



Metabolic memory of β -cells controls insulin secretion and is mediated by CaMKII^a

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ABSTRACT

Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) functions both in regulation of insulin secretion and neurotransmitter release through common downstream mediators. Therefore, we hypothesized that pancreatic β -cells acquire and store the information contained in calcium pulses as a form of “metabolic memory”, just as neurons store cognitive information. To test this hypothesis, we developed a novel paradigm of pulsed exposure of β -cells to intervals of high glucose, followed by a 24-h consolidation period to eliminate any acute metabolic effects. Strikingly, β -cells exposed to this high-glucose pulse paradigm exhibited significantly stronger insulin secretion. This metabolic memory was entirely dependent on CaMKII. Metabolic memory was reflected on the protein level by increased expression of proteins involved in glucose sensing and Ca²⁺-dependent vesicle secretion, and by elevated levels of the key β -cell transcription factor MAFA. In summary, like neurons, human and mouse β -cells are able to acquire and retrieve information.

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Keywords Insulin secretion; Metabolic memory; CaMKII

1. INTRODUCTION

Ionic calcium (Ca²⁺) controls multiple cellular signaling processes in all eukaryotic cells, including proliferation, gene expression, and neurotransmitter release. Ca²⁺-binding proteins such as calmodulin play a pivotal role in Ca²⁺ signal transmission and amplification. Increases in the concentration of intracellular Ca²⁺ activate specific protein targets, among which the Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) is a critical signal mediator [1].

In response to an increase in intracellular Ca²⁺, Thr-286 of CaMKII becomes exposed and phosphorylated in an intraholoenzyme reaction [2]. The coupling of Ca²⁺-calmodulin to one of the CaMKII subunits allows for the phosphorylation of an adjacent subunit at Thr²⁸⁶. This process, known as “calmodulin trapping”, confers Ca²⁺-calmodulin-independent kinase activity to the complex and thus prolongs the Ca²⁺ signal. Thus, calmodulin trapping represents a molecular mechanism of memory [3,4], which is defined as the capacity to acquire, store (consolidate), and retrieve (evocate) information [5].

CaMKII is a major synaptic protein that is activated during the induction of long-term potentiation (LTP) by Ca²⁺ influx through N-methyl-D-aspartate (NMDA) receptors. Calmodulin trapping allows CaMKII to remain activated long after the initial Ca²⁺ signal has

dissipated, suggesting that CaMKII is a memory molecule crucial for LTP [4,5]. Consistent with this notion, CaMKII-null mice present with impaired memory formation, and CaMKII is essential for genesis and maintenance of LTP in postsynaptic neurons [2]. Following presynaptic stimulation, CaMKII is activated in postsynaptic neurons, which creates a physiological imprint of the initial Ca²⁺ signal, and increases translocation of NMDA receptors to the plasma membrane [6]. Because of its capacity to remain activated long after the initial pulse of Ca²⁺ signaling, CaMKII perpetuates Ca²⁺ effects and modulates gene expression and the epigenetic profile of postsynaptic neurons [7].

CaMKII also participates in glucose-stimulated insulin secretion (GSIS), as multiple insulin secretagogues increase CaMKII activity [8]. In the perfused rat pancreas, the dynamics of CaMKII activation correlate with the amplitude of GSIS, and CaMKII activation is temporally associated with insulin secretion [8]. CaMKII is essential for appropriate GSIS and is involved in several steps of this process, including the synthesis of insulin granules, the modulation of cytoplasmic content of ATP, and the activation of Synapsin [8]. Importantly, CaMKII also regulates transcription factors central for β -cell function such as CREB [9] and MafA [10,11].

Here, we investigated whether pancreatic β -cells, like neurons, acquire and store the information contained in calcium pulses as a form of

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“metabolic memory”. Indeed, we find that β -cells retain memory of prior activation, and describe the molecular mechanism that contributes to this memory.

2. MATERIALS AND METHODS

2.1. The glucose pulse paradigm

MIN6 insulinoma cells or mouse or human pancreatic islets were distributed into three groups: control, pulse, and pulse + KN93 (10 μ M). Initially, all groups were maintained in the same medium with 5.6 mM glucose for 24 h. After this acclimation period, the control

group was exposed to 3-mM glucose for 24 h, while the pulse and pulse + KN93 (10 μ M) groups were exposed to 30 mM of glucose for four 1-h periods intercalated with 7-hour periods of 3 mM glucose. KN93 (a CaMKII inhibitor) at 10 μ M was only present during the 30 mM glucose pulses. All groups were then maintained at 3 mM glucose for a 24-h consolidation period.

2.2. Insulin secretion assays

Insulin secretion of MIN6 cells [12] and mouse pancreatic islets [13] was conducted following standard procedures, and insulin was measured by RIA.

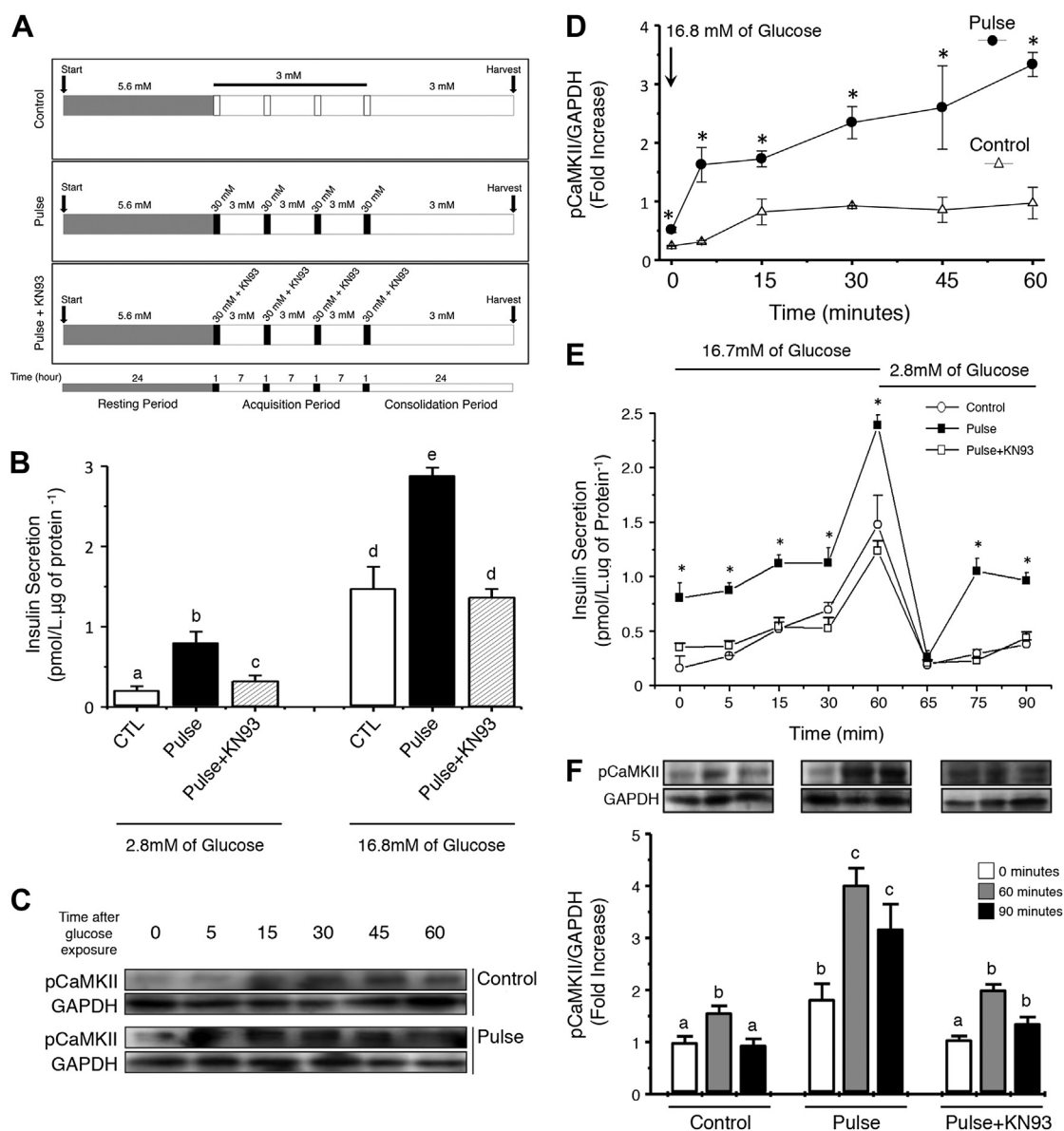


Figure 1: MIN6 insulinoma cells acquire, store, and recall metabolic information. (A) Experimental paradigm to elicit metabolic memory. β -cells were exposed to four pulses of high (30 mM) glucose, interspersed with three intervals of low (3 mM) glucose, followed by a 24-h consolidation period before analysis of insulin secretion. Parallel dishes were cultured in low glucose continuously, or exposed to the CaMKII inhibitor KN93 only during the glucose pulses. (B) Acute insulin secretion of MIN6 β -cells 24 h after the last glucose pulse at 2.8 or 16.8 mM glucose for 1 h. Letters indicate significant differences at $p < 0.05$ between groups. a – CTL 2.8 vs all groups; b – Pulse 2.8 vs all groups; c – Pulse + KN93 vs all groups; d – CTL 16.7/Pulse + KN93 16.7 vs CTL 2.8/Pulse 2.8/Pulse + KN93 2.8/Pulse 16.7; and e – Pulse 16.7 vs all groups. Data represent six independent experiments. (C and D) MIN6 β -cells of the control (white triangle) or glucose pulse group (black circle) were acutely exposed to 16.7 mM of glucose. Phosphorylation of CaMKII (pCaMKII/GAPDH) was assessed at 0, 5, 15, 30, 45, and 60 min of exposure. Values are presented as fold-increase over time zero. * $p < 0.05$. (E) Insulin secretion during the acute stimulus of 16.7 mM glucose over time (0, 5, 15, 30, and 60 min), and after the removal of the stimulus (65, 75, and 90 min). * $p < 0.05$. (F) CaMKII phosphorylation of the three experimental groups in basal conditions, after 60 min of acute glucose stimulus, and 30 min after the removal of glucose (“90 minutes”). Letters denote significant differences between the groups at $p < 0.05$: a – CTL 0’/CTL 90’/Pulse+KN93 0’ vs all groups; b – CTL 60’/Pulse 0’/Pulse+KN93 60’/Pulse+KN93 90’ vs all groups; c – Pulse 60’/Pulse 90’ vs all groups. Data are means \pm SEM of four independent experiments.

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Gender	Age	Height (m)	Weight (kg)	BMI	Diabetes
M	27	1.77	64	20.2	No
M	44	1.70	75	25.8	No
F	36	1.62	92	34.8	No
M	43	1.95	117	30.6	No

Table 1: Parameters of islet donors.

2.3. Human islet perfusion

One hundred and fifty islets were handpicked under a light microscope and pre-incubated for 1 h in KRBB (Krebs bicarbonate buffer) containing 0.3 g/l BSA and 2.8 mM glucose. The medium was discarded and the islets incubated for an additional hour in 500- μ l KRBB containing 2.8 or 16.8 mM glucose. Subsequently, the supernatant was collected to evaluate insulin secretion by RIA. For perfusion assays, 150 treated human islets were placed into a perfusion chamber (Millipore, Billerica, MA, USA). A computer-controlled fast-performance HPLC system allowed programmable rates of flow and concentrations of the appropriate solutions held in a 37 °C water bath. Islets were perfused for 80 min with KRBB in the absence of glucose, followed by KRBB with increasing concentrations of glucose (glucose was ramped from 0 to 30 mM at 0.75 mM/min). At the end of each experiment, islets were tested for maximal insulin secretion by adding 30 mM KCl to the perfusate [14,15]. Samples were collected at 2 ml/min and insulin content was determined by using RIA.

2.4. Statistics

Data are expressed as means \pm SEM. Statistical analyses were performed using Student's *t*-test or a two-way ANOVA with a Bonferroni's posttest, as required. Statistical significance was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Mouse β -cells exhibit long-lasting metabolic memory

In order to investigate metabolic memory of insulin-producing β -cells, we first had to develop a paradigm in which such memory was evident. As detailed below, we established that exposure of cultured MIN6 β -cells to four 1-h pulses of high glucose, interspersed with 7-h intervals of low glucose and thus mimicking postprandial glucose spikes *in vivo*, were able to elicit robust metabolic memory. The detailed experimental paradigm is outlined in Figure 1A. As shown in Figure 1B, MIN6 β -cells exposed to the glucose pulse regimen showed higher insulin secretion both when exposed to low (2.8 mM) or high (16.8 mM) glucose than β -cells, which had been cultured continuously at low glucose. This increase in insulin secretion was accompanied by a significant rise in the levels of phosphorylated CaMKII, consistent with the notion that CaMKII might be a molecular mediator of this metabolic memory (Figure 1C and D). Importantly, both the increases in basal and stimulated insulin secretion in the glucose pulse group were abolished by KN93 (Figure 1B), a specific CaMKII inhibitor.

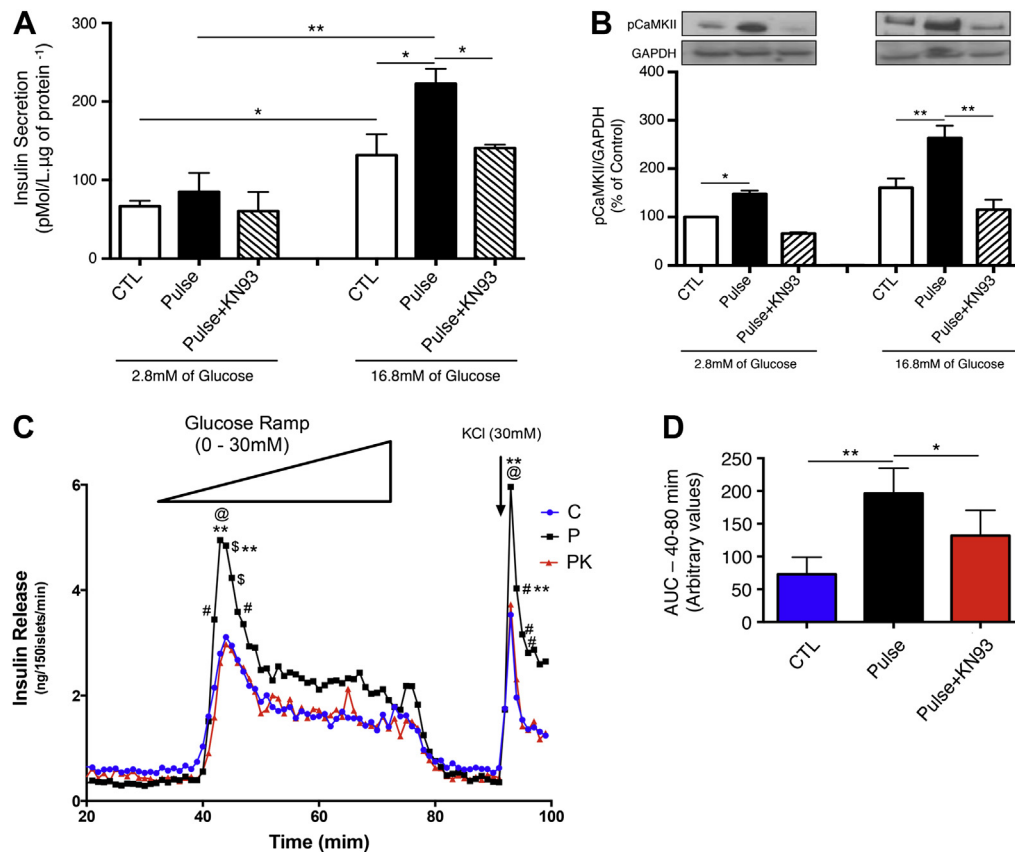


Figure 2: Human pancreatic islets display metabolic memory of prior glucose exposure. Twenty-four hours after the last glucose pulse we determined (A) insulin secretion and (B) phosphorylation of CaMKII in human islets from non-diabetic deceased organ donors exposed to KRBB with 2.8 or 16.8 mM glucose for 1 h. (C) Representative insulin secretion from perfused islets. After the consolidation period, 150 human islet per group were placed into a perfusion chamber and exposed to KRBB without glucose for 30 min, followed by KRBB with increasing concentrations of glucose from 0 to 30 mM, increasing at 0.75 mM/min. Samples were collected at 2 ml/min and insulin levels determined by RIA. (D) The area under the curve (AUC) summarizing insulin secretion during acute glucose exposure. In panel c: # $p < 0.05$ vs Control, @ $p < 0.001$ vs Control, ** $p < 0.05$ vs Pulse + KN93, and \$ $p < 0.01$ vs Control. In panels A, B, D, and E * $p < 0.05$, ** $p < 0.01$. Data are means \pm SEM of 3–5 independent experiments.

Next, we evaluated the kinetics of the β -cell response to acute glucose exposure, and observed that the pulse group secreted more insulin at basal (time zero) and stimulatory concentrations of glucose (16.7 mM) between 5 and 60 min following stimulation (Figure 1E). This effect persisted even after glucose concentrations were returned to 2.8 mM. In parallel, the phosphorylation of CaMKII was higher in the glucose pulse group at both glucose concentrations, and remained elevated even after the glucose concentration was reduced to 2.8 mM

(Figure 1F). Thus, MIN6 β -cells had acquired metabolic memory of prior exposure to glucose pulses, which they retained through a 24-h consolidation period.

3.2. Metabolic memory extends to human β -cells

It was important to determine if metabolic memory is confined to MIN6 insulinoma cells, or if it is also a property of primary human β -cells. Therefore, we cultured human islets from multiple non-diabetic

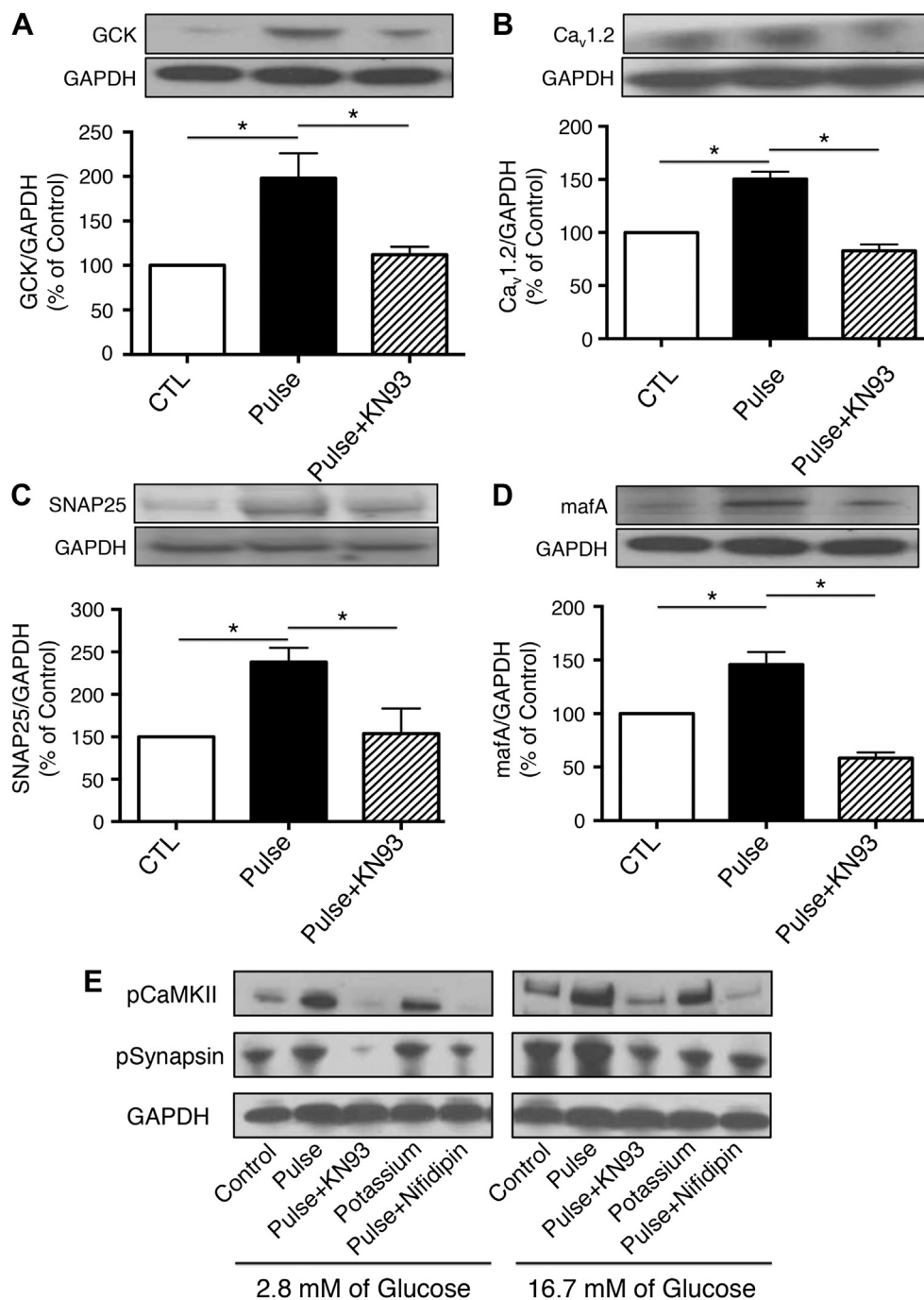


Figure 3: Metabolic memory is reflected in expression changes of key players in insulin secretion. Twenty-four hours after the last pulse of glucose, human islets were harvested and protein extracted to quantify expression of the following proteins: (A) glucokinase (GSK, expression normalized to GAPDH), (B) voltage-dependent Ca²⁺ channel (Ca_v1.2/GAPDH), (C) synaptosomal-associated protein 25 (SNAP-25/GAPDH), and (D) the transcriptional factor MAFA (MafA/GAPDH). Data are means \pm SEM of 3–5 independent experiments. * $p < 0.05$. (E) Expression of pCaMKII, pSynapsin, and GAPDH of human islet from the groups Control, Pulse, Pulse + KN93, potassium chloride, and Pulse + Nifedipin exposed for 1 h to KRBB with 2.8 or 16.8 mM of glucose.

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deceased organ donors (see Table 1 for donor information) and exposed them to pulses of 30 mM glucose in the same paradigm as described above. Pulse treatment increased insulin secretion at basal (2.8 mM) and stimulatory (16.8 mM) glucose concentrations (Figure 2A). As was the case in MIN6 β -cells, the pulse group showed increased CaMKII phosphorylation at both basal and stimulatory glucose concentrations (Figure 2B). Next, we determined the human islet response to a glucose challenge in the islet perfusion assay. Islets from the glucose pulse group showed both increased first and second phase insulin secretion compared with islets from the control group (Figure 2C and D). As before, these effects were abolished by the CaMKII inhibitor KN93 (Figure 2A–D). We also tested metabolic memory of β -cells using isolated mouse pancreatic islets, with similar outcome (Extended Data Figure 1). We also found, using live cell calcium imaging of mouse islets, that the increases in cytoplasmic calcium levels following acute glucose stimulation, representing the penultimate step in insulin secretion, are significantly higher in the pulse group compared with controls (Extended Data Figure 1C).

3.3. Molecular mediators of metabolic memory

To investigate the molecular mechanisms by which the glucose pulse paradigm induces memory in β -cells, we evaluated the expression of essential mediators of the insulin secretory response. The glucose pulse paradigm induced expression of glucokinase (GCK), the main glucose sensor of β -cells (Figure 3A), the voltage-gated Ca^{2+} channel ($\text{Ca}_v1.2$) (Figure 3B), SNAP25, an essential component of the exocytotic

machinery (Figure 3C), and MafA, a key transcription factor of the insulin gene and the mature β -cell phenotype in general (Figure 3D). All the effects of the pulsed glucose paradigm on protein levels were reduced or abolished by treatment with KN93 during the high glucose exposure only.

Finally, we asked if metabolic memory of β -cells is dependent on glucose metabolism itself, or if this memory can be produced by membrane depolarization alone. To this end, we treated human islets with pulses of 30 mM KCl, which causes membrane depolarization, opening of voltage-gated Ca^{2+} channels, and insulin secretion without a prior increase in intracellular ATP levels. As shown in Figure 3E, depolarization by potassium chloride during the acquisition period produced similar, if somewhat lower, increases in pCaMKII and pSynapsin levels 24 h after the last KCl pulse. Thus, increases in intracellular calcium elicited by membrane depolarization are sufficient to activate CaMKII and produce metabolic memory. Total CaMKII protein levels were not affected by any of the treatments used (data not shown).

Glucose-stimulated insulin secretion is a complex process that translates glycolytic flux and elevated ATP production to increased cytoplasmic Ca^{2+} levels and finally fusion of insulin granules with the plasma membrane. This process is accompanied by increased phosphorylation of CaMKII (Extended Data Figure 2), a process now shown to be part of the establishment of metabolic memory in β -cells. Cerasi and colleagues had previously reported time-dependent potentiation of insulin secretion, in that exposure of rat islets to high glucose (27.7 mM) up to 60 min prior to the test stimulation with glucose

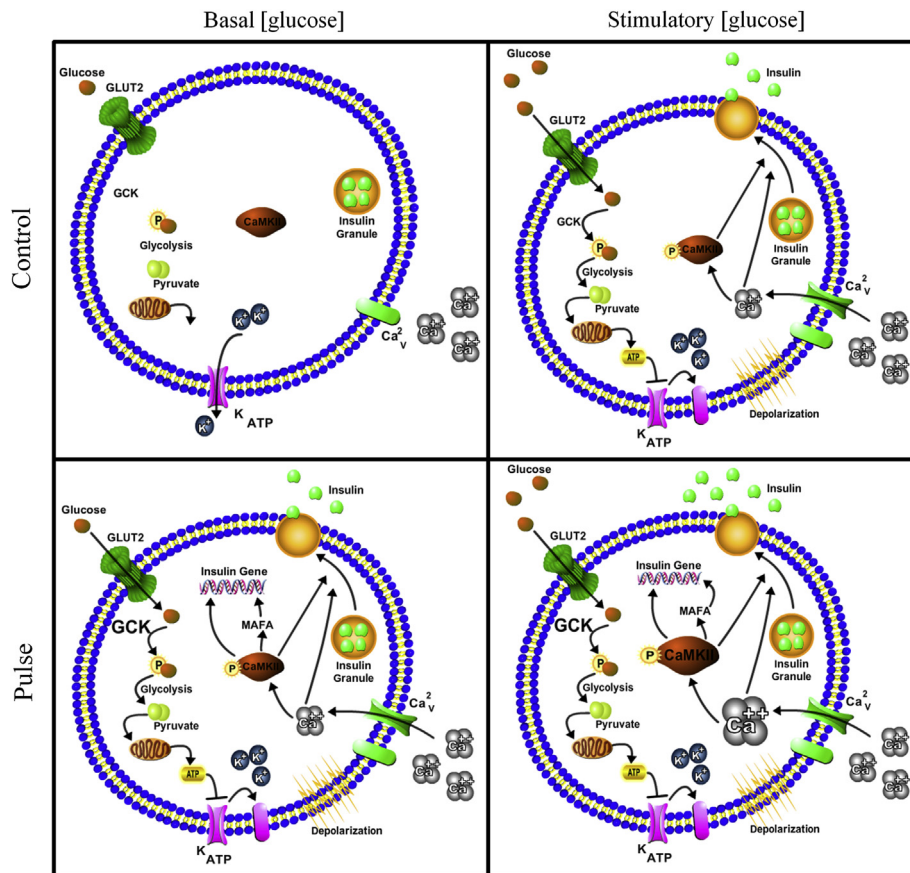


Figure 4: Model of the proposed mechanism of CaMKII-dependent memory formation in pancreatic β -cells. In low glucose concentrations (2.8 mM), control islets exhibit basal insulin secretion. Islets from the glucose pulse group show, even 24 h after the last high glucose pre-treatment, an increase in acute glucose-induced insulin secretion. This is due to the fact that β -cells in the pulse group have increased expression of GCK, calcium channels, and phosphorylation of CaMKII. When exposed to high glucose, islets from the group control increase insulin secretion because of the increased glycolytic flux through GCK and elevated ATP production. β -cells previously exposed to high glucose pulses have higher levels of GCK, calcium channel, Calcium influx, CaMKII phosphorylation, and therefore respond with higher insulin secretion to acute glucose.

increased subsequent insulin secretion [16,17]. However, these studies focused on analyzing acute effects of high glucose pre-treatment, and did not yet investigate the molecular mechanism underlying the phenomenon. Here, we demonstrate that human and mouse pancreatic β -cells are able to acquire, consolidate, and retrieve information, induced by high glucose exposure, and determined that this ability is dependent on CaMKII.

In conclusion, we have shown here that, like neurons, human and mouse β -cells are able to acquire, store, and retrieve information in a process similar to neuronal LTP. This process, presented schematically in Figure 4, is dependent on the activation of CaMKII, as is the case for neuronal memory. These findings provide further evidence that the similarity of neurons and β -cells on the transcriptome and epigenome level is not accidental, but an important aspect of the biology of these embryologically distinct cell types. Metabolic memory of β -cells likely represents a useful evolutionary adaptation to variation in food availability. As periods of food abundance in nature can vary dramatically in length, metabolic memory of repeated carbohydrate loads in β -cells ensures higher insulin secretion and thus more efficient uptake of excess glucose into skeletal muscle and adipose tissue for long-term storage, contributing to the adaptation to periods of starvation. Given the pivotal role of altered insulin secretion in metabolic disorders, future work will need to establish to what extent metabolic memory of pancreatic β -cells contributes to the pathophysiology of these diseases.

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CONFLICT OF INTEREST

None declared.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.molmet.2014.03.011>.

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