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Exenatide and feeding: Possible peripheral neuronal pathways

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ABSTRACT

Intraperitoneal (i.p.) administration of the synthetic agonist of the glucagon like peptide-1 (GLP-1) receptor exenatide reduces food intake. Here, we evaluated possible peripheral pathways for this reduction. Exenatide (0.5 μg/kg, i.p.) was given to three, overnight food-deprived, groups of rats: total subdiaphragmatic vagotomy (VGX, severs the vagus nerve), celiaco-mesenteric ganglionectomy (CMGX, severs the splanchnic nerve) and combined VGX/CMGX. Following the injection, meal sizes (MSs) and intermeal intervals (IMIs) were determined for a total of 120 min. We found that exenatide reduced the sizes of the first two meals but failed to prolong the IMI between them, that VGX attenuated the reduction of the first MS, and that VGX, CMGX and combined VGX/CMGX attenuated the reduction of the second MS by exenatide. Therefore, the vagus nerve appears necessary for the reduction of the first MS by exenatide, whereas both nerves appear necessary for the reduction of the second MS by this peptide.

1. Introduction

Exenatide is a 39-amino-acid synthetic peptide similar in structure to exendin-4, a hormone isolated from the venom of the Gila monster *Heloderma suspectum* [12]. Like exendin-4, exenatide is a potent glucagon-like peptide-1 (GLP-1) agonist. Because of its beneficial effects on glucose metabolism and insulin secretion and its extended circulating half-life and bioactivity levels compared to GLP-1 e.g. hours (2.5 with detectable activity for up to 8 h) versus seconds (2.5 s) [9,10,12,15,52], exenatide was approved by the FDA for the treatment of type II diabetes in 2005 [1].

Intraperitoneal (i.p.) administration of exenatide reduces food intake in rats [2,47,50]. In addition, it has been shown that exenatide activates propiomelanocortin neurons in the arcuate nucleus of the hypothalamus [26], which suppresses appetite through inhibiting neuropeptide Y neurons. Furthermore, intracerebroventricular injections [39] of GLP-1 changed the potencies of both ghrelin and galanin to stimulate food intake, which indicate possible interaction between GLP-1 and these orexigenic peptides also at the hypothalamic level.

On the other hand, how this satiation signal reaches the central nervous system has not been thoroughly investigated. Here, two facts lead us to hypothesize that the sensory innervation of the gastrointestinal tract contributes to this signaling. First, the major source of peripheral GLP is the L cells of the ileum and colon [11,44]. These parts of the gastrointestinal tract communicate with the feeding-control areas of the dorsal vagal complex (DVC) of the hindbrain, including the area postrema (AP), dorsal motor nucleus of the vagus (DMV) and the nucleus tractus solitarius (NTS), via two nerves, vagus and splanchnic [8,45]. The vagus nerve supplies the gut with parasympathetic efferent and vagal afferent innervation, with cell bodies in the DMV and the nodose ganglia respectively, while the splanchnic nerve supplies the gut with sympathetic efferents and spinal afferents, whose cell bodies are in the celiaco-mesenteric ganglia and the dorsal root ganglia, respectively. Both vagal and spinal afferents are in close proximity with the L-cells, and the vagus nerve [43], the nodose ganglia and the sympathetic innervation of the gut also contain GLP-1 receptors [14,18,28].

Second, the gut is equipped with an independent, intrinsic nervous system, which is referred to as the enteric nervous system (ENS, see [37] for review). The ENS consists of two nerve plexuses, myenteric and submucosal, with cell bodies located within these plexuses, and may also communicate information to the DVC through the vagus and the splanchnic nerves. As in the case of the vagal and spinal afferents, the enteric neurons are also in close proximity with the L-cells of the ileum and colon and they also contain GLP-1 receptors [3].

The current work evaluated the peripheral neural pathway through which exenatide reduces food intake by measuring meal sizes (10% sucrose test solution) and determining the intermeal intervals during a 120 min test in response to exenatide (0.5 μg/kg, i.p.) in three groups of Sprague–Dawley rats: total subdiaphragmatic vagotomy (VGX, a surgical procedure which severs the vagal efferent and afferent innervation of the gut), celiaco-mesenteric ganglionectomy (CMGX, a surgical procedure which severs the

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sympathetic and spinal afferent innervation of the gut including the splanchnic nerve) and combined VGX/CMGX. Our previous work showed that this dose of exenatide reduces cumulative food intake [47].

2. Materials and methods

The Tuskegee University Animal Care and Use Committee approved the animal protocols for this study. Adult, male, Sprague–Dawley rats weighing between 350 and 400 g ($n = 40$) and housed in clear cages to allow complete visualization necessary for behavioral rating were used. The rats lived in a controlled environment (12 h dark/12 h light cycle–lights off at 1800 h, 21.5 °C, with ad lib. water and pelleted rodent chow, Teklad, WI).

To habituate the rats to the laboratory environment and experimental design, every day at 0700 h, i.e., 1 h after the beginning of the light cycle, each rat was weighed, handled for 10 min and received an i.p. injection of 0.5 ml saline.

2.1. Surgical procedures

Four groups of rats ($n = 8$ per group) underwent total subdiaphragmatic VGX, CMGX, combined VGX/CMGX and sham surgery as described previously [5,38]. All surgeries were done through a ventral midline celiotomy incision and under general anesthesia. The anesthesia mixture (1 mg/kg body weight intramuscularly; i.m.), prepared in our laboratory, contained 50 mg/ml ketamine, 5 mg/ml xylazine, and 1 mg/ml acepromazine maleate®. The abdominal wall was prepared surgically by clipping and cleaning with betadine solution and alcohol swabs, and a ventral midline celiotomy was performed following the absence of a pedal reflex.

For VGX, the vagus nerve was exposed on both sides of the esophagus, below the diaphragm, and one centimeter sections were removed from each side to insure no regeneration of the nerves, as fully described previously [38]. The CMG were located between the cranial mesenteric artery and celiac artery, branches of the aorta, and removed under 40× magnification using a jeweler's scissors and #5 forceps, as described previously [5]. Celiac branch vagal fibers were identified and spared. Finally, sham surgery was performed by manipulating the tissue without removing the vagus nerve or CMG.

Abdominal wall muscles were closed using polydioxanone II (4-0) absorbable suture material, and the skin was closed using surgical staples. Postoperative care included Metacam® [Meloxicam® (1.1 mg/kg)] subcutaneously for pain control and Baytril® (Enrofloxacin® (0.05 ml)) intramuscularly as an anti-inflammatory. The drugs were given in the initial three days immediately following the surgeries. Rats were allowed seven days for recovery before performing any food intake experiment. The criteria for complete recovery following surgery included absence of clinical signs (e.g. signs of pain, porphyrin secretion around the eye, cold extremities and lethargy) and return of food intake to baseline levels.

2.2. Verification of surgical procedures

Verification of VGX and CMGX was done in two stages as previously described [5,38]. Immediately following surgery, the removed tissues were stained with hematoxylin and eosin (H&E) and evaluated by the senior author who was blinded to all treatments and procedures throughout all experiment. The slides (6–8 slides per animal from each surgery) contained the CMG seen as a distinct neuronal tissue with extensive number of cell bodies or vagus nerve seen as a circular bundle of fibrous connective tissue filled with nerve tissues/axons.

After recovery, based on our previous work [5] the animals received an injection of CCK-33 (3 nmol/kg, i.p.), and the MS (10% sucrose test solution) and the IMI were determined. In the vagotomized rats, CCK-33 failed to reduce the MS and in the celiaco-mesenteric ganglionectomized rats CCK-33 failed to prolong the IMI relative to saline control. In addition, VGX was also verified by measuring intake of 10% sucrose in response to CCK-8 (5 nmol/kg), which also failed to reduce 30 min intake in each of these rats relative to saline vehicle and compared with the sham group.

At the end of the experiment we also verified the surgery site under a surgical microscope (40× magnification) for absence of CMG and vagus nerve in the surgery groups compared to sham rats.

2.3. Establishing baseline food intake

Before the baseline was established, rats were housed in quarantine rooms for one week. Following this week, the rats were removed to the location of the food intake experiment. Each rat was handled for 5–10 min daily and given a saline injection i.p. Food and water were present in their cages all the time. The animals then underwent surgical removal of the vagus and the celiaco-mesenteric ganglia as described previously [5]. We try to perform the surgeries on all animals in approximately the same time to allow timed recovery. Following the surgery, the rats are given pain medication and anti-inflammatory drugs for approximately a week. After complete recovery, each rat starts receiving a saline injection i.p. and presented with a sucrose solution in addition to their food and water in their cages. When the intake of sucrose for each animal is stabilized, the baseline preparation starts. The duration of this stabilization process is variable between animals. It may take between 1 and 3 weeks following full recovery of surgery. When the intake of sucrose is stabilized for all animals the baseline starts.

As previously described by us [23–25,27,46,48] and by others [13,20,21,32–34,40,42], rats were deprived of chow, but not water, overnight on Sundays, Tuesdays and Thursdays beginning at 1700 h. The following morning, at 0700 h (1 h into the light cycle), rats were weighed and then received vehicle injections (0.5 ml of 0.9% saline) i.p. followed immediately by presentation of a 10% sucrose solution; drinking water remained available. The sucrose solution was used because it has comparable responses to solid food, is highly palatable and stimulates large, frequent meals. In addition, it allows monitoring the intake over very short periods of time, 1 min.

Two, well trained examiners equipped with headphones, laptop computers and timers rated the behaviors of rats once each minute and measured their intake as previously described [5,23–25,27,46,48]. The behaviors included feeding (licking the sucrose bottle), licking (licking the water bottle), biting, grooming, locomotion (walking around the cage), rearing up (front paws on side of cage while hind paws on the floor of cage), standing (stationary position in which abdomen and chest are not touching floor of cage, and no other behavior is being exhibited), sniffing, and stretching and resting (stationary position in which the abdomen is on the floor of cage and no other behavior is being exhibited). The total test period lasted 120 min, and chow was then returned immediately after the end of the test period. The first MS was defined as the amount of 10% sucrose consumed immediately following the injection and presentation of sucrose, which also occurred immediately following the injection, until the recording of three consecutive resting periods in three consecutive minutes. The three resting periods were used to make sure that the rat did not come back to consume another meal. Following that the observers continue to rate the behaviors of the rats, including feeding, and measure the sucrose intake until they record another three resting periods, which denoted the end of a second meal. The time between the first minute – not the third minute – of resting in the first meal