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Activation of islet 5-HT_4 receptor regulates glycemic control through promoting insulin secretion

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ABSTRACT

Mosapride, a gastrointestinal prokinetic drug, is an agonist of 5-hydroxytryptamine (5-HT) receptor 4 that also reduces blood glucose. Whether 5-HT_4 receptor is distributed in pancreatic islets and whether mosapride can directly stimulate insulin secretion is unclear. In the present study, the protein expression and cellular location of 5-HT_4 receptor in pancreas was detected through western blotting and immunofluorescence. The acute effects of 5-HT_4 receptor agonists, mosapride and prucalopride, on insulin secretion were investigated *in vivo* and *in vitro* in normal and alloxan-induced diabetes rats. The results indicated that 5-HT_4 receptor immunoreactivity was co-existed in the islets insulin-immunoreactive cells of rat, mouse, pig and human. However the immunoreactive cells of insulin and 5-HT_4 receptor and the protein expression of 5-HT_4 receptor were significantly decreased in the pancreas of alloxan-induced diabetes rats. In normal rats, mosapride and prucalopride decreased blood glucose and increased insulin secretion during glucose tolerance test, in association with an increase in glucose-stimulated insulin secretion, which was abolished by the 5-HT_4 receptor antagonist GR113808. In diabetes rats, mosapride and prucalopride failed to improve blood glucose and insulin levels in the group of 180 mg/kg alloxan, but increased glucose-stimulated insulin secretion in the group of 120 mg/kg alloxan in vitro. We conclude that 5-HT_4 receptor is distributed in the islet β cell. Activation of 5-HT_4 receptor is able to stimulate insulin secretion directly, thereby reduce blood glucose. The study provides important experimental evidences for the 5-HT_4 receptor regulating insulin secretion and acting as a potential drug target in diabetes treatment.

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1. Introduction

Serotonin, also known as 5-hydroxytryptamine (5-HT), is a monoamine neurotransmitter involved in multiple physiological processes, including regulation of pancreatic secretion (Li et al., 2001), epithelial ion transport (Li et al., 2011; Yang et al., 2010), gastrointestinal motility (Mawe and Hoffman, 2013), etc. The subtypes of the 5-HT receptor include 5-HT_1 receptor to 5-HT_7 receptor, of which 5-HT_3 receptor is a ligand-gated ion channel, while the others are G protein-coupled receptors (Fidalgo et al., 2013). Mosapride, a selective agonist of 5-HT_4 receptor and a partial 5-HT_3 receptor antagonist (Liu et al., 2005), is widely used as a gastrointestinal prokinetic agent (Tack et al., 2012).

Diabetic gastroparesis has been found in up to 33% type 1 diabetes patients and 7.5% type 2 diabetes patients (Choung et al., 2012). Delayed gastric emptying leads to fluctuation in blood glucose levels (Vanormelingen et al., 2013). It is reported that mosapride improves glycemic control by increasing glucose utilization in glucose tolerance impaired patients (Nam et al., 2010). In healthy human, mosapride can stimulate insulin secretion (Maruoka et al., 2015). However, in type 1 diabetic patients, another 5-HT_4 receptor agonist cisapride has no effect on glycemic control (Braden et al., 2002). It is unclear whether the 5-HT_4 receptor is *in situ* located in the β cells of the pancreatic islets as well as whether mosapride directly stimulates islet insulin secretion. In the present study, we aim to investigate the cellular localization of 5-HT_4 receptor in the pancreatic islets and the effects of 5-HT_4 receptor agonists on the insulin secretion and glycemic control in both normal and alloxan-induced diabetes rats using western blotting and double-labeling immunofluorescence combined with glucose tolerance test and glucose-stimulated insulin secretion test. The study may provide experimental evidences for the 5-HT_4

Abbreviations: 5-HT, 5-hydroxytryptamine; INS, insulin; GLU, glucagon; PP, pancreatic polypeptide; SST, somatostatin

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receptor regulating the endocrine function of pancreatic islets and acting as a potential drug target.

2. Materials and methods

2.1. Chemicals

Mosapride citrate (mosapride) and GR113808 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Prucalopride succinate (prucalopride) was generously donated by Xian Janssen Pharmaceutical Ltd (P.R. China). Mosapride and GR113808 were dissolved in a solution containing 1% lactic acid (*in vivo* experiment) and were administered intraperitoneally (i.p.) or in 0.1% dimethyl sulfoxide (DMSO) (*in vitro* experiment). Prucalopride was dissolved in saline and was administered orally. The dosages of mosapride and prucalopride were according to the references (Briejer et al., 2001; Johnson et al., 2012; Mine et al., 1997).

2.2. Animal and tissue preparation

Adult male Sprague-Dawley (SD) rats (220–250 g) and C57/BL6 mice (22–25 g) were purchased from the Laboratory Animal Services Center of Capital Medical University. All of the animals were housed in an animal care facility at a temperature of 23 °C on a 12-h light/dark cycle, with free access to standard rodent laboratory food and water. Pig pancreatic tissues were kindly provided by China Agricultural University. All of the experiments were performed according to the guidelines established by the National Institutes of Health and approved by the Animal Care and Use Committee of the Capital Medical University, Beijing, China.

The rats and mice were *killed* by decapitation, the pigs were *killed* by euthanasia and their pancreases were immediately removed. For immunofluorescence staining, pancreatic tissues were placed in 4% paraformaldehyde/phosphate-buffered saline (pH 7.4) for 24 h at 4 °C, dehydrated sequentially in 15% sucrose and 30% sucrose, and embedded in optimal cutting temperature (OCT) solution (Sakura, Finetek, USA).

Human pancreatic specimens were collected from three patients who underwent surgery for pancreatic cancer, according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) and the approval of the Ethics Committee by the Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou, China in January 16, 2014. Informed consent was obtained from all of the patients. The specimens were obtained from macroscopically unaffected areas determined by the Department of Anatomical Pathology of the Affiliated Cancer Hospital of Zhengzhou University. Parts of the specimens were embedded in paraffin using standard methods.

2.3. Alloxan-induced diabetes rat

The procedure to create alloxan-induced diabetes rat was performed as previously described (Hong et al., 2014; Ye et al., 2014). Adult male SD rats (220–250 g) were fasted overnight. To induce diabetes, the alloxan was administered i.p. in a single dosage of 90, 120, 150, 180 mg/kg body weight (alloxan dissolved in cold saline at a concentration of 0.1 g/ml). After 2 h, the rats were given free access to food and water. Two days later, the blood glucose was measured and the pancreatic tissue was harvested after sacrifice.

2.4. Islet isolation

The islet isolation methods followed our published procedures (Ye et al., 2014). Briefly, adult male SD rats (220–250 g) were anesthetized with 4 ml/kg (i.p.) of 10% chloral hydrate. Their pancreases were distended with collagenase V (5–6 ml, 1 mg/ml; Sigma, USA; dissolved in cold Hanks solution) by cannulating them *in situ* via the pancreatic duct. Subsequently, the pancreases were removed and digested in 37 °C in a water bath for 25 min in 50-ml tubes containing 5 ml of collagenase V. Then, 20 ml of cold Hanks solution was added to end digestion. After rinsing with cold Hanks solution two times, the islets were handpicked and stored at −80 °C or cultured in RPMI 1640 medium containing 11.1 mM glucose supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin (all from Gibco) in a 37 °C culture chamber containing 5% CO_2 for further experiments.

2.5. Immunofluorescence

Double-labeling immunofluorescence was performed to determine whether 5-HT_4 receptor and 5-HT_3 receptor were co-localized with any of the four main types of islet cells. OCT-embedded sections were 6-μm thickness. Paraffin-embedded sections of 4-μm thickness were torrefied, dewaxed, and then hydrated as the standard protocol. Prior to staining, microwave heat-induced antigen retrieval was required. Then, the sections were washed with PBS containing 0.3% Triton X-100. After blocking in 5% horse serum, they were incubated with a cocktail of two primary antibodies (Table 1) and the corresponding secondary antibodies (Table 2). Then the nuclei were stained with DAPI (4,6-diamidino-2-phenylindole). Images were analyzed using the TCSSP5 laser confocal microscope and LASAF software (Leica, Germany).

2.6. Western blotting

The rat pancreatic tissue and islets were homogenized in cold lysis buffer supplemented with 0.5% Nonidet P-40, 50 mM Tris–HCl pH 7.5, 150 mM NaCl, 2 mM EDTA, 2% SDS, 1 mM dithiothreitol, 1 mM sodium orthovanadate, 0.5% deoxycholic acid (all from Sigma-Aldrich, USA) and a protease inhibitor cocktail (Roche,

Table 1
Primary antibody.

Primary antibody	Code	Company/catalog no	Host species	Dilution
Monoclonal Anti-Insulin antibody produced in mouse	I2018–2 ml	Sigma	Mouse	1:1000
Anti-Glucagon Antibody	MABN238	Millipore	Mouse	1:2500
PPY Goat anti-Human Polyclonal (Internal) Antibody	LS-B5911	LifeSpanBioSciences	Goat	1:400
Somatostatin Antibody (D-20)	sc-7819	Santa Cruz	Goat	1:200
5-HT4 Antibody	NBP1–19627	Novus	Rabbit	1:50/1:500[a]
Anti-5-Hydroxytryptamine Receptor 3	ASR-031	Alomone	Rabbit	1:50
Anti GAPDH antibody	G9545	Sigma	Rabbit	1:10000[a]

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

[a] Dilution for western blotting.

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