Deregulation of CREB Signaling Pathway Induced by Chronic Hyperglycemia Downregulates NeuroD Transcription.

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Abstract

CREB mediates the transcriptional effects of glucose and insulin hormones in insulin-target cells and insulin-producing islets. Although the inhibition of CREB activity is known to decrease the b-cell mass, it is still unknown what factors inversely alter the CREB signaling pathway in b-cells. Here, we show that b-cell dysfunctions occurring in chronic hyperglycemia are not caused by simple inhibition of CREB activity but rather by the persistent activation of CREB due to increases in protein phosphatase PP2A. When freshly isolated rat pancreatic islets were chronically exposed to 25 mM (high) glucose, the PP2A activity was increased with a concomitant increase in active pCREB. Brief challenges with 15 mM glucose or 20 mM forskolin for 2 hour lasting further increased the level of pCREB and consequently induced the persistent expression of ICER. The excessively produced ICER was sufficient to repress the transcription of NeuroD, insulin, and SUR1 genes. In contrast, when islets were grown in 5 mM (low) glucose, CREB was transiently activated in response to glucose or forskolin stimuli. Thus, CREB expression was transient and insufficient to repress those target genes. Importantly, overexpression of PP2A reversed the adverse effects of chronic hyperglycemia and successfully restored the transient activation of CREB and ICER. Conversely, depletion of PP2A with siRNA was sufficient to disrupt the negative feedback regulation of CREB and reduce hyperglycemic phenotypes even under low glucose conditions. Our findings suggest that the failure of the negative feedback regulation of CREB is the primary cause for b-cell dysfunctions under conditions of pathogenic hyperglycemia, and PP2A can be a novel target for future therapies aiming to protect b-cells mass in the late transition phase of non-maturity dependent type 2 diabetes (NIDDM).

Key words : NeuroD, ICER, CREB, PP2A, b-cell, insulin, glucose.

Introduction

I. NeuroD (BETA2, beta cell E-box transactivator2)

1. A transcription factor in neuroendocrine cells
2. β-cell development, insulin expression
3. Neuronal differentiation

II. CREB, CREM and ICER

1. ICER was induced by CREB/CREM activators.
2. Function as homeostatic mechanism.
3. Melatonin synthesis, circadian rhythm, spermatogenesis and β-cell physiology.
4. Abnormal expression causes various pathologic events.

III. Potential CRE sequence in the proximal promoter of NeuroD.

Putative CRE-response element (CRE) is indicated by asterisk (-75 bp ~ -68 bp) from the transcription initiation site (arrow) of the NeuroD gene.

Results

1. Chronic hyperglycemia induces the β-cell specific gene expression (qPCR) in pancreatic islets.

Fig. 1. Chronic hyperglycemia induces the β-cell specific gene expression (qPCR) in pancreatic islets.

Fig. 2. Chronic hyperglycemia alters the β-cell specific gene expression (semi-quantitative traditional PCR) in pancreatic islets.

Fig. 3. CAMP exerts similar effects in HIT cells after chronic exposure to hyperglycemia.

Fig. 4. ICER binds to a novel CRE sequence in the proximal NeuroD promoter.

Fig. 5. Chronic hyperglycemia persistently activates the basal and forskolin-stimulated pCREB.

Fig. 6. Chronic hyperglycemia reduces the PP2A level.

Fig. 7. Chronic hyperglycemia reduces the PP2A level.

Fig. 8. Reduced activity of PP2A is the primary cause of impaired gene expression.

Summary and Conclusion

1. ICER bind and suppress to CRE of NeuroD promoter in a cell type specific manner.
2. Chronic elevation of pCREB sustained ICER expression in high glucose-cultured HIT cell.
3. Reduced PP2A is involved in these pathogenic process of cAMP-CREB signaling.
4. In pancreatic β-cells, ICER-mediated repression of NeuroD contributes to the glucose toxicity.
5. Vicious cycles of ICER-mediated NeuroD repression is one of critical pathogenic process in hyperglycemia.