, 20) have shown that stimulation of duodenal osmoreceptors, mechanoreceptors, or chemoreceptors induces pancreatic enzyme secretion through a CCK-independent cholinergic pathway. Perivagal and duodenal mucosal pretreatment with capsaicin impaired pancreatic responses to duodenal stimuli such as hyperosmolar NaCl solution or maltose. This indicates that non-CCK-dependent luminal stimuli act through duodenal mucosal vagal afferent fibers to stimulate pancreatic secretion. We showed that ICV administration of CGRP completely abolished the pancreatic secretion induced by intraduodenal infusion of maltose or hyperosmolar NaCl solution. This observation suggests that cerebral CGRP can inhibit pancreatic stimulation, which is mediated by the afferent vagal pathways that can be activated by CCK- and non-CCK-dependent luminal stimuli.

Pancreatic protein secretion induced by electrical stimulation of the peripheral cut end of the vagus nerve was markedly inhibited by atropine, indicating that it is mainly mediated by a cholinergic pathway. This mode of stimulation directly activates the vagal afferent pathway, bypassing the vagal afferent and central sites. Cerebral CGRP at a dose of 0.3 and 0.9 nmol was ineffective in inhibiting pancreatic protein secretion induced by vagal electrical stimulation, suggesting that central CGRP is acting at a site proximal to the vagal afferent pathway and therefore does not inhibit the release of neurotransmitter from the vagus nerve ending in the pancreas.

CGRP is a potent CNS stimulator of noradrenergic sympathetic outflow (7) in the rat. CGRP inhibits duodenal bicarbonate secretion (14) and basal pancreatic secretion in conscious rats (25) by activation of sympathetic efferents with subsequent release of norepinephrine, which acts on α-adrenergic receptors. To determine if the inhibitory actions of central CGRP on stimulated pancreatic secretion are mediated by the sympathetic nervous system, we evaluated the effects of guanethidine and phentolamine on the action of CGRP. Intravenous administration of phentolamine, an α-adrenergic receptor antagonist, or chemical sympathectomy by guanethidine, increased basal pancreatic protein output by 15%, but did not alter the inhibitory effects of CGRP on pancreatic secretion stimulated by 2-DG, CCK-8, intraluminal maltose, or hyperosmolar NaCl. This observation indicates that the inhibitory action of CGRP is not mediated by the sympathetic nervous system. Therefore, the mechanisms that mediate central CGRP to inhibit pancreatic secretion under basal conditions in conscious rats and in response to meal-related stimuli under our experimental conditions are different. It is conceivable that the mechanisms of action of CGRP in conscious and anesthetized rats are different. Alternatively, neural release of norepinephrine may be adequate to inhibit basal secretion but insufficient to reduce stimulated pancreatic secretion. In our present study, central CGRP inhibited pancreatic secretion in response to 2-DG and CCK-8 in rats with intact vagus nerves but failed to inhibit the response in rats with a chronic vagotomy, in which CCK acts through enteropancreatic neuropathways to mediate pancreatic secretion (19). This suggests that central CGRP inhibits the stimulation arising from central or afferent vagal sites through a pathway primarily dependent on the vagus nerves. Central CGRP probably inhibits vagal tone and reduces vagal stimulation to the pancreas. Note that the central inhibitory action of CGRP on gastric acid secretion is also not mediated by the sympathoadrenal axis. A vagally mediated action is suggested by the abolition of inhibitory effects of central CGRP in rats with a vagotomy (15, 33).

Electrophysiological studies in the rat indicate that the central action of CGRP on the stomach is mediated through vagal pathways. Intracisternal injection of α-CGRP inhibits unit efferent discharges recorded from the gastric branch of the vagus nerve in a dose- and time-dependent manner (36). In situ hybridization studies (1, 27, 29) have revealed the distribution and expression of α-CGRP mRNA in the hypothalamus and various brain stem nuclei, including the nucleus ambiguous. The demonstration of CGRP-like immunoreactivity and receptors in the lateral hypothalamus, the nucleus of solitary tract, and the nucleus ambiguus (8, 29, 30, 31) also provides anatomic support for a possible role of CGRP in the modulation of parasympathetic outflow.

In summary, we have demonstrated that CGRP in the CNS inhibits pancreatic secretion stimulated by 2-DG, CCK-8, and non-CCK luminal stimuli, which act through vagal pathways. The inhibitory action of CGRP is not mediated by the sympathetic nervous system but
appears to be mediated by modulation of parasympathetic outflow.

The investigation was supported in part by National Institute of Diabetes and Digestive and Kidney Diseases Grants R01 DK-32838 and P 30 DK-349333.

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Received 3 September 1997; accepted in final form 29 June 1998.

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