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Berberine induces GLP-1 secretion through activation of bitter taste receptor pathways

Yunli Yu[a,*], Gang Hao[b,1], Quanying Zhang[a,*], Wenyan Hua[a], Meng Wang[a], Wenjia Zhou[a], Shunlin Zong[a], Ming Huang[a], Xiaozhou Wen[c]

[a] Department of Clinical Pharmacology, The Second Affiliated Hospital of Soochow University, Suzhou 215004, PR China
[b] Suzhou Institute for Food and Drug Control, 215104, PR China
[c] Jiangsu Province Hospital of Traditional Chinese Medicine, 210029, PR China

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ABSTRACT

Our previous studies revealed that berberine-mediated GLP-1 secretion was a possible mechanism for berberine exerting good effects on hyperglycemia. This study was designed to ascertain whether berberine-induced secretion of GLP-1 was related with activation of bitter taste receptors expressed in gastrointestinal tract. Western blotting results showed that TAS2R38, a subtype of bitter taste receptor, was expressed on human enteroendocrine NCI-H716 cells. GLP-1 secretion induced by berberine from NCI-H716 cells was inhibited by incubation with anti-TAS2R38 antibody. We further performed gene silencing using siRNA to knockdown TAS2R38 from NCI-H716 cells, which showed that siRNA knockdown of the TAS2R38 reduced berberine-mediated GLP-1 secretion. We adopted inhibitors of PLC and TRPM5 known to be involved in bitter taste transduction to investigate the underlying pathways mediated in berberine-induced GLP-1 secretion. It was found that PLC inhibitor U73122 inhibited berberine-induced GLP-1 release in NCI-H716 cells, while TRPM5 blocker quinine failed to attenuate berberine-induced secretion of GLP-1. The present results demonstrated that berberine stimulated GLP-1 secretion via activation of gut-expressed bitter taste receptors in a PLC-dependent manner. Because berberine was found to be a ligand of bitter taste receptor, the results of present study may provide an explanation for some bitter taste substance obtain hypoglycemic effect.

1. Introduction

Bitter (TAS2R) taste receptors are known to detect nutritive and beneficial compounds as well as harmful or toxic substances in gustatory system [1,2]. These receptors are also expressed in gastrointestinal tract [3,4], where they play important roles in nutrient assimilation and endocrine responses [5,6]. The activation of bitter taste receptor has been studied as a potential therapeutic target of the type-2 diabetes mellitus (T2DM) because of its negative influence on glucose homeostatsis [7] and stimulation of GLP-1, an incretin hormone which augments the release of insulin [8]. The correlation between subtypes of TAS2 receptors and GLP-1 secretion is unclear. TAS2R38, a subtype of bitter taste receptor, was reported to be associated with T2DM [7]. Moreover, it was demonstrated to relate with an individual's postprandial response to ingested nutrients [9] and glucose homeostasis [10]. Thus, we speculate that TAS2R38 may be possibly associated with GLP-1 secretion.

Berberine ($[C_{20}H_{18}NO_4]^+$), a major active constituent of Rhizoma coptidis, has been successfully used for diabetes [11,12]. However, the underlying mechanism remains unclear due to the low bioavailability of berberine. Our previous studies found that berberine-induced GLP-1 secretion was a possible mechanism for berberine's hypoglycemic effect [13,14]. The mechanism of regulation of GLP-1 secretion by berberine was not well-elucidated.

Chinese medicines have varied tastes, predominantly sweet, bitter, astringent or pungent. Gut TAS2Rs could be stimulated by a number of compounds and may be important for responses to

Abbreviations: GLP-1, glucagon-like peptide 1; BBR, berberine; PTC, phenylthiocarbamide; PLC, Phospholipase C; TRPM5, transient receptor potential cation channel subfamily M member 5; KRB, Krebs-Ringer bicarbonate buffer; FBS, fetal bovine serum; BSA, bovine serum albumin; HEPES, N-[2-hydroxyethyl piperazine-N]-2-ethanesulfonicacid.

* Corresponding authors at: Department of Clinical Pharmacology, The Second Affiliated Hospital of Soochow University, Suzhou 215004, PR China. Fax: +86 512 67783686.

E-mail addresses: haoyyl0902@163.com (Y. Yu), haogang531@163.com (G. Hao), zhangquanying@163.com (Q. Zhang), huawenyan2010@163.com (W. Hua), enigmatz@163.com (M. Wang), zwjaja@163.com (W. Zhou), zslynsz@126.com (S. Zong), dreamboyhmhm@163.com (M. Huang), 183553076@qq.com (X. Wen).

[1] The two authors contribute equally to this work.

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bitter-tasting stimuli. Berberine is the main component of a number of particularly bitter medicinal plants, such as Coptis Rhizome [15]. Therefore the notion to be discussed in this article is that berberine activated bitter taste receptor and TAS2R38 was involved in berberine-mediated GLP-1 secretion. Consequently, it is expected to obtain an improved understanding of the mechanism regulating GLP-1 secretion by berberine, thereby providing an alternative prospect to the use of berberine in the treatment of diabetes mellitus.

2. Material and methods

2.1. Reagents

Berberine (purity: 98%) was purchased from Nanjing Qingze Pharmaceutical Technology Co., Ltd (Nanjing, China). GLP-1 active ELISA kits were purchased from Millipore (St. Charles, MI, USA). Bovine serum alume (BSA) was obtained from Amresco Co. (Solon, OH, USA). RPMI 1640, DMEM and L-glutamine were purchased from Invitrogen Co. (Carlsbad, CA, USA). Penicillin and streptomycin were bought from Shandong Lukang Pharmaceutical Co., Ltd (Shangdong, China). Matrigel was bought from Becton Dickinson Biosciences (Bedford, MA, USA). Fetal bovine serum (FBS) was bought from PAA Laboratory (Chicago, IL, USA). N-[2-hydroxyethyl piperazine-N]-2-ethanesulfonicacid (HEPES) was obtained from Promega (Madison, WI, USA). Anti-TAS2R38 antibody was bought from Abcam (Cambridge, MA, USA). Phenylthiocarbamide (PTC), U-73122 and quinine were bought from Sigma-Aldrich (St. Louis, MO). Phenylmethylsulfonyl fluoride was provided by Sunshine Biotechnology Co., Ltd (Nanjing, China).

2.2. Cell lines

Human NCI-H716 cells were obtained from the American Type Culture Collection (Manassas, USA). Cell culture was performed according to the method described previously [16]. Briefly, NCI-H716 cells were grown in RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine, 100 IU/ml penicillin and 100 mg/ml streptomycin. Endocrine differentiation was induced by seeding cells in dishes coated with Matrigel, in high-glucose DMEM, 10% FBS, 2 mM L-glutamine, 100 IU/ml penicillin, and 100 mg/ml streptomycin.

2.3. Effect of TAS2R38 on berberine-induced GLP-1 secretion

Human enteroendocrine NCI-H716 cells were maintained in suspension culture and differentiation was performed as described in previous study [13]. Two days before the experiments, 1.5×10^6 cells were seeded in 24-well culture plates coated with Matrigel and containing high glucose DMEM, 10% FBS, 2 mM L-glutamine, 100 IU/ml penicillin and 100 mg/ml streptomycin. On the day of the experiment, medium was replaced by Krebs-Ringer bicarbonate buffer (KRB) buffer (128.8 mmol/l NaCl, 4.8 mmol/l KCl, 1.2 mmol/l KH_2PO_4, 1.2 mmol/l $MgSO_4$, 2.5 mmol/l $CaCl_2$, 5 mmol/l $NaHCO_3$, and 10 mmol/l HEPES, pH 7.4) [17] containing berberine (1 μM, 10 μM, 100 μM and 200 μM), PTC (1 mM, 3 mM and 10 mM) and differently diluted TAS2R38 antibody (1:200 and 1:400 diluted). Following incubation at 37 °C for 2 h, the supernatants were collected with the addition of 50 mg/ml phenylmethylsulfonyl fluoride and stored at −80 °C for analysis. GLP-1 was measured by a GLP-1 active ELISA kit according to the manufacturer's protocol. The GLP-1 content was normalized for the total protein of the cells. At the same time, the cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay [18]. No damage in cells was found for all the tested agents within tested concentrations.

2.4. Western blotting

NCI-H716 cells were harvested and seeded into 75 cm^2 cell culture flasks and treated with berberine (0, 1, 10, 100 μM and 200 μM). After 24 h, cellular proteins were prepared using KeyGen cellular proteins isolation kit according to the manufacturer's instructions. For western blot analysis, an equal amount of protein was separated on an 8% SDS-PAGE and transferred onto a PVDF membrane (Millpore, USA). The membrane was blocked for 1 h at 37 °C with 5% non-fat milk, then incubated at 4 °C over night with primary polyclonal antibody to TAS2R38 (1:800, Abcam, USA) and β-actin (1:200, Boster Biological Technology, China).

After being washed, the membrane was incubated with secondary antibody horseradish peroxidase-conjugated goat anti-mouse IgG (1:8000, Boster Biological Technology, China) for 1 h at 37 °C. The signals were detected by an enhanced chemiluminescence kit (Pierce Chemical, Rockford, IL, USA). The TAS2R38 protein band intensity was normalized to that of β-actin.

2.5. RNA interference assay

NCI-H716 cells were seeded in cell culture flasks. TAS2R38 was transiently knocked down in NCI-H716 cells by TAS2R38 siRNA (OriGene Technologies, Inc.). A non-targeting siRNA was used as a negative control. The transfections were performed for 48 h according to the manufacturer's instructions for the Fugene HD Transfection Reagent. Then the cells were differentiated by Matrigel and incubated with different concentration of berberine (0, 1, 10, 100 μM) for 2 h. The supernatants were collected with the addition of 50 mg/ml phenylmethylsulfonyl fluoride and stored at −80 °C for GLP-1 analysis.

2.6. Bitter taste receptor pathway in berberine-induced GLP-1 secretion

Signaling pathway participated in berberine-induced GLP-1 secretion via bitter taste receptor was investigated by using U-73122, an inhibitor of G-protein-mediated activation of PLC, and quinine, a blocker of transient receptor potential cation channel subfamily M member 5 (TRPM5) [19]. Two-days before experiments, cells were seeded into 24-well culture plates precoated with Matrigel as described above. On the day of the experiments, differentiated cells were starved in FBS-free DMEM containing 0.2% BSA for 1.5 h, then incubated with KRB containing 0.2% BSA and 100 μM berberine co-administrated with U-73122 (0.4 μM, 2 μM and 10 μM) or quinine (25 μM, 50 μM and 100 μM) for 2 h. GLP-1 in supernatant was measured as described above. Effects of U-73122 and quinine on GLP-1 secretion were also investigated.

2.7. Data analysis

Results were presented as the mean ± S.E.M.. One-way analysis of variance (ANOVA) was used to evaluate the statistical significance of the differences among groups. If analysis was significant, the differences between groups were estimated using Student–Newman–Keuls multiple comparison post hoc test. The differences were considered significant when $p < 0.05$.

3. Results

3.1. Effect of TAS2R38 on berberine-induced GLP-1 secretion

We have demonstrated that berberine stimulated GLP-1 secretion in STZ-induced diabetic rats and NCI-H716 cells in previous studies [13,14]. In present study, we further investigated whether berberine-induced GLP-1 secretion was related to bitter