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Effects of β_3-adrenoceptor activation on expression of pancreatic adrenoceptors and angiotensin II receptors in ApoE$^{-/-}$ mice

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ABSTRACT

Hyperlipidemia can be harmful to the pancreas and β_3-adrenoceptor agonist can improve lipid metabolism disorder. We aimed to study the effects of β_3-adrenoceptor activation on glucose, insulin and the expression of pancreatic adrenoceptors and angiotensin II receptors. Ten C57BL/6J mice at the age of 10 weeks served as normal control, and forty age-matched apolipoprotein E knockout (ApoE$^{-/-}$) mice were randomly divided into hyperlipidaemia model group, low-dose and high-dose β_3-adrenoceptor agonist group and β_3-adrenoceptor antagonist group. After 26 weeks of high-fat diet, treatments were given for 12 weeks. Serum glucose and insulin levels in 48 weeks old mice were measured using an automatic biochemical detector. Quantitative rt-PCR and Western blot were used to analyze the mRNA and protein expression of α_{1A}-, α_{2A}-, β_2-, β_3-adrenoceptors and angiotensin II type 1 and type 2 receptors in pancreas. We found that β_3-adrenoceptor agonist could decrease serum glucose and insulin levels in aged ApoE$^{-/-}$ mice ($P<0.01$) and down-regulate the expression of α_{1A}-adrenoceptor and angiotensin II type 1 receptor which were significantly increased in model mice ($P<0.05$, $P<0.01$). Compared with the model mice, α_{2A}-, β_2-, β_3-adrenoceptor and angiotensin II type 2 receptor expression were up-regulated in β_3-adrenoceptor agonist treat mice ($P<0.05$, $P<0.01$). These results suggest that chronic β_3-adrenoceptor activation regulated the expression of adrenoceptors and angiontensin II receptors towards contrary direction, which indicates that there are interactions between β_3-adrenoceptor and subtypes of adrenoceptor and angiotensin II receptor, and these interactions may play a protective role in pancreas and improve glucose metabolism disorders.

1. Introduction

Pancreatic insulin secretion is modulated by sympathetic nervous system and rennin-angiotensin system (Chiu et al., 2012). Studies have demonstrated that catecholamine, which is released after sympathetic nervous system activation, either inhibits or stimulates insulin and glucagon secretion through its interaction with adrenoceptors expressed at pancreatic α and β cell surface (Rodriguez-Diaz et al., 2012). Studies also reported that, in condition of rennin-angiotensin system activation, angiotensin II acts on angiotensin II type 1 and type 2 receptors located on pancreatic β cell and vascular smooth muscle, to regulate pancreatic blood flow and hormone synthesis and secretion (Leung, 2007a, 2007b). Our previous research found that β_3-adrenoceptor activation played a role in delaying the formation of atherosclerotic plaque by improving lipid and glucose metabolism disorders (Wang et al., 2013). In the present study, we used ApoE$^{-/-}$ mice fed a high fat diet as a model of hyperlipidaemia, and observed the effects of β_3-adrenoceptor agonist and antagonist on glucose metabolism, and the expression of adrenoceptor and angiotensin II receptor subtypes in pancreas, to establish whether excited β_3-adrenoceptor could improve glucose metabolism by regulating expression of adrenoceptors and angiotensin II receptors.

2. Materials and methods

2.1. Ethics statement

All animals were housed and handled according to the standards for laboratory animals established by the People's Republic of China (GB14925-2001), and all animal work was approved by the Committee on the Ethics of Animal Experiments of Capital Medical University (Permit number: SCXK2006-0008). To minimize animal suffering, the number of animals used was based on

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the minimum required for statistically valid results. All experiments were performed according to institutional guidelines. All work was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

2.2. Animals

Ten-week-old male homozygous ApoE$^{-/-}$ mice and wild type C57BL/6J mice were provided by Beijing Vital River Laboratory Animal Technology Co Ltd. (Certificate no: SCXK 2006-0008) and the weight was (22.85 ± 0.45) g. After a one week adaptation period, all mice were barrier housed in an air-conditioned room with a 12 h light-dark cycle under specific pathogen free conditions, and maintained in static isolator cages. ApoE$^{-/-}$ mice were given a high-fat diet containing 0.15% (*w*/*w*) cholesterol and 21% (*w*/*w*) fat from 11 weeks to 48 weeks of age (Beattie et al., 2013). Autoclaved food and water were provided ad libitum.

At 36 weeks of age, ApoE$^{-/-}$ mice (n=40) were randomized into the following 4 treatment groups (n=10 per group): (i) hyperlipidaemia model, (ii) low-dose β_3-adrenoceptor agonist, (iii) high-dose β_3-adrenoceptor agonist and (iv) β_3-adrenoceptor antagonist groups. Mice in the hyperlipidaemia model group received vehicle (0.9% saline, by intraperitoneal injection, twice a week). Low-dose β_3-adrenoceptor agonist mice received the β_3-adrenoceptor agonist BRL37344 at a dose of 1.65 μg/kg body weight by intraperitoneal injection, twice a week. High-dose β_3-adrenoceptor agonist mice received the β_3-adrenoceptor agonist BRL37344 at a dose of 3.30 μg/kg body weight by intraperitoneal injection, twice a week. β_3-adrenoceptor antagonist mice received the β_3-adrenoceptor antagonist SR59230A at a dose of 50 μg/kg body weight by intraperitoneal injection, twice a week. All mice were treated for 12 weeks.

2.3. Reagents and drugs

The β_3-adrenoceptor agonist BRL37344 and the β_3-adrenoceptor antagonist SR59230A were purchased from Sigma Chemical Co. (St Louis, MO, USA). Trizol reagent was obtained from Invitrogen Co. (Carlsbad, CA, USA). Reverse transcription-polymerase chain reaction (RT-PCR) kit A3500 was produced by Promega Co. (Madison, WI). Random primers were obtained from Invitrogen Co. (Shanghai, China). SYBR Green Real-Time PCR Master Mix kit was purchased from Katara Bio. (Shiga, Japan). Antibodies against α_{1A}-, α_{2A}-, β_2-, β_3-adrenoceptors, angiotensin II type 1 receptor and β-actin were produced by Abcam Co. (California, USA). Secondary antibody was obtained from Sigma Chemical Co. (St Louis, MO. USA).

2.4. Measurement of glucose and insulin

At 48 weeks of age, all animals were fasted for 6 hs and anesthetized using 1% sodium pentobarbital by intraperitoneal injection, and then blood samples were collected by retro-orbital sinus puncture. Glucose (Glu) and Insulin (INS) were determined using a Beckman CX7 (Beckman Coulter, Fullerton, CA, USA), and ISI was calculated according to ISI=Ln [1/(INS × Glu)].

2.5. Quantitative real-time PCR

Pancreatic tissues were immediately removed, frozen in liquid nitrogen, and stored at −80 °C until extraction of RNA. Total RNA was extracted with TRIZOL Reagent, which was then reverse transcribed into double-stranded cDNA using RT-PCR kit. Templates were subjected in triplicate to quantitative real-time PCR using a thermocycler. All reactions were performed utilizing the following conditions: 40 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. Sequence-specific PCR primers were quoted from literatures based on the known mouse cDNA sequenc. Please see Table 1 for details of gene-specific primers. The housekeeping gene β-actin was used as control. Results were expressed as fold differences for each gene after normalization against β-actin using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

2.6. Western blot

Protein expression of α_{1A}-, α_{2A}-, β_2-, β_3-adrenoceptors and angiotensin II type 1 receptor in pancreas was determined by western blot analysis. The pancreas tissues were homogenized in RIPA buffer (50 mM Tris/HCl pH 7.4, 150 mM NaCl, 2 mM EDTA, 1% NP-40, 0.1% SDS) and total protein was extracted from the homogenates. The protein concentration of the pancreas was quantified using the Bradford protein assay. The samples were boiled for 5 min followed by loading on a 10% sodium dodecylsulfate polyacrylamide gel (SDS-PAGE) and transferred to polyvinylidene difluoride membranes. After transfer, the membrane was blocked for 2 h at room temperature with 5% skim milk in Tris-buffered saline-Tween (TBST; 20 mM Tris, 500 mM NaCl, pH 7.5, 0.1% Tween 20). The membranes were then incubated with primary antibodies against α_{1A}-adrenoceptor (rabbit polyclonal, 1:250 dilution), α_{2A}-adrenoceptor (rabbit polyclonal, 1:500 dilution), β_2-adrenoceptor (rabbit polyclonal, 1:1000 dilution), β_3-adrenoceptor (rabbit polyclonal, 1:1000 dilution), AT_1R (rabbit polyclonal, 1:1000 dilution) in 5% skim milk in TBST for 2 h at room temperature. The membranes were washed three times with TBST at room temperature and then incubated with a secondary antibody (goat anti-rabbit IgG-HRP, 1:5000) conjugated to horseradish peroxidase in 2.5% skim milk in TBST for 1 h at room temperature. The membranes were washed three times and the immunoreactive proteins were detected by enhanced chemiluminescence (ECL). The results of western blot analysis were quantified by measuring the relative intensity compared with the control using Quantity one 4.6 (Bio-Rad, California, USA).

Table 1
Sequences of the primers used in this study. The sizes of the PCR products are given in base-pairs (bp).

Genes	NCBI number	Forward primers (5′–3′)	Reverse primers (5′–3′)	Sizes (bp)
α_{1A}-R	NM_013461	GCAGCGGAGTAAGCAGTG	AGCCAGCAGAGGACGAAG	124
α_{2A}-R	NM_007417	CAAGATCAACGACCAGAAGT	GTGCGACGCTTGGCGATCT	120
β_2-AR	NM_007420	GGACAACCTCATCCCTAA	AGAGTAGCCGTTCCCATA	169
β_3-AR	NM_013462	CAGTCCCTGCCTATGTTTG	TTCCTGGATTCCTGCTCT	165
AT_1R	NM_177322	TGCTCAGAAACGGGGACAC	CTCTGAAGTAGCCCACCTGTTA	177
AT_2R	NM_007429	GATGGAGGGAGCTCGGAACT	AATTTGGAGTTGCTGCAGTTCAA	143
β-Actin	NM_007393	TCCATCATGAAGTGTGACGT	GAGCAATGATCTTGATCTTCAT	112