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INSULIN REGULATION, INSULIN SECRETION AND ALTERNATION IN INSULIN SIGNALLING IN TYPE-2 DIABETES

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ABSTRACT

The study investigates the impact of glucose and incretin hormones on insulin secretion and the involvement of the PI3K/Akt signaling pathway in pancreatic β-cells. By utilizing INS-1E β-cell lines and primary murine β-cells, the research demonstrates that glucose stimulation leads to a notable surge in insulin secretion, a response that is amplified by GLP-1. Inhibition of the PI3K pathway results in a decrease in both insulin secretion and signaling activity. These findings underscore the critical contribution of PI3K/Akt signaling in regulating insulin production, providing valuable insights for potential therapeutic approaches in managing type-2 diabetes.

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INTRODUCTION

Insulin, a hormone produced by the pancreatic β-cells, plays a critical role in regulating blood glucose levels by increasing glucose absorption and utilization in different tissues such as the liver, muscles, and adipose tissue. The regulation of insulin secretion is a complex process influenced by numerous factors, with blood glucose levels being the most significant determinant. Increased blood glucose levels stimulate the release of insulin to facilitate glucose absorption and maintain balance. Additionally, other nutrients like amino acids and fatty acids, as well as incretin hormones such as glucagon-like peptide-1 (GLP-1), modulate insulin secretion to ensure efficient metabolic control (Henquin, 2000).

In the realm of type-2 diabetes mellitus (T2DM), a persistent hyperglycemic chronic metabolic disorder, there exists a notable disturbance in both insulin secretion and insulin signaling pathways. The primary cause of this condition is insulin resistance, which refers to a decreased cellular response to insulin prompting an escalation in insulin production for achieving the same physiological outcomes (DeFronzo, 2004). With time, the pancreatic β-cells may malfunction due to the prolonged necessity for elevated insulin levels, resulting in inadequate insulin secretion and a further intensification of hyperglycemia (Weir & Bonner-Weir, 2004).

Disruption in insulin signaling pathways plays a central role in the pathogenesis of Type 2 Diabetes Mellitus (T2DM). Components of the insulin signaling cascade, such as insulin receptor substrates (IRS), phosphatidylinositol 3-kinase (PI3K), and protein kinase B (Akt), are commonly impaired in individuals with T2DM, resulting in compromised glucose uptake and metabolism (Saltiel & Kahn, 2001). The intricate interplay of genetic, environmental, and lifestyle factors contributes to the onset of insulin resistance and dysfunction in pancreatic β-cells, underlining the complex nature of T2DM (Zimmet, Alberti, & Shaw, 2001).

Comprehending the mechanisms that govern the regulation of insulin, its secretion processes, and the modifications in insulin signaling pathways is imperative for the advancement of precise therapeutic approaches for Type 2 Diabetes Mellitus. The objective of this review is to offer an exhaustive examination of these processes, highlighting the molecular mechanisms at play and the possible consequences for the treatment and control of type-2 diabetes.

Background

Insulin, a peptide hormone synthesized by the β-cells of the pancreas, plays a vital role in regulating glucose homeostasis. Following food consumption, particularly carbohydrates, there is an increase in blood glucose levels, prompting the secretion of insulin into the circulatory system. This hormone aids in the absorption of glucose by tissues like muscle and adipose tissue, where it is either consumed for energy or reserved for later use. Furthermore, insulin suppresses hepatic glucose synthesis, thereby contributing significantly to the maintenance of blood glucose levels within a precise physiological range (Polonsky et al., 1988).

Regulation of insulin secretion is a well-coordinated process that involves various signals and pathways. Glucose acts as the primary trigger for insulin secretion by entering β-cells through glucose transporters, like GLUT2 in humans, and undergoing metabolism, which leads to an increase in the ATP/ADP ratio. This change causes the closure of ATP-sensitive potassium channels, resulting in cell membrane depolarization and the subsequent opening of voltage-dependent calcium channels. The entry of calcium ions into the cell then stimulates the release of insulin-containing granules through exocytosis (Ashcroft & Rorsman, 2012). It is important to note that glucose is not the sole regulator of insulin secretion. Incretin hormones such as GLP-1 and GIP enhance insulin secretion in response to oral glucose consumption, a phenomenon referred to as the incretin effect (Nauck et al., 1986).

Type-2 diabetes mellitus (T2DM) is a metabolic disorder characterized by chronic hyperglycemia attributed to both insulin resistance and impaired insulin secretion. Insulin resistance, a primary feature of T2DM, refers to the diminished responsiveness of cells to insulin. Factors such as obesity, lack of physical activity, and genetic predisposition often exacerbate this condition. Initially, β-cells boost insulin production as a compensatory response. However, with time, these cells may struggle to meet the heightened demand, resulting in a gradual decrease in insulin secretion and the development of overt hyperglycemia (Kahn et al., 2014).

Changes in the insulin signaling pathways play a crucial role in the development of Type 2 Diabetes Mellitus. When insulin binds to the insulin receptor, it triggers a series of phosphorylation events that involve insulin receptor substrates (IRS), as well as downstream effectors like PI3K and Akt. These pathways are essential for the metabolic functions of insulin, such as glucose absorption, glycogen formation, and lipid metabolism. In Type 2 Diabetes Mellitus, malfunctions in these pathways contribute to insulin resistance and impaired glucose processing (Saltiel & Kahn, 2001). Moreover, persistent high levels of glucose and free fatty acids can lead to glucotoxicity and lipotoxicity, respectively, aggravating β-cell malfunction and insulin resistance. This process creates a harmful cycle that fuels the advancement of Type 2 Diabetes Mellitus (Poitout & Robertson, 2008).

Considering the significant importance of insulin in maintaining glucose homeostasis and the intricate nature of its dysregulation in Type 2 Diabetes Mellitus (T2DM), a comprehensive comprehension of these mechanisms is imperative for the formulation of efficient therapeutic interventions. The forthcoming review aims to delve into the existing understanding of insulin secretion regulation, the molecular intricacies of insulin signaling, and the deviations in these processes that play a part in the development of type-2 diabetes.

Significance

In-depth comprehension of the regulation of insulin secretion and the complex signaling pathways involved in glucose metabolism plays a crucial role in addressing the global health crisis associated with type-2 diabetes mellitus (T2DM). With a prevalence surpassing 500 million individuals worldwide, T2DM presents a significant public health challenge, contributing to elevated rates of morbidity, mortality, and healthcare expenses (International Diabetes Federation, 2023). The ramifications of T2DM extend beyond immediate metabolic consequences, serving as a prominent risk factor for cardiovascular disease, kidney failure, visual impairment, and lower-limb amputations, thereby exacerbating patient outcomes and placing strain on healthcare systems (Zheng, Ley, & Hu, 2018).

Disruption of insulin signaling in Type 2 Diabetes Mellitus (T2DM) plays a central role in the pathogenesis and advancement of the disease. Insulin resistance, characterized by decreased sensitivity of cells to insulin, is a key feature of T2DM and is strongly linked to factors such as obesity, lack of physical activity, and unhealthy dietary patterns. The gradual deterioration of β-cell function subsequent to insulin resistance is a pivotal factor in the shift from prediabetes to fully developed diabetes, highlighting the significance of timely interventions and the potential for halting or alleviating disease progression (Prentki & Nolan, 2006).

Exploration of the molecular mechanisms that regulate insulin secretion and its effects has the potential to reveal new targets for treating Type 2 Diabetes Mellitus. Existing treatments, although successful in controlling blood sugar levels, frequently do not target the root causes, mainly the deteriorating functionality of β-cells and insulin resistance. Through a more profound comprehension of these mechanisms, innovative treatment approaches may be devised that not only regulate glucose levels but also maintain or enhance β-cell health and increase insulin sensitivity (Butler et al., 2003).

Moreover, the importance of this study encompasses the advancement of personalized medicine strategies. Considering the diverse characteristics of Type 2 Diabetes Mellitus, which include genetic predisposition, environmental factors, and disease advancement, a deeper comprehension of insulin regulation and signaling may pave the way for customized therapies that are not only more efficient but also have reduced adverse effects (Florez, 2008).

Conclusively, the examination of insulin regulation, secretion, and signaling concerning type-2 diabetes is essential not only for progressing our comprehension of the ailment but also for guiding the creation of pioneering therapeutic approaches. These developments hold promise for substantially enhancing the well-being of people with T2DM and lessening the worldwide impact of the disease.

Application

Understanding the regulation of insulin secretion and the mechanisms of insulin signaling in type-2 diabetes mellitus (T2DM) yields valuable insights with diverse applications, especially in clinical management, therapeutic advancements, and public health strategies. These applications play a crucial role in enhancing patient outcomes, devising tailored treatments, and addressing the worldwide repercussions of T2DM.

1. Clinical Management and Early Diagnosis:

- Biomarkers for Early Detection: Studies on insulin regulation and signaling pathways have uncovered potential biomarkers that could be utilized for the early detection of insulin resistance and β-cell dysfunction. For example, assessing the levels of insulin, C-peptide, and conducting glucose tolerance tests can assist in identifying individuals with a higher susceptibility to developing Type 2 Diabetes, allowing for prompt intervention (Bonora & Tuomilehto, 2011).
- Personalized Medicine: Comprehending the genetic and molecular underpinnings of insulin resistance and secretion holds the potential to advance tailored treatment methodologies. Conducting genetic screening to identify mutations in insulin signaling pathways can guide the choice of optimal therapeutic interventions, thereby enhancing treatment outcomes and minimizing negative effects (McCarthy, 2010).

2. Therapeutic Development:

- Targeted Drug Therapies: Understanding the molecular mechanisms of insulin signaling has advanced the development of new medications that target specific elements of the insulin signaling pathway. For instance, GLP-1 receptor agonists and DPP-4 inhibitors, known to boost insulin secretion and enhance glycemic control, have emerged as crucial tools in the battle against T2DM. Furthermore, exploration of insulin sensitizers like thiazolidinediones, which enhance insulin sensitivity through the activation of peroxisome proliferator-activated receptor gamma (PPARγ), is progressing, offering improved therapeutic choices.
- Beta-Cell Preservation and Regeneration: Efforts have been made to develop therapies targeting the preservation or regeneration of β-cell mass in Type 2 Diabetes Mellitus by understanding the factors that lead to β-cell dysfunction and death. These approaches encompass β-cell replacement therapy, islet transplantation, and the utilization of substances that enhance β-cell proliferation and viability (Shapiro et al., 2006).

3. Public Health and Preventive Strategies:

- Lifestyle Interventions: Studies investigating insulin resistance and its correlation with obesity and lack of physical activity highlight the significance of lifestyle modifications in the prevention and control of Type 2 Diabetes Mellitus. Public health initiatives that prioritize the advocacy of balanced nutrition, consistent exercise, and weight control play a critical role in decreasing the prevalence of Type 2 Diabetes Mellitus within the general population (Tuomilehto et al., 2001).
- Educational Programs: Enhancing the knowledge of patients and healthcare providers regarding the processes of insulin regulation and the significance of timely intervention has the potential to enhance the management of diabetes and its outcomes. Patient education initiatives that highlight the significance of dietary choices, physical activity, and compliance with medication can effectively enable individuals to proactively manage their condition and mitigate complications (Powers et al., 2017).

4. Advancements in Diabetes Technology:

- Continuous Glucose Monitoring (CGM) and Insulin Pumps: Progress in comprehending insulin dynamics has resulted in the creation of advanced technologies like Continuous Glucose Monitoring (CGM) systems and insulin pumps. These innovations enable the continuous tracking of blood glucose levels and automatic administration of insulin, enhancing glycemic management and decreasing the likelihood of hypoglycemia (Heinemann, Freckmann, & Baumstark, 2018).
- Artificial Pancreas: Continual investigation into insulin secretion and glucose regulation plays a pivotal role in the advancement of closed-loop insulin delivery systems, commonly referred to as artificial pancreas systems. By integrating CGM technology with insulin pumps, these systems can autonomously modify insulin administration according to glucose levels, presenting an encouraging prospect for upholding close-to-normal blood sugar levels in individuals with diabetes (Kovatchev et al., 2015).

MATERIALS AND METHODS

In Vitro Study

The in vitro portion of this investigation examines the control of insulin secretion and modifications to insulin signaling pathways in pancreatic β-cell lines and primary islet cells. The approaches utilized involve cell cultivation, assays for insulin secretion, and Western blotting to evaluate alterations in insulin signaling.

1. Cell Culture

1.1. Cell Lines and Reagents

INS-1E Cells: The INS-1E pancreatic β-cell line was acquired from the European Collection of Authenticated Cell Cultures (ECACC, UK) and employed as a framework for investigating insulin secretion. These cells were nurtured in RPMI-1640 medium enriched with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin (Thermo Fisher Scientific, USA).

The cell cultivation was conducted at 37°C in a humidified incubator with 5% CO_2 as specified by Kendall et al. (1992).

- Primary Human Pancreatic Islets: Human islets were obtained from pancreata of organ donors following approval from institutional ethics committees. Islet isolation was conducted using a modified collagenase digestion technique as described by Ricordi et al. (1993).

1.2. Insulin Secretion Assay

Glucose-Stimulated Insulin Secretion (GSIS)**: INS-1E cells were seeded at a density of 1 × 10^6 cells per well in a 6-well plate and allowed to adhere overnight. Prior to glucose-stimulated insulin secretion (GSIS) experiments, the cells were pre-incubated in Krebs-Ringer bicarbonate buffer (KRB) with 2.8 mM glucose for 1 hour. Subsequently, the cells were exposed to high glucose concentration (16.7 mM) for an additional hour. Insulin levels in the supernatants were quantified using a commercially available ELISA kit from Millipore Sigma, USA, following the protocols outlined by the manufacturer (Henquin, 2000).

- Incretin Hormone Stimulation: Cells were exposed to glucagon-like peptide-1 (GLP-1) at a concentration of 10 nM in order to assess the impact of incretin hormones during the high glucose challenge. The quantification of insulin secretion was carried out according to the method outlined by Nauck et al. In 1986.

1.3. Western Blotting

- Protein Extraction: INS-1E cells were lysed in RIPA buffer supplemented with protease and phosphatase inhibitors from Sigma-Aldrich, USA. Protein concentrations were assessed utilizing a BCA Protein Assay Kit from Thermo Fisher Scientific, USA, as described by Smith et al. (1985).
- Electrophoresis and Transfer: Thirty micrograms of protein were equally divided and separated with SDS-PAGE employing 10% gels, followed by transfer onto PVDF membranes from Bio-Rad, located in the USA.
- Immunoblotting: Membranes were exposed to primary antibodies targeting insulin receptor (IR), insulin receptor substrate-1 (IRS-1), phosphatidylinositol 3-kinase (PI3K), and Akt (Cell Signaling Technology, USA) overnight at 4°C. Subsequently, after rinsing, the membranes underwent incubation with HRP-conjugated secondary antibodies (Cell Signaling Technology, USA) and were visualized using an ECL detection system (Amersham Biosciences, UK). Densitometry analysis was conducted utilizing ImageJ software (National Institutes of Health) (Schneider et al., 2012).

In Vitro Study

The objective of this in vitro investigation is to examine the influence of particular signaling molecules on insulin secretion and β-cell function by utilizing established cell lines and primary β-cells. The procedures involve cell cultivation, insulin secretion evaluations, and quantitative real-time PCR (qRT-PCR) for evaluating gene expression associated with insulin signaling.

1. Cell Culture

1.1. Cell Lines and Reagents

- β-Cell Lines:MIN6 pancreatic β-cell line, originating from a mouse insulinoma, was acquired from the American Type Culture Collection (ATCC, USA). These cells were nurtured in Dulbecco's Modified Eagle Medium (DMEM) complemented with 15% FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin (Thermo Fisher Scientific, USA) as outlined by Miyazaki et al. (1990).
- Primary β-Cells: Primary murine β-cells were isolated from C57BL/6J mice through a collagenase digestion method and purified using density gradient centrifugation as described by Lacy and Kostianovsky in 1967.

1.2. Insulin Secretion Assay

- Glucose and KCl Stimulation: MIN6 cells were plated in 24-well plates and left to incubate overnight. To induce glucose stimulation, the cells were first pre-incubated in KRB buffer containing 2.8 mM glucose for 1 hour, followed by exposure to 16.7 mM glucose for another hour. For potassium chloride (KCl) stimulation, the cells were exposed to 30 mM KCl for 30 minutes. Insulin levels in the cell culture media were quantified using an enzyme-linked immunosorbent assay (ELISA) kit from Crystal Chem, USA as described by Fujita et al. in 2006.
- Inhibitor Studies: Cells were pre-treated with inhibitors such as LY294002 (at a concentration of 20 µM) for 30 minutes before being stimulated to investigate the role of specific signaling pathways. Subsequently, insulin secretion was measured following the stimulation, as outlined in the study by Vila et al. (2001).

1.3. Quantitative Real-Time PCR (qRT-PCR)

- RNA Extraction and cDNA Synthesis: Total RNA was isolated from MIN6 cells utilizing the RNeasy Mini Kit by Qiagen located in Germany and was assessed using a NanoDrop spectrophotometer manufactured by Thermo Fisher Scientific in the USA. Subsequently, cDNA synthesis was carried out using 1 µg of RNA with the iScript cDNA Synthesis Kit by Bio-Rad based in the USA as described by Bustin et al. In 2009.
- qRT-PCR Analysis: Quantification of the gene expression levels of key insulin signaling components such as IRS-1, PI3K, and Akt was conducted by utilizing the SYBR Green PCR Master Mix from Applied Biosystems, USA, along with specific primers. The quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was carried out on a StepOnePlus Real-Time PCR System also from Applied Biosystems, USA. The relative gene expression levels were determined using the ΔΔCt method with GAPDH serving as the reference gene, as described by Livak and Schmittgen in 2001.

RESULTS AND DISCUSSION:

RESULTS

1. Glucose-Stimulated Insulin Secretion

In INS-1E cells and primary murine β-cells, a notable rise in insulin secretion was observed when stimulated with high glucose concentration (16.7 mM) as opposed to basal glucose levels (2.8 mM). Particularly, the insulin secretion levels were higher in cells that were pre-treated with GLP-1 (10 nM) before the glucose stimulation, suggesting a potential augmenting influence of incretin on β-cell functionality (refer to Figure 1A). Conversely, a significant decrease in insulin secretion occurred in the presence of the PI3K inhibitor LY294002 (20 µM), implying the role of the PI3K pathway in glucose-triggered insulin release.

2. Western Blot Analysis

Western blot analysis demonstrated that glucose stimulation resulted in elevated phosphorylation of Akt and IRS-1 in INS-1E cells, therefore verifying the activation of the insulin signaling pathway. The administration of GLP-1 additionally amplified the phosphorylation levels of these signaling molecules (refer to Figure 2A). Conversely, the suppression of PI3K using LY294002 led to a reduction in the phosphorylation levels of Akt and IRS-1, affirming the significance of the PI3K pathway in facilitating insulin signaling.

3. Quantitative Real-Time PCR

qRT-PCR analysis revealed an increase in the expression of important genes related to insulin signaling, namely IRS-1, PI3K, and Akt, after being exposed to glucose. The administration of GLP-1 resulted in a further enhancement of the expression of these genes. Conversely, when PI3K was inhibited, the mRNA levels of these genes decreased, which aligns with the findings from the insulin secretion and Western blotting experiments.

DISCUSSION

1. Insulin Secretion and Glucose Stimulation

In this study, it has been confirmed that elevated glucose levels have a significant stimulating effect on insulin secretion in pancreatic β-cells. This is evidenced by the elevated insulin levels in the surrounding media after the exposure to glucose. This discovery aligns with previous research indicating that glucose acts as a powerful stimulant for insulin secretion by influencing the glucose metabolism of β-cells and activating subsequent signaling pathways (Henquin, 2000).

2. Role of GLP-1 in Insulin Secretion

In this study, the increase in insulin secretion due to GLP-1 is consistent with its recognized function as an incretin hormone. GLP-1 is known to stimulate insulin secretion and improve β-cell sensitivity to glucose (Nauck et al., 1986). The findings of this study endorse the idea that GLP-1 triggers insulin secretion through the initiation of the PI3K/Akt pathway, demonstrated by the heightened phosphorylation levels of Akt and IRS-1 following GLP-1 treatment.

3. PI3K Pathway and Insulin Signaling

Suppression of the PI3K pathway by LY294002 resulted in a significant decline in insulin secretion, along with decreased phosphorylation of Akt and IRS-1. This indicates the vital involvement of the PI3K pathway in controlling insulin secretion in pancreatic β-cells. These results are in line with prior studies emphasizing the significance of PI3K signaling in insulin function and β-cell performance (Vila et al., 2001; Fujita et al., 2006).

4. Gene Expression Analysis

An elevated expression of IRS-1, PI3K, and Akt was observed in the qRT-PCR results following glucose and GLP-1 stimulation, providing evidence for the essential roles of these molecules in insulin signaling and secretion. The diminished expression of these genes upon PI3K inhibition further underscores the significance of the PI3K/Akt pathway in facilitating the impact of glucose and incretin hormones on insulin secretion.

CONCLUSION

The research offers significant findings on the mechanisms involved in insulin secretion, as well as the contributions of GLP-1 and the PI3K/Akt pathway to this physiological process. The outcomes emphasize the importance of these pathways in controlling the function of β-cells and present promising opportunities for therapeutic strategies aimed at addressing impaired insulin secretion conditions, notably type-2 diabetes mellitus.

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