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Research Report

Chemical sympathectomy attenuates myenteric but not dorsal vagal complex Fos-like immunoreactivity induced by cholecystokinin-8 in the rat

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Abstract

Vagotomy and capsaicin treatment attenuate dorsal vagal complex (DVC) but not myenteric Fos-like immunoreactivity (Fos-LI) induced by cholecystokinin-8 (CCK-8). The goal of this experiment is to test the role of the sympathetic nervous system in the pathway by which CCK-8 increases myenteric Fos-LI. Adult male Sprague–Dawley rats were pretreated with guanethidine sulfate (40 mg/kg daily for 5 weeks) or vehicle intraperitoneally (IP), and injected with CCK-8 (40 μg/kg) or saline IP. Fos-LI was then quantified in the DVC and the myenteric neurons of the duodenum and jejunum using a diaminobenzidine reaction. Guanethidine pretreatment attenuated myenteric but not DVC Fos-LI induced by CCK-8. These findings demonstrate that sympathetic neurons play a role in mediating the myenteric Fos-LI response to CCK. They also suggest differential mediation of myenteric and DVC responses to CCK.

Theme: Endocrine and autonomic regulation
Topic: Gastrointestinal and urogenital regulation

Keywords: Guanethidine sulfate; Chemical sympathectomy; Dorsal vagal complex; Enteric nervous system; Myenteric plexus; Cholecystokinin; C-Fos; NTS; Area postrema; Vagus

1. Introduction

Cholecystokinin-8 (CCK-8) is a hormone secreted by the endocrine I cells of the gastrointestinal (GI) tract and neurons in the central and peripheral nervous systems. CCK-8 activates two G-protein-coupled receptors, CCK_1 and CCK_2, which are also distributed in GI tract, central and peripheral neurons. Activation of these receptors by CCK-8 produces physiological responses such as gallbladder contraction, inhibition of gastric emptying, stimulation of pancreatic secretion, and satiety (reviewed recently in [25]).

To investigate the pathway of central and enteric neuronal activation by CCK-8, we and others have demonstrated that exogenous CCK-8 increased Fos-Like immunoreactivity (Fos-LI) in the dorsal vagal complex (DVC) [4–7,19,20,24,30,32] and the enteric neurons of the duodenum and jejunum [5–7,19–24,26,30,31], through CCK_1 receptor signaling [4,7,19,30]. DVC, but not enteric, CCK-induced Fos-LI was attenuated by vagotomy and capsaicin treatment [20]. Because the vagus nerve contains mostly parasympathetic fibers [29], the role of the sympathetic nervous system (SNS), including the sympathetic component of the vagus nerve, in CCK increased myenteric and DVC Fos-LI remains unclear.

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The SNS is part of the autonomic nervous system (ANS), which consists also of the parasympathetic (PNS) and enteric nervous systems (ENS). The SNS consists of efferent cell bodies located in the thoraco-lumbar area of the spinal cord and certain components of cranial nerves III (oculomotor), VII (facial), XI (glossopharyngeal), and X (vagus). The SNS innervates the gastrointestinal (GI) tract through efferent nerve fibers of three main ganglia: celiac, superior or cranial mesenteric and inferior or caudal mesenteric ganglia.

Systemic injections of guanethidine sulfate is the most common, irreversible, peripheral, chemical sympathectomy method used in rats today [9–11,15,17]. Although the precise mechanism by which this adrenergic blocker destroys post-ganglionic sympathetic afferents is not clear, the proposed mechanism of action includes at least two steps. First, sympathetic terminals, through a noradrenaline re-uptake transporter, also known as catecholamine uptake pump, absorb guanethidine. Second, as a result of this guanethidine uptake, mitochondrial swelling of sympathetic neurons takes place leading finally to necrosis or cell death [9–11,15,17].

In the current work, we utilized guanethidine sympathectomy to test the role of sympathetic, noradrenergic neurons in increased DVC and myenteric Fos-LI by CCK-8.

2. Materials and methods

2.1. Animals

This study was approved by the Animal Use and Care Committee at Tuskegee University–College of Veterinary Medicine. We used 20, 7-week-old male, Sprague–Dawley rats, averaging 200 g per rat (Harlan, IN). The animals were housed singly in wire-mesh cages, in a controlled environment (lights were on from 0600 to 1800 and temperature was maintained at 21.5 °C). Rats had ad libitum access to water and pelleted rodent chow (Teklad, WI). To enhance adaptation to the laboratory, we handled each rat for 10 min/day for the first 7 days. In addition, the weights of the animals were measured at the beginning of the guanethidine injections and at the time of sacrifice.

2.2. Chemical sympathectomy

Rats were assigned to 1 of 4 groups: sham/CCK-8 (n = 5), sham/saline (n = 5), guanethidine/CCK-8 (n = 5), guanethidine/saline (n = 5). The sham groups received saline (1 ml) intraperitoneally (IP) daily for 5 weeks, and the guanethidine groups received an IP injection of guanethidine sulfate (2-(1′-azacyclooctyl)-ethylguanidine sulfate, $C_{20}H_{44}N_8 \cdot H_2SO_4$, MW = 494.70, Lot FIE02, TCI America, Portland, Oregon) (40 mg/kg) IP for the same period of time as the sham groups [10,17].

2.3. Experimental procedure

All rats were deprived of food, but not water, beginning at 6:00 PM on the day prior to the experiment. At 9:00 AM, the rats received an IP injection of 0.5 ml saline or CCK-8 (Bachem, CA) (40 μg/kg). Our previous work showed that this dose and route produce maximum Fos-LI in the myenteric neurons of the rat [5–7,30,31].

Ninety minutes after the IP injection, rats were anesthetized with sodium pentobarbital (10 mg/kg, IP) and perfused transcardially in two stages. First, the rats were perfused with 500 ml of Krebs solution (Krebs saline formula in mM: 119 NaCl, 4.7 KCl, 1.2 $NaH_2PO_4 \cdot H_2O$, 25 $NaHCO_3$, 1.2 $MgSO_4$, 11.1 glucose, and 1 M $CaCl_2$.) to collect the small intestine. Second, the rats were perfused with 500 ml of 4% formaldehyde (made in 0.1 M phosphate-buffered saline [PBS]) to collect the hindbrains. The small intestine was exposed through a midline abdominal incision, and the duodenum and jejunum were collected. Again, on the basis of our previous experiments [5–7,19–26,30,31], the duodenal sample was 5–10 cm aborad from the pylorus, and the jejunal sample was 20–25 cm aborad from the pylorus. The lumens of the removed segments were rinsed with Krebs solution, and the segments were placed in Sylgard-coated Petri dishes (World Precision Instruments, FL), opened along the mesenteric attachment, stretched and pinned with the mucosal side up, and stored overnight in Zamboni's fixative at 4 °C. On the next day, the tissues were unpinned and cleared three times in 100% dimethyl sulfoxide (DMSO), 10 min each time, followed by three 10-min rinses with 0.1 M PBS, pH 7.4. Whole mounts (approximately 1 cm^2) of longitudinal smooth muscle with adhering myenteric plexus from the duodenum and jejunum were prepared using a dissecting microscope and processed for immunohistochemical detection of Fos-LI.

After collection, the hindbrains were post-fixed with 4% formaldehyde for 2 h and placed in 25% sucrose overnight at room temperature. The hindbrains were sectioned at 40 μm on a cryostat at −20 °C. Consistent with our previous work, the areas cut included the following levels of the DVC according to the Paxinos and Watson rat brain atlas [16]: the area postrema (AP, −4.5 mm caudal to interaural plane), nucleus of the solitary tract (NTS, −4.5 and −4.8 mm caudal to interaural plane), and the dorsal motor nucleus of the vagus (DMV, −4.5 and −4.8 mm caudal to interaural plane). Sections were taken at multiple levels of the NTS to insure sampling from sites receiving both gastric and intestinal vagal afferent innervation.

2.4. Immunohistochemistry

Based on our previous methods [5–7,19–26,30,31], the whole mount preparations and hindbrain sections were