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Gastric emptying in response to IAPP and CCK in rats with subdiaphragmatic afferent vagotomy

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ABSTRACT

In the subdiaphragmatic vagal deafferentation procedure (SDA), the afferent fibers of the vagus are surgically severed unilaterally where they enter the brain stem. The technique includes a subdiaphragmal truncal vagotomy performed on the contralateral side. This procedure has been used to study the control of food intake, but it has not been used previously to investigate the role of vagal afferent fibers in the control of gastric emptying (GE). The current experiment studied the effect of SDA on the inhibition of GE by islet amyloid polypeptide (IAPP or amylin) and cholecystokinin (CCK) in awake, unrestrained rats with gastric cannulas. The experimental group underwent subdiaphragmatic vagal deafferentation; the control group had sham operations. All rats received 20-min intravenous infusions of IAPP (1, 3, 9, 27, and 81 pmol/kg/min), CCK (3, 30 and 90 pmol/kg/min), and normal saline in random order. Gastric emptying of saline was measured by the phenol red method during the last 5 min of each infusion period. CCK dose-dependently inhibited gastric emptying in both the control and SDA animals. The inhibition of GE by CCK was significantly attenuated by SDA ($p<0.01$). IAPP also inhibited gastric emptying dose-dependently, but the difference between the SDA and control groups was not significant. The current experiment, which used a different methodology than previous studies, provides support for the hypothesis that the inhibition of gastric emptying by CCK, but not by IAPP, is mediated partly by afferent vagal fibers.

1. Introduction

The physiological control of gastric emptying is complex and has been shown to include both neural and hormonal mechanisms. The neural pathways involved have been investigated using a number of techniques, including bilateral gastric vagotomy and administration of the neurotoxin capsaicin [1–4]. In the subdiaphragmatic vagal deafferentation procedure (SDA), the afferent fibers of the vagus are severed as they enter the brain stem proximal to the lacerated foramen, and a subdiaphragmal truncal vagotomy is performed on the contralateral side [5–7]. The SDA technique has been used to study the role of vagal afferent fibers in the inhibition of food intake by a number factors including cholecystokinin, interleukin-1β, and glucose preloads [8–10]. However, this procedure has not previously been used to study the control of gastric emptying (GE). In the current study, we used SDA to investigate the role of vagal afferent fibers in the inhibition of GE by islet amyloid polypeptide (IAPP or amylin) and cholecystokinin (CCK) in awake, unrestrained rats.

2. Materials and methods

2.1. Experimental design

At intervals of at least 48 h, all animals underwent nine gastric emptying experiments using 3 concentrations of CCK, 5 concentrations of IAPP, or vehicle alone in random order.

2.2. Animals and surgical procedures

Male Sprague-Dawley rats weighing approximately 250 g were purchased from Scanbur BK, Norrviken, Sweden. They were housed in individual cages in the animal facility of the hospital, with free access to food and water except as noted. Gradual training familiarized the rats with experimental procedures such as attachment to tubing. The experiment followed the American Physiological Society's guidelines for animal research and was approved by the Animal Experiment Ethics Committee in Stockholm.

Animals underwent subdiaphragmatic vagal deafferentation as described by Walls et al. [6,7]. Briefly, this consisted of two surgical procedures: unilateral intracranial selective afferent vagotomy (AV) on the right side using the dorsal approach and a subdiaphragmal truncal vagotomy (SV) on the left side. One week of recovery was allowed

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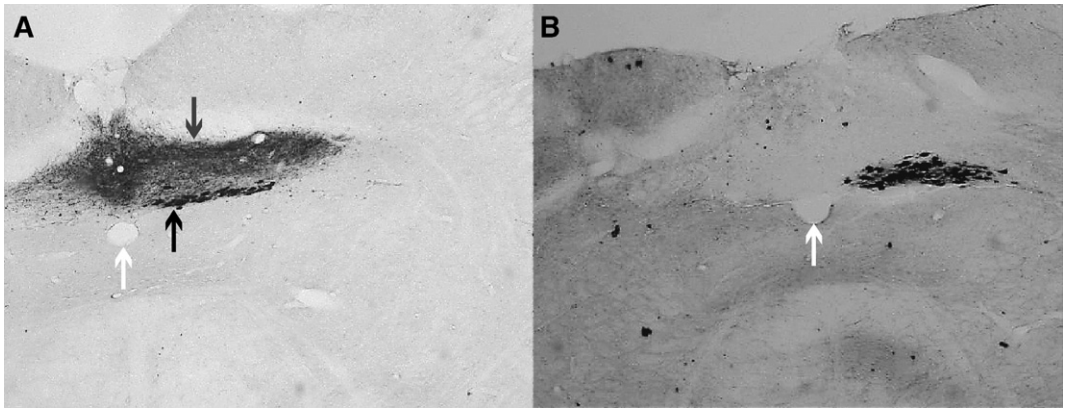

Fig. 1. Photomicrographs of brain stem specimens from a control rat (A) and an SDA rat (B). Central canal, white arrow; Dorsal Motor Nucleus of the Vagus (DMV), black arrow; Nucleus of the Solitary Tract (NTS), grey arrow. In B, note the strong staining in the DMV that is combined with absence of staining in the NTS.

between the AV and SV procedures, which were performed in random order. At the time of the SV procedure, a stainless steel gastric cannula was implanted as previously described [11]. The AV procedure was performed in combination with the placement of an indwelling external jugular vein catheter that was exteriorized at the neck. The control animals received gastric cannulas and jugular vein catheters, but the vagus bundles were not disturbed and the intracranial procedure was interrupted after lancing the dura mater.

2.3. Anesthesia and analgesia

Isoflurane was delivered by a Univentor 400 anesthesia unit (Univentor, Zejtun, Malta) when the SV/cannula procedure was performed and when the vagotomy was verified by vagus injection. Buprenorphine (0.022 mg/rat) was given after induction of anesthesia and repeated 12 h after surgery. In the AV/venous-catheter procedure, anesthesia was induced with 50 mg/kg sodium pentobarbital i.p., followed by 50 mg/kg ketamine i.m. Ketamine was given as needed during the 2–4 h procedure. Buprenorphine was given postoperatively and repeated after 12 h. Bupivacaine (1 mg/rat) was given s.c. along the suture lines on completion of each surgery.

2.4. Verification procedures

Two tracer techniques were employed to verify the completeness of the vagotomies [5]. Briefly, animals received an intraperitoneal injection of 1 ml Fluorogold® (1 mg/ml, Fluorochrome, Denver, CO) 2 days before euthanasia and then received an intraneural injection of 0.2 µl wheat germ agglutinin-horseradish peroxidase (WGA-HRP, 0.2 g/l, Vector Laboratories, Burlingame, CA) in the cervical vagus, ipsilateral to the AV laceration, 1 day before euthanasia. An i.p. injection of a lethal dose of sodium pentobarbital was used for euthanasia. The rats were then perfused through the heart with formaldehyde fixative (Histofix®; Histolab, Vastra Frolunda, Sweden). The brainstems were harvested and kept at 4 °C in 20% sucrose-PBS for up to 3 days until sectioning and histology.

2.5. Histology

Serial 50-µm cryostat sections were made from the brain stem specimens. Two adjacent sections were collected every 300 µm throughout the brain stem specimen. The sections were saved in refrigerated PBS until further processing. The first set of sections was processed for WGA-HRP using the Tetra Methyl Blue kit (TBM; Vector Laboratories, Buringame, CA, USA), and then air-dried, cleared in xylene, and mounted using Mountex (Histolab, Västra Frölunda; Sweden). Cover slips were placed on the second set of sections (consecutive to the first set) and the slides were examined in a fluorescence microscope equipped with filters for Fluorogold. Rats were excluded from the final analysis if (1) any sign of TMB staining in the afferent vagal nuclei or related fiber was present or (2) Fluorogold was present in the vagal efferent nuclei ipsilateral to the SV lesion.

2.6. Gastric emptying experiments

Animals were fasted for 18 h before the experiments, with free access to water. Their stomachs were then rinsed with saline, and the rats were placed in the experimental cages approximately 45 min later. The gastric cannulas were connected to tubing in preparation for the introduction of phenol red. The i.v. catheters were connected to a syringe infusion pump (Harvard Apparatus, South Natick, MA) after flushing and verification of patency by observation of blood backflow. No harnesses were used. A 0.125-ml/kg/min infusion rate was used throughout the experiment to deliver rat IAPP (Bachem, Bubendorf, Switzerland) at doses of 1, 3, 9, 27, or 81 pmol/kg/min or sulphated CCK-8 (Sigma-Aldrich, St. Louis, MO, USA) at doses of 3, 30, or 90 pmol/kg/min. Normal saline was used as the vehicle and zero concentration. After 15 min of infusion, 5 ml of phenol red solution (60 mg/l, Merck, Darmstadt, Germany) was injected into the stomach through the cannula, and the cannula was then closed. The infusion was stopped 5 min later. The gastric contents were then aspirated and saved. The stomach was rinsed with 5 ml of normal saline, which was also collected. The two gastric samples were frozen separately until analysis. In rats without blood backflow when the tubing was being connected, catheter patency was checked by injection of 0.25 ml 25 mg/ml thiopental sodium (Abbot Scandinavia, Solna, Sweden) i.v. after that day's experiment. Data from that infusion were analyzed only if the animal fell asleep within 10 s.

Phenol red concentrations were measured with a spectrophotometer after centrifugation and alkalinization, and the amount of phenol red emptied from the stomach was calculated, allowing for volumes retained in the gastric cannula and the amount of dilution occurring through gastric secretion.

2.7. Statistical methods

The data were analyzed with SAS software (SAS Institute Inc. NC, USA) using the procedure Mixed. The model was set up as a repeated measures design. Two different covariance pattern models were tested: compound symmetry and heterogeneous compound symmetry. The latter model is appropriate when the variances differ between the repeated measurements. Between-subject heterogeneity was also incorporated in the ANOVA model. The model with the smallest value of the Akaike's Information Criterion was chosen: compound symmetry for CCK and heterogeneous compound symmetry for IAPP. Group, Concentration, and Group*Concentration effects were fitted as fixed effects. Group effect