Original Article

Inhibition of cholinergic potentiation of insulin secretion from pancreatic islets by chronic elevation of glucose and fatty acids: Protection by casein kinase 2 inhibitor

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ABSTRACT

Objectives: Chronic hyperlipidemia and hyperglycemia are characteristic features of type 2 diabetes (T2DM) that are thought to cause or contribute to β-cell dysfunction by "glucolipotoxicity." Previously we have shown that acute treatment of pancreatic islets with fatty acids (FA) decreases acetylcholine-potentiated insulin secretion. This acetylcholine response is mediated by M3 muscarinic receptors, which play a key role in regulating β-cell function. Here we examine whether chronic FA exposure also inhibits acetylcholine-potentiated insulin secretion using mouse and human islets.

Methods: Islets were cultured for 3 or 4 days at different glucose concentration with 0.5 mM palmitic acid (PA) or a 2:1 mixture of PA and oleic acid (OA) at 1% albumin (PA/BSA molar ratio 3.3). Afterwards, the response to glucose and acetylcholine were studied in perifusion experiments.

Results: FA-induced impairment of insulin secretion and Ca^{2+} signaling depended strongly on the glucose concentrations of the culture medium. PA and OA in combination reduced acetylcholine potentiation of insulin secretion more than PA alone, both in mouse and human islets, with no evidence of a protective role of OA. In contrast, lipotoxicity was not observed with islets cultured for 3 days in medium containing less than 1 mM glucose and a mixture of glutamine and leucine (7 mM each). High glucose and FAs reduced endoplasmic reticulum (ER) Ca^{2+} storage capacity; however, preserving ER Ca^{2+} by blocking the IP3 receptor with xestospongin C did not protect islets from glucolipotoxic effects on insulin secretion. In contrast, an inhibitor of casein kinase 2 (CK2) protected the glucose dependent acetylcholine potentiation of insulin secretion in mouse and human islets against glucolipotoxicity.

Conclusions: These results show that chronic FA treatment decreases acetylcholine potentiation of insulin secretion and that this effect is strictly glucose dependent and might involve CK2 phosphorylation of β-cell M3 muscarinic receptors.

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Keywords Pancreatic islets; Fatty acids; Glucolipotoxicity; Acetylcholine; Insulin secretion

1. INTRODUCTION

Neuro-endocrine regulation of the insulin producing pancreatic β-cells is considered a key aspect of the physiology, pathology and pharmacology of glucose homeostasis [14,15,21,36,41]. Numerous neural and hormonal factors are involved in this regulation including the neuro-transmitters acetylcholine and nor-epinephrine and the enteric hormones GLP1 (glucagon like peptide 1) and GIP (glucose dependent insulinotropic peptide). Evidence is mounting that defects in the neuro-endocrine regulation of β-cells participate in the molecular pathogenesis of T2DM. For example, GLP1 mediated potentiation of insulin release physiologically associated with food intake is impaired in T2DM [55]. This defect is, at least in part, the result of reduced expression of β-cells GLP1 receptors, which may limit the efficacy of the widely used antidiabetic drugs acting via GLP-1 receptor activation [15,44]. GLP1 receptor loss may be triggered by glucolipotoxicity [50], a process that is known to play a key role in the molecular pathogenesis of T2DM. The concept of "glucolipotoxicity" implies that repeated or continued exposure to high glucose and lipids (specifically to elevated free fatty acids (FAs)) impacting at the same time cause β-cell damage and dysfunction [38,42,43]. The literature data on acetylcholine mediated potentiation of insulin release in obesity and T2DM are very limited [5–7]. Previously, we have shown that FAs interfere acutely with acetylcholine potentiation of glucose stimulated insulin release in studies with isolated mouse islets [13]. It was suggested that this effect of FAs is due to acute emptying of the ER calcium stores,

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because it was prevented by inhibition of IP3 receptors [13]. To our knowledge, the chronic effect of glucolipotoxicity on acetylcholine-potentiated insulin secretion in pancreatic islets has not been studied yet.

Although cholinergic regulation of insulin release has been known for many years, the mechanisms by which acetylcholine stimulates insulin secretion are still debated. In their classical paper [2], Ahren and colleagues demonstrated that the non-specific muscarinic receptor agonist carbachol, when administered intravenously, not only potentiated glucose-stimulated insulin secretion in mice fed with either a control or a high-fat diet, but also normalized glucose-stimulated insulin secretion and glucose tolerance in mice subjected to a high-fat diet. This study led to the proposal for the development of islet-specific muscarinic agonists, with lesser general muscarinic activity, to improve insulin secretion in T2DM [2]. Since then novel muscarinic receptor downstream signaling pathways were discovered which might represent promising new targets for the treatment of T2DM [48]. There are five cholinergic muscarinic receptor subtypes (M_1—M_5), and the work of Gautam and colleagues using transgenic and gene knockout technology have determined that the M_3-muscarinic receptor (M3R) is the main acetylcholine receptor that is responsible for enhancing glucose-dependent insulin release in β cells [18]. The classical mechanism by which M3R regulates insulin release was thought to be primarily via G-protein depending signaling to the calcium and PKC pathways. As a prototypical $G_{q/11}$-coupled receptor, activation of M3R induces the hydrolysis of membrane phospholipid phosphatidylinositol-4,5-bisphosphate (PIP_2), catalyzed by phospholipase C (PLC). This generates two second messengers, inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3, in turn, mobilizes calcium from the IP_3-sensitive stores while DAG activates PKC. Both of these pathways have been thought to play an important role in M3R-mediated insulin secretion [21,48]. However, recent molecular and genetic studies have pointed to further mechanisms. In particular, sustained insulin release associated with the enteric phase appears to be mediated by a process that is independent of G-protein signaling. This is evidenced by studies from the Tobin laboratory and others, which demonstrated that protein kinase D1 (PKD1) is one of the key components by which M3R regulates glucose-dependent insulin release [29,52,53]. M3R is phosphorylated by various kinases, including GPCR kinases and casein kinase 2 (CK2). This process not only leads to the uncoupling of the receptor from its cognate G proteins but also allows for the activation of G protein-independent signaling, a process that is driven largely by the recruitment of β-arrestin adaptor proteins via a G-protein-independent, β-arrestin-dependent process that results in activation of PKD1 and secretary vesicle priming [29]. It is still unclear how G-protein-dependent and independent pathways contribute under different physiological conditions. In particular, the potentiation of GSIR by GPR40 ligands, such as oleic acid [17], occurs due to the G-protein independent pathway [17]. However, recent results from the Wess laboratory showed that M3R in β-cells is specifically phosphorylated by CK2, and this negatively regulates the receptor-mediated insulin release in vitro and in vivo [47]. Preventing M3R phosphorylation, either by inhibiting CK2 in pancreatic β-cells, knocking down CK2α expression, or genetic deletion of CK2α in β-cells of mutant mice, selectively augmented Ca^{2+} signaling and M3R-stimulated insulin release in vitro and in vivo [47]. In support of these results, analysis of human microarray data revealed a significant increase in CK2 expression in human β-cells isolated from T2D subjects, compared with β-cells from nondiabetic donors [32]. It is also possible that receptor phosphorylation by a defined kinase determines a specific signaling outcome and can lead to different physiological effects [53]. Finally, CX4945 treatment protected mice against diet-induced hyperglycemia and glucose intolerance in an M3R-dependent fashion [47]. Based on these data, the goal of the present study was to test whether cholinergic potentiation of insulin secretion is altered due to chronic exposure to elevated glucose and lipids (two major characteristics of T2D) and whether Ca^{2+} signaling or phosphorylation of M3R by CK2 is involved in this effect and whether a selective CK2 inhibitor could prove beneficial under these conditions.

2. RESEARCH DESIGN AND METHODS

2.1. Animals

B6D22F1 male mice (from Jackson Laboratory) were used throughout. The mice were maintained on a 12:12 h light—dark cycle and were fed a standard rodent chow diet. All research was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania (protocol no. 803719).

2.2. Mouse islet: isolation and culture

Mouse islets from male mice were isolated from fed animals using collagenase (EC 3.4.24.3 Serva, 17449) digestion in Hanks buffer followed by separation of islets from exocrine tissue in a Ficoll (Sigma, F-9378) gradient. Isolated islets were cultured for 3—5 days in RPMI 1640 medium (Sigma) containing 10% undialized fetal bovine serum, 10 ml/l penicillin-streptomycin-amphotericin B solution (GIBCO BRL) and 10, 16, or 25 mM glucose with or without free fatty acids noting that undialized bovine albumin contributes variable amounts, but at most 1 mM glucose to the culture medium.

2.3. Human islets: source and culture

Human islets were received from the accredited Human Islet Resource Center at the University of Pennsylvania. The pancreata were procured individually and the isolation performed according to previously described protocols [10,11]. Altogether, we studied pancreatic islets isolated from 18 individuals, all were normoglycemic at organ isolation and ranged in age from 19 to 51 years. Isolated human islets were kept under culture conditions in medium at 5 mM glucose and 25 °C before they were transferred to our laboratory and cultured the same way as mouse islets. Time elapsed from the isolation to transferring the tissue to our laboratory ranged from 1 to 3 days. The culture conditions were similar to those for mouse islets.

2.4. Preparation of fatty acid solution

A 5 mM stock solution of sodium palmitate (Sigma—Aldrich) was prepared by dissolving the fatty acid salt in 10% of bovine serum albumin (BSA, Sigma—Aldrich, fraction V, fatty acid free) in Krebs buffer by continuous stirring for ∼4 h in a 37 °C water bath. In case of a mixture of palmitate and oleate (2:1; final concentration 5 mM), the sodium palmitate was dissolved first and then sodium oleate was added to the stock solution of 10% BSA. The stock solution was then diluted by Krebs buffer to obtain the final concentration of 0.5 mM sodium palmitate or 0.5 mM of a mixture of palmitate and oleate and 1% of BSA (a molar FA/BSA ratio of 3.3 as seen in extremes of fasting or in diabetic states).

2.5. Perifusion of islets for measurement of insulin release

Cultured islets (130 islets) were placed on a nylon filter in a plastic perifusion chamber (Millipore, Bedford, MA) and were perfused with a flow rate of 1.5 ml per min. The perifusion apparatus consisted of a computer-controlled low pressure chromatography system (BIO-RAD Econo system) that allowed programmable rates of flow and glucose concentration in the perfusate, a water bath (37 °C), and fraction

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