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Brief Report

Acute metabolic amplification of insulin secretion in mouse islets: Role of cytosolic acetyl-CoA

*Uwe Panten**, *Eike Früh*, *Kirstin Reckers*, *Ingo Rustenbeck*

Institute of Pharmacology, Toxicology and Clinical Pharmacy, Technical University of Braunschweig, Mendelssohnstrasse 1, D-38106 Braunschweig, Germany

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ABSTRACT

Objective. Stimulation of the ß-cell metabolism by glucose and other fuels triggers insulin release by enhancing the mitochondrial ATP production and acutely amplifies the secretory response by increase in mitochondrial export of metabolites. We aimed to narrow down the uniform final reaction steps mediating fuel-induced acute amplification of insulin secretion.

Material/Methods. Insulin secretion and metabolic parameters were measured in isolated mouse islets exposed to the sulfonylurea glipizide in high concentration (closing all ATP-sensitive K^+ channels) during the entire experiment. Fuel-induced effects were examined after treating the islets for one hour with medium devoid of fuels. This experimental design prevented acute amplification, but only when glucose was the sole fuel.

Results. Strong amplification of insulin secretion by α-ketoisocaproate or glucose combined with α-ketoisovalerate (supplying mitochondrial oxaloacetate) was abolished within 14 min after transition to medium devoid of fuels. After transition from medium containing glucose plus α-ketoisovalerate to medium containing solely glucose or α-ketoisovalerate, amplification (strong or weak, respectively) occurred until the end of the experiment. Glucose (alone or combined with α-ketoisovalerate) increased the total acetyl-CoA content as intensely as α-ketoisocaproate. Low concentrations of α-ketoisovalerate or α-ketoisocaproate were sufficient for saturation of acetyl-CoA increase, but caused no or only weak amplification, respectively. No acetyl-CoA increases occurred in the absence of glipizide.

Conclusions. Glucose and other fuels regulate acute amplification of insulin secretion by controlling the supply of acetyl-CoA to the ß-cell cytosol. Cytosolic acetyl-CoA does not amplify by serving as substrate for syntheses of metabolic intermediates, but amplifies by acting as substrate for cytosolic protein acetylation.

1. Introduction

Stimulation of the ß-cell metabolism by glucose and other fuels triggers insulin release and amplifies the secretory response [1]. Triggering results from increase in the cytosolic Ca^{2+} concentration, initiated by enhanced mitochondrial ATP production, whereas the action of elevated cytosolic Ca^{2+} is amplified by a mechanism incompletely understood. Many metabolic path-

Abbreviations: de-SUMOylation, enzymatic removal of SUMO peptides from proteins; Golgi/ER, lumen of Golgi apparatus and endoplasmic reticulum; KIC, α-ketoisocaproate; KIV, α-ketoisovalerate; RhoGDI, Rho guanine nucleotide dissociation inhibitor.

* *Corresponding author*. Tel.: +49 531 3915669; fax: +49 531 3918182.
E-mail address: u.panten@tu-bs.de (U. Panten).

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ways have been suggested to mediate fuel-induced amplification of insulin secretion [1–6]. We previously presented evidence that acute metabolic amplification results from mitochondrial export of metabolites, but not from mitochondrial energy generation [7]. This finding suggested that export of citrate and acetoacetate, both sources for cytosolic acetyl-CoA in ß-cells, mediates acute metabolic amplification. There were objections against mediation by malate, succinate, α-ketoglutarate or isocitrate in the cytosol [7]. Recently, cytosolic isocitrate has been reported to amplify by activating a de-SUMOylation pathway [8]. But this pathway mediated at most a small proportion of glucose-induced amplification (Fig. 8C in [8]), and recording of exocytotic responses to metabolic intermediates included intracellular application of 125 mmol/L glutamate. Among other things, this extremely high concentration activates cytosolic transaminations and in this way traps oxaloacetate and generates α-ketoglutarate. Thereby acetyl-CoA production by citrate lyase is favored and production of α-ketoglutarate and NADPH by cytosolic isocitrate dehydrogenase is inhibited. These experiments did not support amplification via isocitrate-induced generation of cytosolic NADPH. To further narrow down the uniform final reaction steps mediating fuel-induced acute amplification of insulin secretion, we examined which metabolic pathways involving cytosolic acetyl-CoA might mediate acute metabolic amplification of insulin secretion.

2. Methods

2.1. Chemicals and Media

Sigma/Fluka (Taufkirchen, Germany) provided N-ethylmaleimide, glutathion, oxaloacetate, acetylphosphate, phosphotransacetylase, citratesynthase and acetyl-CoA (sodium salt). Sources of other chemicals and composition of basal medium were previously described [7]. Amplification of insulin secretion was examined by exposing isolated islets to a high (2.7 μmol/L) glipizide concentration (closing all ATP-sensitive K^+ channels) during the entire experiment.

2.2. Islet Isolation

Albino mice (NMRI) were purchased and bred as described [7]. The study was conducted in accordance with the Principles of Laboratory Care, approved by the responsible authorities. Pancreatic islets from female mice (12–15 weeks old, fed an unrestricted diet) were isolated by collagenase digestion (presence of 5 mmol/L glucose) and hand-picked under a stereomicroscope.

2.3. Insulin Secretion

Insulin secretion from freshly isolated islets was measured by perifusion at a flow rate of 0.9 mL/min as described [7].

2.4. ATP/ADP Ratio

Preincubation and incubation of freshly isolated islets and measurement of the islet ATP and ADP content were performed as described [7]. For each single experiment, the ATP/ADP ratio was calculated from the measured ATP and ADP content.

2.5. Acetyl-CoA Content

Freshly isolated islets were preincubated and incubated as described for measurement of citrate content [7]. Incubations were stopped by centrifuging the incubation tube and washing the islets once with basal medium [7]. To the 10 μl of medium containing the islets were added 40 μl of 15 mmol/L HCl, followed by vortex mixing, 5 min at 97 °C, cooling for 5 min (water of room temperature) and centrifuging for 5 min (20,000 g, 2 °C). In aliquots of the supernatant the acetyl-CoA content was measured (modified version of an enzymatic cycling method [9]). 20 μl of supernatant (or appropriate dilutions) + 5 μl N-ethylmaleimide (0.1 mmol/L, dissolved in 0.5 mol/L Tris–HCl-buffer, pH 7.6) were vortexed, incubated for 12 min at 30 °C and cooled (ice bath). Cycling followed without delay and was started by adding 25 μl cycling reagent (100 mmol/L Tris–HCl-buffer, pH 7.4, 40 mmol/L NH_4^+, 10 μmol/L glutathion, 2.4 mmol/L oxaloacetate, 4 mmol/L acetylphosphate, 0.04% BSA, 32 U/ml phosphotransacetylase, 4 U/ml citratesynthase) and vortex mixing. Cycling at 30 °C lasted 60 min and was stopped by 5 min at 97 °C, followed by cooling (ice bath) and centrifuging for 5 min (20,000 g, 2 °C). The citrate in aliquots of the supernatant was measured as described [7]. Appropriate controls and standards were run in parallel.

2.6. Statistical Analysis

Values are presented as mean ± SEM. Differences between means were analyzed by ANOVA, followed by post-hoc tests (t-test and t-test for paired observations, both two-tailed, including the Bonferroni-Holm procedure for multiple comparisons). Statistical significance was assumed at $P < 0.05$.

3. Results and Discussion

As regulation of insulin secretion by acute metabolic amplification requires quick reversal of amplification when the stimulating exogenous fuel is reduced, we examined the secretory kinetics caused by finishing application of amplifying fuels. Glucose (30 mmol/L) combined with KIV (10 mmol/L, supplying mitochondrial oxaloacetate) or KIC (10 mmol/L) amplified the insulin secretion from time 68 min to time 80 min in each single experiment (Fig. 1A). These amplifications were abolished within 14 min after transition to medium devoid of fuels. After transition from medium containing glucose plus KIV to medium containing solely glucose or KIV, amplification decreased, but took place until the end of the perifusion (Fig. 1A). Catabolites inhibiting pyruvate dehydrogenase (observed in non-ß-cells [10]) are generated by the strong KIV oxidation in mouse islets [11] and might explain why KIV alone maintained low mitochondrial acetyl-CoA production, thus inducing only weak amplification (Fig. 1A, [7]). The decrease in amplification after transition to glucose alone (Fig. 1A) might have been caused by suboptimal supply of oxaloacetate after removal of KIV, the metabolism of which provides oxaloacetate (supporting amplification by acetyl-CoA produced via pyruvate dehydrogenase [7]). But due to the previous replenishment of citrate cycle intermediates by KIV, pyruvate carboxylase activity was probably high enough to