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Diabetes attenuates the inhibitory effects of endomorphin-2, but not endomorphin-1 on gastrointestinal transit in mice

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ABSTRACT

Diabetes affects the entire gastrointestinal tract from the esophagus to the anus. In the present study, the charcoal meal test was undertaken to evaluate and compare the effects of intracerebroventricular (i.c.v.) administration of endomorphins (EMs) on gastrointestinal transit in non-diabetic and diabetic mice. Significantly delayed gastrointestinal transit was found in both 4 and 8 weeks alloxan-induced diabetes compared to non-diabetes. Moreover, i.c.v. EM-1 and EM-2 dose-dependently delayed gastrointestinal transit in non-diabetes and diabetes. The EM-1-induced inhibitory effects of gastrointestinal transit in 4 weeks diabetes were qualitatively similar to those of non-diabetes. However, at higher doses, the EM-1-induced effects in 8 weeks diabetes were largely enhanced. Different to EM-1, the EM-2-induced inhibition of gastrointestinal transit in diabetic mice was significantly attenuated compared to non-diabetic mice. Moreover, these effects were further decreased in 8 weeks diabetes. The delayed gastrointestinal transit effects caused by EM-1 may be primarily mediated by μ_2-opioid receptor in both non-diabetes and 4 weeks diabetes. Interestingly, in 8 weeks diabetes, these effects were mediated by μ_2- and δ-receptors. However, the inhibitory effects of EM-2 were mediated by μ_1-opioid receptor, which exerted a reduced function in diabetes. Also, poor blood glucose control might result in the attenuated effects of EM-2. Our present results demonstrated that diabetes attenuates the inhibitory effects of EM-2, but not EM-1 on gastrointestinal transit in mice. The different effects of EM-1 and EM-2 on gastrointestinal transit in diabetes may be due to changes of opioid receptor subtypes and their functional responses.

1. Introduction

Endomorphin-1 (EM-1) and endomorphin-2 (EM-2) are two endogenous, potent and highly selective μ-opioid receptor agonists (Zadina et al., 1997). Centrally administered EMs produced potent antinociception in rodent models of acute (Stone et al., 1997; Tseng et al., 2000) and neuropathic (Przewłocki et al., 1999) pain, with less side effects (Czapla et al., 2000; Wilson et al., 2000).

Opioid receptors are most abundant in the central and peripheral nervous systems (Wittert et al., 1996). There are three well-defined types of opioid receptors: the μ-, δ- and κ-opioid receptors (Clark et al., 1989; Harrison et al., 1998; Kieffer, 1999). The μ-opioid receptor mediates the most potent antinociceptive activity, accompanied, however, by the development of tolerance, dependence and opioid bowel dysfunction (OBD). It is now thought that OBD occurs as a result of opioid-induced inhibition of gastrointestinal motility and alterations in fluid balance through activation of μ-receptor (Pappagallo, 2001). Thus, it is important to evaluate the gastrointestinal functions of μ-opioid ligands. As both EMs are endogenous μ-agonists, they play important roles in the regulation of gastrointestinal functions (Fichna et al., 2007). Previous studies indicated that both EMs inhibited gastric emptying and intestinal propulsion (Bihm et al., 1998). Moreover, intracerebroventricular (i.c.v.) administration of EMs significantly inhibited the gastrointestinal transit (Goldberg et al., 1998), similar to the actions of morphine and other μ-drugs (Mori et al., 2013). Our previous study demonstrated that EMs induced significant contractions in isolated mouse colon preparations, and the activation of multiple subtypes of opioid receptors was required (Yu et al., 2007).

As we know, gastrointestinal disorders are common in diabetic patients and animals (Battle et al., 1980; Shakil et al., 2008). It has been estimated that more than 75% of patients visiting diabetes clinics report gastrointestinal symptoms (Zhao et al., 2009). It is thought that the functional abnormality in the opioidergic system may alter the modulation of gastrointestinal activities of opioids in diabetes. Previously, we have demonstrated that

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diabetes significantly attenuated the modulation of EMs on the mouse colonic contractions (Wang et al., 2008a). The constipating effects of μ-opioid ligands are considered to be predominantly of colonic origin, since the retention time of digesta in the large bowel takes up about 80% of the oro-anal transit time (Banta et al., 1979; Arhan et al., 1981). Recently, we examined the effects of EMs on colonic bead expulsion and large intestinal transit in diabetic mice, which were colonic origin. Our results indicated that diabetes attenuated the inhibition of distal colonic propulsion induced by EMs (Wang et al., 2013).

Diabetes affects the entire gastrointestinal tract from the esophagus to the anus. However, the influence of diabetes on the EMs-induced effects on gastrointestinal transit is still unknown. Thus, our present study was undertaken to evaluate and compare the effects of i.c.v. EM-1 and EM-2 on gastrointestinal transit in diabetic and non-diabetic mice, using the charcoal meal test. Also, the specific opioid receptor antagonists were used to examine whether functional abnormalities in the opioidergic system of diabetes may alter the EMs-induced gastrointestinal effects.

2. Materials and methods

2.1. Animals

Diabetes was induced by alloxan (Fluka) (260 mg/kg, i.p.) in male Kunming strain mice (4–5 weeks old and 20–25 g in weight). Age-matched normal mice served as control. Animals were housed in an animal room that was maintained at 22 ± 2 °C with a 12-h light:12-h dark cycle. Food and water were available ad libitum. The experiments began after 4 weeks for one group and 8 weeks for the other. The serum glucose was determined the day before the experiments began. Mice with a random serum glucose level above 20 mmol/l were used. As for the insulin treatment group, 3 days after the injection of alloxan, the mice were administered with intermediate-acting insulin (Novolin N, Novo Nordisk) by daily s.c. injection of 1.2 unit/kg for about 4 weeks. Control mice were daily s.c. injected by saline for about 4 weeks. All animals were cared for and experiments were carried out in accordance with the principles and guidelines of the Ethics Committee of Harbin Institute of Technology.

2.2. Drugs

Peptides used in this study: EM-1 and EM-2 were synthesized by a liquid-phase method and characterized by RP-HPLC, NMR and ESI-MS as described in our previous study (Wang et al., 2008b). Both EMs were purified by column chromatography on silica gel with the purities more than 95%. Their bioactivity was recorded by a model BL-420F system (Taimeng Technology & Market Corporation of Chengdu, China). Naloxone hydrochloride, β-funaltrexamine hydrochloride (β-FNA), naloxonazine dihydrochloride, naltrindole isothiocyanate hydrochloride (NTI) and nor-binaltorphimine hydrochloride (nor-BNI) were purchased from Sigma-Aldrich. All compounds were dissolved in sterile saline and stored at −20 °C. The aliquots were thawed and used on the day of the experiment. During an experiment, the drug solutions were kept on crushed ice.

2.3. I.c.v. administration

I.c.v. administration was performed in conscious mice following the method previously described (Haley and Mccormick, 1957). The injection site was 1.5 mm from the middle, 1 mm from the bregma and 3 mm from the surface of the skull. Drugs were administrated in a volume of 4 μl at a constant rate of 10 μl/min attached to a 10-μl Hamilton microsyringe. Vehicle control animals received appropriate normal saline. The proper injection site was verified in pilot experiments by administration and localization of methylene blue dye.

2.4. Gastrointestinal transit study

Gastrointestinal transit was determined using the charcoal meal test according to the procedures as described previously (Broccardo et al., 2003; Niijima et al., 2000). Mice were fasted individually for 20 h in wire-bottom cages to prevent coprophagy with free access to water before the experiment, and then dosed orally with 0.2 ml/mouse of a suspension of charcoal meal (10% activated charcoal in 5% gum arabic). Then the animals were killed by cervical dislocation 30 min later, as at this time point maximal differences between saline and drug groups were observed. The abdomen was opened and the intestine was removed from the pyloric junction to the cecal end. The omentum was carefully separated avoiding stretching. The total length of the small intestine as well as the length traveled by the charcoal was measured for each mouse. Controls were given saline. The propulsive activity of the gastrointestine was calculated as the percent of the distance traveled by the charcoal relative to the total length of the small intestine. The data are expressed as the percent of gastrointestinal transit measured as a quotient of the propulsion value in drug-treated mice and that in saline-treated mice (% of saline effects = 100%). Each value represents the mean with S.E.M. for 10–15 mice. The lower the quotient, the stronger the inhibition of transit. Drugs or saline (0.5, 1.5 and 5 nmol/mouse) were injected i.c.v. in a volume of 4 μl 5 min before the charcoal meal. The opioid receptors antagonists naloxone, β-FNA, naloxonazine, NTI or nor-BNI were administered concurrently with EM-1 or EM-2 at the dose of 20 nmol/mouse.

2.5. Statistical analysis

Values were expressed as the mean ± S.E.M. Responses were analyzed with a one-way analysis of variance (ANOVA) followed by Scheffe's test. $P < 0.05$ was used as the criterion for statistical significance.

3. Results

3.1. Gastrointestinal transit in diabetic mice

The effects of i.c.v. injected saline on gastrointestinal propulsive activity in diabetic and non-diabetic mice are shown in Fig. 1. The propulsive activities of the gastrointestine (%) of non-diabetic, 4 weeks and 8 weeks diabetic mice were 70.01 ± 3.23%, 42.11 ± 2.63% and 33.23 ± 3.02%, respectively. Significantly delayed gastrointestinal transit was found in both 4 weeks and 8 weeks diabetic mice compared to non-diabetic mice. Moreover, the gastrointestinal propulsive activity in 8 weeks diabetes was greater than that of 4 weeks diabetes.

3.2. Effects of EM-1 and EM-2 on gastrointestinal transit in diabetic and non-diabetic mice

As shown in Fig. 2, i.c.v. administration of EM-1 and EM-2 (0.5, 1.5 and 5 nmol/mouse) dose-dependently delayed gastrointestinal transit in both non-diabetic and diabetic mice. As for EM-1 in non-diabetes, the quotients of gastrointestinal transit were 68.5 ± 5.92%, 55.93 ± 4.12% and 45.43 ± 3.75% at the dose of 0.5, 1.5 and 5 nmol/mouse, respectively. At 4 weeks after the onset of diabetes, there was no significant difference in the EM-1-induced inhibition of gastrointestinal transit compared to that of non-diabetes. Moreover,