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A role for trans-caryophyllene in the moderation of insulin secretion

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ABSTRACT

Glucose-stimulated insulin secretion (GSIS) is essential for the control of metabolic fuel homeostasis and its impairment is a key element in the failure of β-cells in type 2 diabetes. Trans-caryophyllene (TC), an important constituent of the essential oil of several species of plants, has been reported to activate the type 2 cannabinoid receptor (CB2R). The effects of TC on GSIS are still unknown. Our results demonstrate that administration of TC in MIN6 cells promotes GSIS in a dose dependent manner. However, inhibition of CB2R by a specific inhibitor or specific RNA interference abolished the effects of TC on GSIS, which suggests that the effects of TC on GSIS are dependent on activation of CB2R. Further study demonstrated that treatment with TC leads to the activation of small G protein Arf6 as well as Rac1 and Cdc42. Importantly, Arf6 silencing abolished the effects of TC on GSIS, which suggests that Arf6 participates in mediating the effects of TC on GSIS. We conclude from these data that TC has a novel role in regulating GSIS in pancreatic β-cells.

1. Introduction

Type 2 diabetes has been considered one of the most prevalent chronic diseases in the world. Insulin resistance is essential for the pathogenesis of this disease. Glucose-stimulated insulin secretion (GSIS) plays a critical role in the control of metabolic fuel homeostasis and its impairment is a key element involved in β-cell failure in type 2 diabetes [1]. Coordinated signaling events participate in regulating trafficking of insulin-laden secretory granules to plasma membranes for docking and fusion. Multiple lines of evidence have demonstrated that small G-proteins (e.g., Arf6, Cdc42, and Rac1) play a critical role in controlling such signaling events [2]. Arf6 is an important member of the Arf family which has been well-documented as a protein that plays positive modulatory roles in multiple cell types. Importantly, Arf6 has been verified to be expressed in islets and insulin-secreting RINm5f cells [3]. Up to now, a growing body of evidence has demonstrated that Arf6-regulated insulin secretion is mediated by glucose, GTPγS, and membrane depolarization. Importantly, Arf6 is considered to be the upstream signaling factor of Rac1 and CDC42 involved in the process of GSIS regulation [4]. However, the precise molecular and cellular mechanisms underlying GSIS still need to be elucidated.

Trans-caryophyllene (TC), an important constituent of the essential oils derived from several species of plants, has been reported to possess many pharmacological properties. Previous studies have demonstrated its antimicrobial [5] and analgesic effects [6]. In addition, it also possesses an anti-inflammatory effect [7]. Importantly, TC has been shown to be a specific agonist of the type 2 cannabinoid receptor (CB2R). Through activation of CB2R, TC is able to promote fatty acid oxidation in mouse myoblast cell line C2C12 [8] and reduce cerebral ischemic injury in rodents [9]. It is reportedly accepted that CB2R are present in pancreatic islets. However, the effects of TC in GSIS are still unknown. In this study, we investigated the effects of TC on GSIS and explored its underlying mechanisms.

2. Materials and methods

2.1. Cell culture

MIN6 β-cells (passage 22–30) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 2 mM glutamine, 10% fetal bovine serum (FBS), 100 units/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a 5% CO_2 humidified atmosphere.

2.2. Determination of insulin secretion

In order to determine insulin secretion, MIN6 cells were preincubated in Krebs–Ringer HEPES buffer (KRHB) containing 0.1% BSA

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and 2 mmol/l glucose for 1 h at 37 °C. After that, cells were incubated for 1 h at 37 °C with KRHB containing either 2 or 20 mmol/l glucose. An aliquot of the buffer was taken and insulin release was measured by RIA.

2.3. Cellular transfection and knockdown

CB1R, CB2R, and Arf6 protein expression was performed using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's instructions [10]. Briefly, 24 h before transfection, cells (5×10^5) were plated in 0.5 ml cultural medium and allowed to grow overnight at 37 °C and 5% CO_2. The small interfering RNA (siRNA) transfection complex, formed by combining transfection reagent and 50 nM CB1R, CB2R, or Arf6 siRNA in serum-free Opti-MEM medium, was added to cells dropwise. The successful knockdown of CB2R or Arf6 was verified using Western blot analysis.

2.4. Quantitation of Arf6-GTP, Rac1-GTP and Cdc42-GTP in MIN6 cells

Active Arf6, Rac1, and Cdc42 were quantitated by pull-down assay. Recombinant protein PAK1-PBD-GST, which binds specifically to the GTP-bound forms of Rac and Cdc42, was prepared as described previously [11]. After having been washed with ice-cold PBS, cells were lysed with lysis buffer and lysate was then clarified by centrifugation at 16,000*g* at 4 °C for 15 min. For active Arf6, 400 μg protein was incubated with 100 μl of glutathione resin and 100 μg of GST-GGA3-PBD beads at 4 °C for 1 h with gentle rocking. For active Rac1 and Cdc42, 400 μg protein was incubated with PAK1-PBD-GST. The resulting proteins were separated by SDS–PAGE and activated Arf6, Rac1, and Cdc42 were identified using Western blot analysis.

2.5. Western blot analysis

Samples were subjected to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE). Proteins were transferred to polyvinylidene fluoride microporous membranes (Millipore, USA) as described previously [12]. Membranes were then blocked with 5% non-fat milk in TBST (150 mM NaCl, 25 mM Tris–HCl (pH 8.0), and 0.1% Tween 20) for 1 h at room temperature followed by incubation with Arf6, Rac1, and Cdc42 primary antibodies for 2 h at room temperature. After being washed 3 times, membranes were incubated with either HRP-conjugated secondary anti-mouse antibody or HRP-conjugated secondary anti-rabbit antibody for 1 h at room temperature. Blots were developed using the ECL technique (Santa CruzBiotechnology, USA) [13].

2.6. Statistical analysis

Data are represented as mean ± S.E.M. One way analysis of variance (ANOVA) was used to determine areas of statistical significance between different groups. A p-value < 0.05 was considered as the minimum level of statistical significance.

3. Results

One of the objectives of this study was to investigate the contributory roles of TC in GSIS. To address this, MIN6 cells were treated with TC at concentrations ranging from 0.1 to 1 μM. Insulin secretion was quantitated in these cells in the presence of 2.0 or 20 mM glucose. The molecular structure of TC is shown in Fig. 1A. Data in Fig. 1B shows that TC promoted GSIS in a dose dependent manner.

In previous studies, TC has been shown to be an important agonist of CB2R [9]. We next investigated whether the effects of TC on GSIS are dependent on CB2R. MIN6 cells were treated with either 1 μM CB2R inhibitor SR144528 or 1 μM CB1 inhibitor AM251. Results indicate that 1 μM CB2R inhibitor SR144528 abolished the effects of TC on GSIS. However, 1 μM CB1R inhibitor AM251 did not produce this effect (Fig. 2A). In order to further confirm this finding, expression of CB1R and CB2R was knocked down by being transiently transfected with CB1R and CB2R siRNA, respectively. Results indicating that the two proteins were successfully silenced are shown in Fig. 2B. Consistently, knockdown of CB2R but not CB1R abolished the effects of TC on GSIS (Fig. 2C). These findings suggest that the effects of TC on GSIS are dependent on CB2R.

Activation of CB2R has been reported to stimulate activation of small G proteins, including RhoA, Rac1, Rac2, and Cdc42 [14], activation of which is involved in GSIS. Recent studies identified activation of Arf6 as an upstream signaling event that leads to activation of Cdc42 and Rac1 [4]. Therefore, we investigated the possibility of TC playing a role in the activation of small G-proteins, specifically Arf6, in MIN6 cells. To address this, glucose-induced Arf6 activation was quantitated in cells that were treated with TC. The results listed in Fig. 3 indicate that administration of TC markedly promoted the ability of glucose to induce Arf6 activation, which was abolished by pretreatment with CB2R silence.

In order to ensure that the effects of TC on GSIS are indeed mediated by Arf6, cells were transfected with Arf6 siRNA. Successful knockdown of Arf6 is shown in Fig. 4A. Importantly, GSIS was significantly inhibited in Arf6 knockdown cells (Fig. 4B). In addition, TC treatment also promoted Cdc42 activity as well as Rac1 activity, which was abolished by knockdown of Arf6 (Fig. 4C and D). These data suggest that the effects of TC on GSIS are mediated by Arf6.

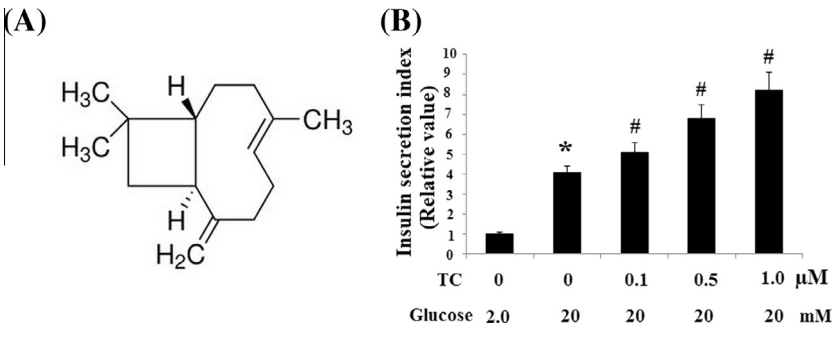

Fig. 1. Impact of Trans-caryophyllene (TC) on insulin secretion in MIN6 cells. (A) Molecular structure of Trans-caryophyllene (TC); (B) MIN6 cells were pretreated with TC at a variety of concentrations for 24 h. Cells were challenged with either 2.0 or 20 mM glucose. Insulin secretion determination displayed that TC promoted glucose-stimulated insulin secretion (GSIS) in a dose-dependent manner (*p < 0.01 vs. 2 mM Glucose treated group; #p < 0.01 vs. 20 mM Glucose only treated group).