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Effects of centrally injected glucagon-like peptide-2 on gastric mucosal blood flow in rats: Possible mechanisms

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ABSTRACT

"Glucagon-like peptide-2" (GLP-2) is a peptide that is released from the enteroendocrine L cells in response to food in the gastrointestinal tract. Peripheral injection of GLP-2 has been shown to increase gastrointestinal blood flow, but effects of central GLP-2 on any vascular bed has not been studied yet. The aim of this study is to investigate the effects of various doses of intracerebroventricularly (i.c.v.)-injected GLP-2 on gastric mucosal blood flow (GMBF) and contribution of calcitonin gene related peptide (CGRP), nitric oxide synthase-nitric oxide (NOS-NO) and cyclooxygenase-prostaglandin (COX-PG) systems to the possible effect. The gastric chamber technique was used to determine GMBF. Urethane anesthesia was used throughout the recording procedure. Male Wistar rats were treated with GLP-2 (100, 150 ve 200 ng/10 μl; i.c.v.) or saline (10 μl; i.c.v.) in order to find out the effective dose of i.c.v. GLP-2 on GMBF. Then, CGRP receptor antagonist CGRP-(8-37) (10 μg/kg; s.c.), NOS inhibitor NG-nitro-L-arginine methyl ester (L-NAME; 30 mg/kg; s.c.) or COX inhibitor indomethacin (5 mg/kg; i.p.) was injected before the effective dose of i.c.v. GLP-2. GMBF was measured continuously for 35 min following GLP-2 and recorded every fifth minute. Non-parametric Kruskal–Wallis test was used for statistical analysis. Differences were considered to be significant at $p < 0.05$. GMBF increased rapidly following 100 ng GLP-2 injection and did not fall to the basal levels during 35 min. Other doses of i.c.v. GLP-2 did not produce any significant difference in GMBF. CGRP receptor antagonist, CGRP-(8-37) (10 μg/kg; s.c.) and COX inhibitor indomethacin (5 mg/kg; i.p.) significantly prevented the increase in GMBF due to GLP-2 (100 ng; i.c.v.), while L-NAME (30 mg/kg; s.c.) was ineffective. None of the drugs produced a significant change in GMBF when administered alone. Thus we suggest that, i.c.v. GLP-2 increases GMBF and CGRP and endogenous prostaglandins but not NO, contribute to this effect.

Introduction

"Glucagon-like peptide-2" (GLP-2) is an important specific biological peptide which is produced through posttranslational processing of proglucagon in mammals. It is released together with "Glucagon-like peptide-1" (GLP-1) from the mucosal L-cells of the ileum and the large intestine and from the preproglucagonergic (PPG) cells of the brain [1–3]. GLP-1 is a glucose-dependent insulinotropic hormone and it also regulates various gastric and cardiovascular functions [4–7]. On the other hand, the physiological role of GLP-2 has not been discovered until 1996, when it has been shown to stimulate the mucosal epithelial proliferation in the rat small intestine [8,9]. Due to its intestinotrophic effects, GLP-2 has been suggested to have a potential therapeutic role in "the short bowel syndrome" [8,10,11]. Beneficial effects have been observed due to administration of GLP-2 itself or its analogs, when used in several models of intestinal damage or dysfunction, such as inflammatory bowel syndrome [11], massive small intestine resection [12], colitis [13], ischemic bowel [14] and chemotherapeutic damage [15]. It has also been suggested that, it inhibits the motility of the whole gastrointestinal tract by decreasing the gastric tone and gastric emptying [16–18] and by inhibiting the myoelectrical activity of the small intestine [19]. A number of studies suggesting hemodynamic effects of GLP-2 are also present [20–26]. GLP-2 increases mesenteric blood flow in both rats [20] and humans [23,25,26]. It also mediates the stimulation of portal blood flow in TPN-fed neonatal pigs [21]. Blood flow in the superior mesenteric artery (SMA) increases rapidly and dose-dependently following GLP-2 administration and blood flow in the inferior mesenteric and carotid arteries exhibit less significant elevation [20,26]. On

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the other hand, blood flow in the celiac, renal and left gastric arteries does not significantly change after GLP-2 infusion [20,26]. Also, GLP-2 has either no effect or produces a modest suppression of blood flow in the stomach and colon, and slightly decreases spleen and kidney blood flow [22]. Thus, GLP-2 has different vasoactive effects on the gastrointestinal tract as well as the other vascular beds [20–24]. However these findings are all due to the peripheral administration of GLP-2 and no data exists related to the central effects of GLP-2 on blood flow. Although the effects of GLP-2 seem to be limited to the gastrointestinal tract, its effects have been also shown in various organs. GLP-2 receptors are expressed in neurons (enteric neurons, vagal afferent neurons and central nervous system neurons) as well as endocrine cells [18,20,27–32]. GLP-2 shares the same pathway with GLP-1 for synthesis and signaling in the brain and also they have similar effects on gastric motility and eating behavior [3,17,33]. Previously, we have shown that peripherally injected GLP-1 increases gastric mucosal blood flow and thus prevents gastric mucosal damage [34,35]. In addition, we have suggested that intracerebroventricular (i.c.v.) GLP-1 also has a protective role in several models of gastric mucosal damage, especially which are related to the decreased gastric mucosal blood flow [36–38]. Here, we suggest that GLP-2 may also have similar effects, since it is both synthesized and also has specific receptors in brain areas which contribute to the central regulation of gastrointestinal function. Therefore we aimed to investigate (1) the effects of various doses of i.c.v. GLP-2 on gastric mucosal blood flow (GMBF) and (2) the contribution of CGRP, NOS-NO and COX-PG systems to the possible role of GLP-2 on GMBF.

Materials and methods

Animals

Male Wistar rats (Experimental Animals Breeding and Research Center, Uludag University, Bursa, Turkey), weighing 200–300 g were used in this study. Rats were housed 4–6 in a cage under constant environmental conditions (20–24 °C; 12-h light–dark cycle) with food and water ad libitum. The animals were fasted for 24–26 h before the experiments with free access to tap water until 1 h before testing. The surgical and experimental protocols used were approved by the Animals Care and Use Committee of Uludag University (reference number: 2011-08/02).

Gastric chamber preparation

The gastric chamber technique, first described by Mersereau and Hinchey, was used to study gastric mucosal blood flow [39]. Each animal was anaesthetized with urethane (1.5 g/kg; i.p.). The body temperature was maintained at 36 °C using a homeothermic blanket (WBC3044 water bath and circulator-therapy pad) and core temperature was monitored by a rectal thermometer (SS7L temperature transducer) throughout the experiment. In order to record the arterial blood pressure simultaneously with the gastric blood flow, rats were implanted through the right femoral artery with a PE 50 tubing filled with heparinized saline (100 U/ml). The arterial catheter was connected to a blood pressure transducer (BPT300) which is attached to "MP30 Data Acquisition System" to record arterial blood pressure and heart rate. Mean arterial pressure was expressed in mmHg, and heart rate was expressed in beats/min. The same system was used for the monitorization of the rectal temperature.

Laparotomy was performed through a midline epigastric incision. The stomach was exposed and brought to the abdominal surface by gentle traction. It was then drawn through the center hole of the plastic cylindrical chamber (inner diameter 2.4 cm), which was secured to a plastic platform placed over the animal. The stomach was opened along the greater curvature and the mucosal edges pinned to a rubber diaphragm on the platform surface. Gastric mucosa was washed three times with saline (% 0.9 sodium chloride) and then bathed with 1.5 ml of saline. The ex vivo gastric chamber allowed access to the gastric lumen and mucosa for GMBF measurement.

I.c.v. injections

For i.c.v. injections, a burr hole was drilled through the skull 1.5 mm lateral to the midline and 1–1.5 mm posterior to bregma on the right side. Through this hole, a 10 mm length of 20 gauge stainless steel hypodermic tubing was directed toward the right lateral ventricle. The cannula was lowered 4.2–4.5 mm below the surface of the skull perpendicularly and was fixed to the skull with acrylic cement. Animals were housed individually and allowed to recover for 5 days. At the end of the experiments, 5 μl of a methylene blue solution was injected into the cerebral ventricle through the cannula, and the placement of the inner end of the cannula was verified for each rat. After decapitation, the brains were removed and sections were observed macroscopically to ascertain whether the cannula had been correctly placed into the lateral cerebral ventricle.

Blood flow measurement

GMBF was measured by a laser Doppler flowmeter (OxyLab LDF laser doppler flowmeter), connected to computer. Data were recorded continuously in tissue perfusion units (ml/min/100 g of tissue) by using "MP30 Data Acquisition System". The probe (MNP100XP standard needle probe) was placed just above and perpendicular to the corpus mucosa and its position maintained by a micromanipulator. Measurements were made continuously at the same site on the anterior part of the mucosal surface of the gastric corpus.

Experimental protocols

Each rat was equipped with an i.c.v. cannula 5 days before the experiments and with an arterial catheter on the experiment day. Then gastric chamber was prepared for GMBF recording. Drugs were administered in different groups as indicated below.

Effect of i.c.v. GLP-2 on GMBF

Following 30-min calibration period, basal GMBF of rats were recorded, which then received either GLP-2 (100, 150 or 200 ng/10 μl; i.c.v.) or saline (10 μl; i.c.v.). Measurements were made continuously for 35 min following the injections and recorded every fifth minute. GMBF values were expressed as percent change from basal values.

Contribution of CGRP to the effect of GLP-2 on GMBF

Rats were pretreated with a CGRP receptor antagonist CGRP-(8-37) (10 μg/kg; s.c.), 15 min before GLP-2 (100 ng/10 μl; i.c.v.) or saline (10 μl; i.c.v.) injection. Each group consisted of 6–8 rats. GMBF was measured for 35 min and recorded every fifth minute following the injections.

Contribution of endogenous NO to the effect of GLP-2 on GMBF

Rats were pretreated with a NOS inhibitor, NG-nitro-L-arginine methyl ester (L-NAME) (30 mg/kg; s.c.) 30 min before GLP-2 (100 ng/10 μl; i.c.v.) or saline (10 μl; i.c.v.) injection. Each group consisted of 6–8 rats. GMBF was measured for 35 min and recorded every fifth minute following the injections.