ORIGINAL RESEARCH

GLUCAGON-LIKE PEPTIDE–2 INDUCED HEMODYNAMIC ALTERATIONS IN THE RAT SMALL INTESTINE

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ABSTRACT

Objective: Glucagon-like peptide–2 (GLP–2) is a 33 amino acid peptide produced in endocrine L-cells of the intestinal mucosa. The aim of the present study was to characterize whether GLP–2 alters the hemodynamic parameters of the small intestine and to find out possible mediators.

Methods: After an overnight fasting, the rats were anesthetized with urethane. The right carotid artery and jugular vein were cannulated and a midline abdominal incision was made to isolate the superior mesenteric artery (SMA) for flow probe placement. GLP–2 at doses of 1, 5 and 10 μg/rat were infused into the jugular vein for 120 minute. SMA blood flow and resistance were measured and calculated. In an additional group, the animals were pretreated with atropine sulfate (1 mg/kg), teophylline (25 mg/kg) and ramosetron (100 μg/kg) given 20 minutes before the infusion of GLP–2 (5 μg/rat).

Results: There was no significant difference in the mean arterial pressure of the untreated or GLP–2 treated rats throughout the experiments. GLP–2 administration at all doses increased the SMA blood flow compared with the untreated values. Neither atropine sulfate and ramosetron nor theophylline pretreatment significantly changed the blood flow responses obtained from GLP–2 infusion.

Conclusion: These results demonstrated that GLP–2 induced changes in blood flow are not mediated by muscarinic, adenosine or serotonergic 5-HT_3 receptors.

Keywords: GLP–2, Blood flow, Small intestine

SIÇAN İNCE BARSAK HEMODİNAMİĞİNDE GLUKAGON BENZERİ PEPTİD–2 ARACILI MEYDANA GELEN DEĞİŞİKLİKLER

ÖZET

Amaç: İntestinal L-hücrelerinden salınan GLP–2, besin absorbsiyonu ve epiteliyal permeabilitenin düzenlenmesinde önemli bir rol oynamaktadır. GLP–2'nin bilinen bu özelliklerine rağmen barsak kan akımı üzerindeki etkileri ve bunlardan sorumlu olası aracı moleküller bilinmemektedir. Bu çalışmanın amacı GLP–2'nin intestinal hemodinamik parametreler üzerindeki etkilerini karakterize etmek ve bu etkilere aracılık eden mekanizmaları aydınlatmaktır.

Yöntem: Bütün deneyler kurumsal etik komite tarafından onaylanmıştır. Üretan anestezisi altında sıçanların sağ karotid arter ve juguler venleri kanüle edildikten sonra süperior mesenterik arter'e (SMA) akım probu yerleştirildi. GLP–2 (%0.1 sığır serum albumini içinde), 1, 5 ve 10 μg/rat dozlarında, 0.016 ml/dk hızında juguler venden 120 dakika boyunca infüze edildi. Kan basıncı eş zamanlı olarak kaydedildi, SMA rezistansı hesaplandı. Diğer gruplarda, GLP–2 (5 μg/rat) infüzyonundan 20 dk önce atropin sülfat (1 mg/kg), ramosetron (100 μ/kg) ve teofilin (25 mg/kg) tedavileri uygulandı. Sonuçlar ortalama ± standart hata şeklinde ifade edildi ve ANOVA ile analiz edildi.

Bulgular: GLP–2 kan basıncını anlamlı olarak değiştirmemiştir. SMA kan akımını anlamlı bir şekilde arttırırken rezistansı düşürmektedir ($p<0.01$ kontrole göre). Ön tedavilerin hiçbiri GLP–2 ile meydana gelen SMA kan akımı değişikliklerini anlamlı olarak değiştirmemiştir.

Sonuç: Bu sonuçlar GLP–2 ile meydana gelen hemodinamik değişikliklerde muskarinik, adenozin veya ve serotonerjik 5-HT_3 reseptörlerinin sorumlu olmadığı düşündürmektedir.

Anahtar Kelimeler: GLP–2, Kan akımı, İnce barsak

INTRODUCTION

Many different growth factors (GLP–2, EGF, TGF-a) take part in the growth, development and repair of the gut. Glucagon like peptide 2 (GLP–2) is a 33 amino acid peptide produced in endocrine L-cells of the intestinal mucosa. Both GLP–1 and GLP–2 are secreted from gut endocrine cells in the small and large intestine.

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Secretion of these peptides is predominantly regulated by nutrient intake[1]. GLP–2 stimulates intestinal growth[2,3,] crypt cell proliferation[4,] decreases intestinal apoptosis in rats and mice[5,] regulates gastric motility and acid secretion[6] and increases intestinal hexose transport[7].

GLP–2 has also been implicated as a therapeutically useful agent in various experimentally-induced gut inflammations, including dextran sulfate-induced colitis[8,] indomethacin-induced gastrointestinal injury[9] and intestinal ischemia-reperfusion injury[10,11,12]. These inflammatory conditions are associated with alternations in the gut hemodynamics. Although beneficial actions of GLP–2 during inflamatory conditions are attributed to its proliferative and anti-apoptotic properties, it is unknown whether some of these actions are associated with the changes in intestinal hemodynamics. Therefore, the objectives of the present study were to characterize whether GLP–2 alters the hemodynamics of the small intestine and to find out whether muscarinic, adenosine and serotonergic 5-HT_3 receptors were involved in the intestinal hemodynamic responses induced by GLP–2

METHODS

Animals

Sprague-Dawley rats of both sexes (250-300g) were kept at a constant temperature of 22±2 ºC with light-dark cycles of 12 h and fed a standard diet and water ad libitum. Studies were approved by the Marmara University Animal Care and Use Committee. After an overnight fast, the rats were anesthetized with urathane (1.2 g/kg, ip.) and a tracheotomy was performed to facilitate breathing. The right carotid artery was cannulated for arterial pressure recording (Nihon Kohden polygraph, model AP-621G). The right jugular vein was also cannulated for the injection of various compounds. A thermometer was inserted into the rectum, and the body temperature was maintained at 37 ºC by use of a heating pad.

Hemodynamic measurements.

After laparotomy, blood flow in the superior mesenteric (SMA) artery was measured by the ultrasonic transit time technique for 60 or 120 min as previously described[13]. Briefly a 0.7 VB 156 probe (Transonics Systems, Ithaca, NY) was used for the blood flow measurements. The probe houses two ultrasonic transducers and fix acoustic reflector. The transducers pass ultrasonic signals back and forth, alternately intersecting the flowing liquid in upstream and downstream directions. A short length (~5 mm) of the SMA was isolated for flow probe placement. All fatty tissue was removed from the isolated segment of the SMA to avoid obstruction of the ultrasonic signal. The flowmeter (model T106; Transonic Systems) subtracts the downstream transit time, estimating the volume of blood flow (ml/min), normalized for per 100 g of tissue weight at the end of the experiments. Mean arterial pressure (MAP) was recorded simultaneously with the blood flow and the resistance of arteries was calculated by dividing MAP in millimeters of mercury by blood flow. Following 20 min of stabilization, baseline recordings of blood flow were collected for 5 min. All subsequent changes in blood flow and resistance caused by various treatments were calculated as a percentage of the baseline recording.

Administration of drugs

GLP–2 (Sigma Chemical, St. Louis, MO) prepared in 0.1% bovine serum albumin (BSA) and doses of 1, 5 and 10 μg/rat (20 % of the total dose as bolus and the rest as infusion) were given and infusions were continued for 60 minutes. Control animals received the same volume of (1 ml/rat) vehicle (0.1% BSA in saline). In a group of animals, non selective muscarinic acetylcholine receptor antagonist atropine sulfate (1 mg/kg; iv, Sigma Chemical, St. Louis, MO), 5-HT_3 receptor antagonist ramosetron (100mg/kg; iv Gift) and adenosine A_1 and A_2 receptor antagonist theophylline (25 mg/kg; iv) were used 20 minutes before the infusion of GLP–2.

Statistics:

All values were reported as means ± SE. One-way analysis of variance with the Tukey-Kramer (post hoc) test was used for multiple comparisons. Differences were considered statistically significant if $p<0.05$.

RESULTS

Effects of GLP–2 infusion

The administration of GLP–2 at various doses did not cause any significant change in mean arterial pressure. The mean arterial pressure before and after GLP–2 infusion (5 mg/rat) was 82.1±5.18 mmHg and 79.33±6.5 mmHg, respectively, which slowly declined to 76.88±4.2 mmHg 60 minutes later. Figure 1 illustrates the time-dependent

changes in SMA blood flow (Fig. 1A) and resistance (Fig. 1B) induced by the administration of different doses of GLP–2. Basal values of the groups were found to be not statistically different. Administration of GLP–2 at all doses significantly increased SMA blood flow compared to baseline ($p<0.01$), in which GLP–2 infusions significantly reduced the SMA resistance in all groups.

Effects of treatments

The effects of the pretreatment of animals with various doses of atropine sulfate, ramosetron and theophylline on GLP–2 induced alterations in blood flow are shown in Fig. 2A. Atropine sulfate (1 mg/kg), ramosetron (100 µg/kg) and theophylline (25 mg/kg) did not change the GLP–2 induced increases in blood flow compared with the untreated group.

Similar results were obtained from the resistance data (Fig. 2B), in which pretreatment with atropine sulfate, ramosetron or theophylline did not significantly alter the SMA resistance.

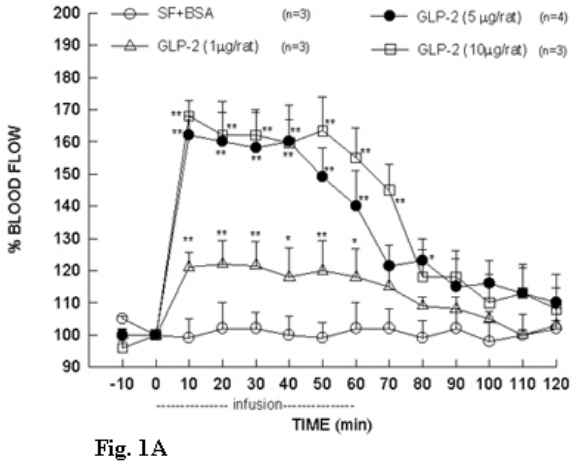

Fig. 1A

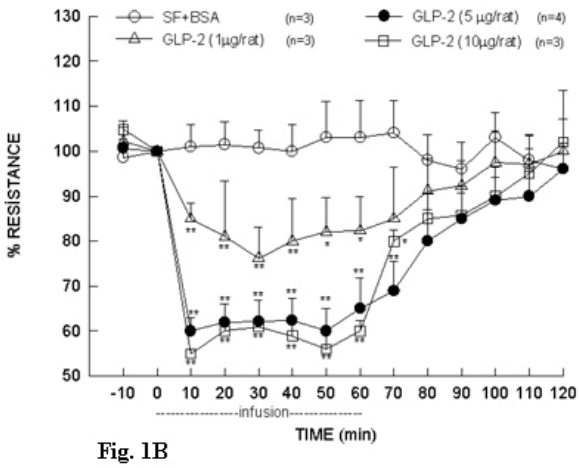

Fig. 1B

Fig. 1: Time-dependent changes in superior mesenteric artery blood flow (A) and resistance (B) induced by administration of different doses of GLP–2. *p<0.05, **p<0.01; compared to basal time point value.

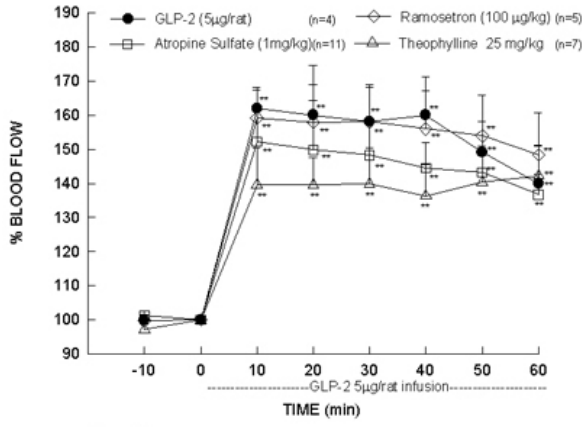

Fig. 2A

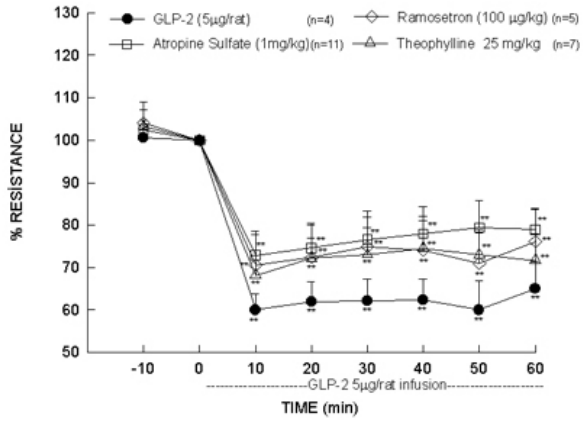

Fig. 2B

Fig. 2: Changes in SMA blood flow (A) and resistance (B) after GLP–2 administration (5 µg/rat) and GLP–2 plus atropine sulfate (1 mg/kg), ramosetron (100 µg/kg) and theophylline (25 mg/kg) **p<0.01; compared with basal time point values.

DISCUSSION

An intact mucosal barrier is vitally important for the separation of the luminal contents from the underlying mucosal interstitial fluid. The factors important for barrier function and maintenance of epithelial mucosal integrity in the small intestinal epithelium are complex and include local and systematically derived growth factors, prostaglandins, endogenous gut bacterial flora and mucins. Recent studies have addressed the possibility that GLP–2 may be therapeutically useful for enhancing the endogenous reparative response to mucosal epithelial damage. Investigators administered GLP–2 to mice with dextran sulfate-induced colitis. It has been shown that a human GLP–2 analogue (Gly^2)GLP–2 significantly reverses weight loss, reduces interleukin-1 expression and increases colon length, crypt depth and mucosal area in the colon of mice with acute dextran sulfate colitis [8]. In another study, the administration of human (Gly^2)GLP–2 significantly improved survival, reduced histological evidence of disease activity, myeloperoxidase activity, cytokine induction and bacteremia after indomethacin-induced gastrointestinal injury [9]. The beneficial effects of GLP–2 are also observed in animals after I/R injury. In these studies, a synthetic protease-resistant analogue of GLP–2, GLP–2a significantly increases mucosal mass, mucosal DNA content and absorptive function in rats after reperfusion followed by 30 minutes of intestinal ischemia [10, 11].

In the present study the administration of different doses of GLP–2 directly into SMA significantly elevated the intestinal blood flow. The observations that GLP–2-induced increases in SMA flow are accompanied by decreases in vascular resistance suggests that elevations in blood flow are associated with decreased vascular tonus, rather than the alterations in blood pressure. It has been generally accepted that reductions in tissue blood flow are associated with the inflammation-induced tissue injury. It has been shown that the administration of vasodilator agents such as nitric oxide or inhibition of vasoconstrictor agents such as endothelin-1 by receptor blockers has been documented to be beneficial following acute episodes of ischemia reperfusion injury, indomethacin induced intestinal damage or colitis [8,9,12]. Thus, it is conceivable to suggest that in addition to proliferative [2] and anti apoptotic [14] actions, some of the beneficial actions of GLP–2 observed after various models of the intestinal inflammation [8,9,14] can be attributed to the restorations in blood flow.

In order to address the mechanism by which GLP–2- induced increases in intestinal blood flow, we pretreated the animals with atropine, theophylline and ramosetron 20 minutes before the infusion of GLP–2. None of the pretreatments significantly changed GLP–2-induced alterations in hemodynamics, indicating that muscarinic receptors, 5-HT_3 and adenosine A_1 and A_2 receptors are not involved in this response. Other mediators such as nitric oxide, prostaglandins and vasoactive intestinal polypeptide should be considered regarding the GLP–2-induced increases in SMA blood flow.

In conclusion, GLP–2 dose dependently increased intestinal blood flow and decreased vascular resistance without changing the blood pressure. The effect of GLP–2 on intestinal blood flow is not mediated by muscarinic receptors, adenosine and 5-HT_3 receptors.

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