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Vitamin D_3 supplementation increases insulin level by regulating altered IP3 and AMPA receptor expression in the pancreatic islets of streptozotocin-induced diabetic rat

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Abstract

Pancreatic islets, particularly insulin-secreting β cells, share common characteristics with neurons. Glutamate is one of the major excitatory neurotransmitter in the brain and pancreas, and its action is mediated through glutamate receptors. In the present work, we analysed the role of vitamin D_3 in the modulation of AMPA receptor subunit and their functional role in insulin release. Radio receptor binding study in diabetic rats showed a significant increase in AMPA receptor density. Insulin AMPA colabelling study showed an altered AMPA GluR2 and GluR4 subunit expression in the pancreatic beta cells. We also found lowered IP3 content and decreased IP3 receptor in pancreas of diabetic rats. The alterations in AMPA and IP3 receptor resulted in reduced cytosolic calcium level concentration, which further blocks Ca^{2+}-mediated insulin release. Vitamin D_3 supplementation restored the alteration in vitamin D receptor expression, AMPA receptor density and AMPA and IP3 receptor expression in the pancreatic islets that helps to restore the calcium-mediated insulin secretion. Our study reveals the antidiabetic property of vitamin D_3 that is suggested to have therapeutic role through regulating glutamatergic function in diabetic rats.

Keywords: AMPA receptor; IP3; Vitamin D_3; Pancreatic islets; Diabetes; Insulin

1. Introduction

Diabetes mellitus is a clinically and genetically heterogeneous group of metabolic disorders manifested by abnormally high levels of glucose in the blood. Hyperglycemia is a result of defective insulin secretion or resistance to the action of insulin in liver and muscle or a combination of these. The insulin is produced from pancreas, a mixed gland, with a large exocrine and a much smaller endocrine gland. The endocrine cells are arranged into small islands of cells called the islets of Langerhans. The interactive functions of both the exocrine and the endocrine parts are particularly important for the normal functioning of the body.

Insulin secretion is controlled by a variety of neurotransmitters and hormones [1–3]. The insulin-secreting pancreatic β cells share many features with other neuroendocrine cells. A growing body of evidence suggests that glutamate, the major excitatory neurotransmitter in the central nervous system, acts as a signalling molecule in peripheral tissues [4–6]. In the cells of the endocrine pancreas, glutamate is stored in glucagon or insulin-containing granules [7,8] and, once secreted, acts extracellularly to regulate hormone secretion [9,10]. Various reports suggest that glutamate stimulates insulin release in rat pancreas, by acting on excitatory AMPA receptor subtypes [11,12]. Weaver *et al.* reported that the AMPA receptors were located in the α, β and pancreatic polypeptide-producing cells but were generally absent from the δ cells [13]. AMPA receptor is composed of different combinations of GluR1, GluR2, GluR3 and GluR4 subunits, and these subunits have a modular organisation [14,15].

Vitamin D_3 is well known to function in calcium homeostasis [16]. Role for vitamin D in endocrine pancreas function was first suggested by Boquist *et al.* [17]. Further studies revealed that vitamin D deficiency leads to lowered insulin secretion and impaired glucose tolerance [18,19]. Vitamin D_3 is either synthesised in the epidermis from 7-dehydrocholesterol by absorption of ultraviolet light or obtained from diet in a limited number of foods such as eggs, fish oils and fortified milk. An increased prevalence of diabetes has been described in vitamin-deficient individuals [20]. Insulin synthesis and secretion has been shown to be impaired in β cells of animals deficient in vitamin D [21–23].

Many studies revealed that vitamin D_3 has antidiabetic effect [24,25]. Previous study from our laboratory showed that vitamin D_3 can modulate the altered cholinergic neurotransmitter receptor function in the pancreas [26]. The present study was designed to analyse AMPA and IP3 receptor alterations in the pancreas of diabetic rats and to examine the therapeutic role of vitamin D_3 in modulating insulin secretion mediated by the AMPA and IP3 receptor.

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2. Materials and methods

Biochemicals used in the present study were purchased from Sigma Chemical Co, St. Louis, MO, USA. All other reagents of analytical grade were purchased locally. [^{3}H]AMPA (specific activity, 45.8 Ci/mmol) and AMPA were purchased from American Radiolabeled Chemicals. Rat primary antibodies for AMPA GluR2 (BD Pharmingen), insulin (Abcam), IP3 receptor 3 (IP3R3) (BD Pharmingen), AMPA GluR4 and vitamin D receptor (VDR) (Pierce Antibodies) and secondary antibodies of Alexa Fluor 488 (Life Technologies) and CY5 (Chemicon) were used for immunohistochemistry studies using confocal microscope. Vitamin D_3 (Cholecalciferol) and Tri-reagent kit were purchased from Sigma Chemicals USA.

Male adult Wistar rats of 200–250 g body weight were used for all experiments. They were housed in separate cages under 12 h light and 12 h dark periods. Rats had free access to standard food and water *ad libitum*. All animal care and procedures were done in accordance with the Institutional and National Institute of Health guidelines. Diabetes was induced in rats by single intrafemoral injection of streptozotocin (STZ) freshly dissolved in 0.1 M citrate buffer, pH 4.5, under anaesthesia [27]. STZ was given at a dose of 55 mg/kg body weight [28,29]. Animals were divided into the following groups: (i) control (C), (ii) diabetic (D), (iii) insulin-treated diabetic (D+I) and (iv) diabetic rats treated with vitamin D_3 (D+V). The insulin-treated diabetic group received subcutaneous injections (1 IU/kg body weight) of Lente and Plain insulin (Boots India) daily during the entire period of the experiment. The last injection was given 24 h before sacrificing the rats. Groups treated with vitamin D_3 received orally 12 μg/kg vitamin D_3 dissolved in 0.3 ml of coconut oil [30]. Rats were sacrificed on 16th day by decapitation. The pancreas was dissected out quickly over ice and was stored at −80°C until assayed.

2.1. Estimation of blood glucose

Blood glucose was estimated by spectrophotometric method using glucose oxidase–peroxidase reaction. Blood samples were collected from the tail vein at 0 h (before the start of the experiment) and 3rd, 8th, 12th and 16th day, and the glucose levels were estimated subsequently. Along with this, blood samples were collected 3 h after the administration of a morning dose. The results were expressed in terms of milligramme per deciliter of blood.

2.2. Estimation of circulating insulin level

Circulating insulin level was measured using radioimmunoassay kit for insulin purchased from Baba Atomic Research Centre, Mumbai, India.

2.3. AMPA receptor binding studies in the pancreas

AMPA receptor assay was done using the modified protocol of Mutel *et al.* [31]. We homogenised 200–250 mg of pancreas tissue in 5–6.25 ml (25 volumes) of homogenisation buffer with a polytron homogeniser. The homogenate was centrifuged at 1000*g* for 10 min and the supernatant was centrifuged at 40,000*g* for 1 h. The pellet was rehomogenised in 50 volumes of 50 mM Tris–HCl, pH 7.1, containing 0.04% Triton X-100. The homogenate was incubated for 30 min at 37°C, then washed three times with 50 mM Tris–HCl (pH 7.1) binding buffer and centrifuged at 40,000*g*. The final pellet was homogenised in 50 volumes of binding buffer (original wet weight) and used as such in the assay. The final concentration of membrane in the assay was 250 μg per assay medium. The incubation was performed in the presence of 0.5–10 nM [^{3}H]AMPA. Nonspecific binding was determined in the presence of 1 mM AMPA. After 1 h of incubation at 4°C, the suspension was filtered (Whatman GF/C) and washed five times with 3 ml of cold washing buffer. Bound radioactivity was counted with cocktail-T in a Perkin Elmer Tri-Carb 2810 TR liquid scintillation analyser.

2.4. Analysis of the receptor-binding data

The receptor binding parameters were determined using Scatchard analysis [32]. The specific binding was determined by subtracting nonspecific binding from the total. The binding parameters, maximal binding (B_{max}) and equilibrium dissociation constant (K_d), were derived by linear regression analysis by plotting the specific binding of the radioligand on the *x*-axis and bound/free on the *y*-axis using Sigma plot software (version 11.0, Systat Software Inc, California).

2.5. Protein determination

The amount of protein was measured by the method of Lowry *et al.* [33].

2.6. Calcium content in the pancreatic islets of control and experimental rats

Pancreatic islets were isolated from experimental rats by standard collagenase digestion procedures [34] (Howell and Taylor, 1968). The islets were homogenised in a polytron homogeniser. Homogenate was centrifuged at 600*g* for 3 min and removed the pellet and cytosolic fraction was taken for calcium assay using calcium colorimetric assay kit (Sigma-Aldrich)

2.7. IP3 content in the pancreas

The pancreas of the experimental rats were homogenised in a polytron homogeniser with 20 volumes of cold 50 mm Tris–HCl buffer containing 1 mM EDTA. The homogenate was then centrifuged at 30,000*g* for 30 min and the supernatant was transferred to fresh tubes for IP3 assays using [^{3}H]IP3 Biotrak assay system kits purchased from GE Healthcare, UK. The unknown concentrations were determined from the standard curve using appropriate dilutions and calculated for picomoles per milligramme of protein.

2.8. Vitamin D and IP3 receptor expression studies in the pancreas of control and experimental rats using confocal microscope

Control and experimental rats were deeply anesthetised. The rat was transcardially perfused with PBS, pH 7.4, followed by 4% paraformaldehyde in PBS (pH 7.4). After perfusion, the pancreas was dissected and immersion fixed in 4% paraformaldehyde for 1 h and then equilibrated with 30% sucrose solution in 0.1 M PBS. We cut 10-μm sections using cryostat (Leica, CM1510 S). To block unspecific binding, the sections were incubated for 1 h at room temperature with blocking solution (5% bovine serum albumin in PBST). Pancreatic sections were incubated overnight at 4°C with rat primary antibody for IP3 receptor (diluted in PBST at 1:500 dilutions) and VDR (diluted in PBST at 1:500 dilution) individually. After incubation and PBST wash, immunodetection was done using proper secondary antibody (for IP3 receptor CY5 and VDR Alexa Flour 594) in blocking solution at 1:1000 dilutions.

2.9. Insulin AMPA GluR4 and insulin AMPA GluR2 receptor colabelling studies in the pancreas of control and experimental rats using confocal microscope

A total of 10-μm pancreatic sections were cut using cryostat (Leica, CM1510 S). To block nonspecific binding, the sections were incubated for 1 h at room temperature with blocking solution. For AMPA receptor subunit–insulin colabelling studies, the pancreatic sections were incubated overnight at 4°C with primary antibody for AMPA GluR4 receptor (diluted in PBST at 1:500 dilution) and AMPA GluR2 receptor (diluted in blocking solution at 1:500 dilution) subunits individually. After incubation, the sections were rinsed with PBST and proper secondary antibody (CY5 for AMPA GluR2 and Alexa Flour 594 for AMPA GluR4) diluted in blocking solution at 1:1000 dilution was added and kept for 2 h at room temperature. The sections were then washed with PBST and incubated overnight with rat primary antibody for insulin (diluted in blocking solution at 1:500 dilutions). Proper secondary antibody Alexa Fluor 488 (diluted in PBST at 1:1000 dilutions) was added after overnight incubation and PBST wash followed by incubation for 2 h at room temperature. Pancreatic sections were thoroughly washed, mounted, observed and photographed using confocal imaging system (Leica SP 5). Images (1024×1024 pixels) were obtained with 20× and 40× lenses. Expression was analysed using pixel intensity method [35].

2.10. Statistics

Statistical evaluations were done with analysis of variance (ANOVA), using GraphPad Instat (version 2.04a, San Diego, USA). Student Newman–Keuls test was used to compare different groups after ANOVA.

3. Results

3.1. Blood glucose level in control and experimental groups

Blood glucose level of all groups before STZ administration was within the normal range. STZ administration leads to a significant increase ($P<.001$) in blood glucose level. Insulin and vitamin D_3 treatment significantly reversed ($P<.001$) the high blood glucose level when compared to diabetic group (Fig. 1).

3.2. Circulating insulin level

There was a significant decrease in the serum insulin level of the diabetic group when compared to control ($P<.001$). Insulin and Vitamin D_3 treatment for 14 days significantly increased ($P<.001$) the serum insulin level when compared to diabetic group (Fig. 2).

3.3. AMPA receptor binding studies in the pancreas

Scatchard analysis of AMPA receptor using [^{3}H]AMPA binding against AMPA in the pancreas of diabetic group showed a significant increase in B_{max} ($P<.01$) compared to control. This result showed increased AMPA receptor density in the pancreas of diabetic compared