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Metabolic amplification of insulin secretion is differentially desensitized by depolarization in the absence of exogenous fuels

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ABSTRACT

Objective. The metabolic amplification of insulin secretion is the sequence of events which enables the secretory response to a fuel secretagogue to exceed the secretory response to a purely depolarizing stimulus. The signals in this pathway are incompletely understood. Here, we have characterized an experimental procedure by which the amplifying response to glucose is reversibly desensitized, while the response to α-ketoisocaproic acid (KIC) is unchanged.

Materials/Methods. Insulin secretion, NAD(P)H- and FAD-autofluorescence, Fura-2 fluorescence and oxygen consumption were measured in perifused NMRI mouse islets. The ATP- and ADP-contents were measured in statically incubated mouse islets. All islets were freshly isolated.

Results. While the original observation on the dissociation between glucose- and KIC-amplification was obtained with islets that had been exposed to a high concentration of the sulfonylurea glipizide in the absence of glucose, we now show that in the absence of exogenous fuel a moderate depolarization, irrespective of its mechanism, progressively decreased the amplification in response to both glucose and KIC. However, the amplification in response to glucose declined faster, so a time window exists where glucose was already inefficient, whereas KIC was of unimpaired efficiency. Measurements of adenine nucleotides, NAD(P)H- and FAD-autofluorescence, and oxygen consumption point to a central role of the mitochondrial metabolism in this process. The desensitization could be quickly reversed by increasing oxidative deamination of glutamate and consequently anaplerosis of the citrate cycle.

Conclusion. Depolarization in the absence of exogenous fuel may be a useful model to identify those signals which are indispensable for the generation of metabolic amplification.

1. Introduction

The mechanism by which the pancreatic beta-cell recognizes its main physiological stimulus, glucose, and transforms glucose concentrations into rates of insulin secretion requires the metabolic breakdown of the stimulus [1]. Early on, it was hypothesized that the mitochondrial metabolism of glucose is of decisive importance, supported by the observation that leucine

Abbreviations: $[Ca^{2+}]_i$, cytosolic Ca^{2+} concentration; BCH, 2-Amino-2-norbornanecarboxylic acid; K_{ATP} channel, ATP-sensitive K^+ channel; KIC, α-ketoisocaproic acid.

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and α-ketoisocaproic acid (KIC), which generate reducing equivalents in the mitochondria, stimulate insulin secretion in the absence of glucose or other exogenous fuels [2–4]. Together, these stimuli can be classified as fuel secretagogues.

The mitochondrial metabolism and the fuel-induced electrical activity of the beta-cell are linked by the ATP-sensitive K+ channel (K_{ATP} channel) of the plasma membrane [5]. In intact beta-cells both glucose and KIC inhibit this channel [6,7], glucose most likely via increased ATP generation, KIC probably also via a direct inhibitory effect [8]. The ensuing depolarization leads to Ca^{2+} influx via voltage-dependent Ca^{2+} channels and to Ca^{2+}-triggered exocytosis [9]. However, glucose is able to increase insulin secretion beyond the extent established by plasma membrane depolarization alone. Two different experimental approaches were used to establish a depolarization prior to the exposure to fuel secretagogues: either K_{ATP} channels were blocked by a sulfonylurea [10] or K_{ATP} channels were opened by diazoxide to permit a depolarization by high extracellular potassium [11]. The name "amplifying pathway" has become widely accepted for this signaling, based on the evidence that it does not increase insulin secretion if the plasma membrane is not depolarized, which under physiological conditions requires the closure of the K_{ATP} channels [12]. The pathway leading to the K_{ATP} channel closure and Ca^{2+} influx, in contrast, was termed "triggering pathway" [12].

While a consensus exists on the general outline of the triggering pathway, a generally accepted model of the amplifying pathway has not yet emerged. There is a consensus that the export of metabolites from the mitochondrial matrix into the cytosol (cataplerosis) is of importance [13]. Some authors view the cataplerosis as part of one or more cycles of export and re-import of metabolites to convey reducing equivalents out of the mitochondria to ultimately increase the cytosolic levels of NADPH [14,15]. NADPH is discussed as regulator of exocytosis in beta-cells via the SUMO protein [16,17]. However, the high activity of the anaplerotic enzyme pyruvate carboxylase in beta-cells [18] is not necessarily linked to such metabolite cycling, but could as well serve to compensate for the export of metabolites. This would be the case when cytosolic levels of such compounds as glutamate [19], α-ketoglutarate [20] or short-chain acylCoA [21] would confer the signal of metabolic amplification. The recent availability of metabolomic data [22–24] has not yet led to a clarification in this respect.

To identify metabolites which confer the amplification signal we have investigated the phenomenon that exposure of islets to the sulfonylurea glipizide in the absence of glucose abolishes the insulinotropic effect of a subsequent stimulation by glucose but not KIC [25]. Originally, this protocol was conceived to test whether only glucose or fuel secretagogues in general increase insulin secretion beyond the extent established by K_{ATP} channel closure. Thus, glucose was removed one hour before the stimulation by KIC to minimize the metabolic interference. Unexpectedly, 30 mM glucose turned out to be ineffective after this pretreatment, whereas 10 mM KIC strongly increased insulin secretion, which in view of the pre-existent depolarization must be ascribed to metabolic amplification [25]. However, the mechanisms, by which the pretreatment leads to the desensitization to glucose but not to fuel secretagogues in general, have not yet been characterized. The observations in this study suggest that any moderate depolarization in the absence of exogenous fuel progressively decreases the activity of the citrate cycle. This becomes faster critical for the insulinotropic effect of glucose than for that of KIC. This functional difference offers the perspective to identify the metabolites that convey the signal of metabolic amplification [26].

2. Methods

2.1. Chemicals

Collagenase NB8 for islet isolation was purchased from Serva (Heidelberg, Germany). Fura-2 LeakRes (AM) was obtained from TEF-Labs (Austin, TX, USA). Luciferase kit for ATP determination, pyruvate kinase, tolbutamide and (±)-BCH (2-Amino-2-norbornanecarboxylic acid) were from Sigma. All other reagents of analytical grade were from E. Merck (Darmstadt, Germany).

2.2. Tissue

Islets were isolated from the pancreas of SUR1 (–/–) mice [27] or NMRI mice (12–16 weeks old, fed ad libitum) by a collagenase digestion technique and hand-picked under a stereomicroscope. For all experiments freshly isolated islets were used. Animal care was supervised by the regional authority (LAVES, Lower Saxony, Germany) and conformed to the current EU regulations.

2.3. Measurement of Insulin Secretion

Batches of 50 islets were introduced into a purpose-made perifusion chamber (37 °C) and perifused at 0.9 ml/min with a HEPES-buffered Krebs-Ringer medium which was saturated with 95% O_2 and 5% CO_2 and contained the respective secretagogue and 2 mg/ml bovine serum albumin (for details see ref. 28). The insulin content in the fractionated efflux was determined by ELISA according to the manufacturer's protocol (Mercodia, Uppsala, Sweden).

2.4. Measurement of Islet NAD(P)H- and FAD-Autofluorescence

Autofluorescence of NAD(P)H (=NADH + NADPH) and FAD [3,29,30] was simultaneously recorded. Freshly isolated islets were placed on a nylon mesh in a purpose-made perifusion chamber on the stage of an Orthoplan epifluorescence microscope (Leitz, Wetzlar, Germany) and perifused at 0.2 ml/min and 35 °C. Fluorescence was excited with a mercury arc (Hanau ST41) using the following filter combinations (Omega Optical, Brattleboro, VT, USA): for NAD(P)H, excitation 366 ± 15 nm bandpass, dichroic separation 405 nm, emission 450 ± 32 nm bandpass; for FAD, excitation 440 ± 21 nm bandpass, dichroic separation 455 nm, emission 520 ± 20 nm bandpass. The filter cubes were switched every 2.5 s with an exposure time of 0.1 s. The use of freshly isolated islets, which were not attached to a glass cover slip, required the use of a low magnification objective with a long working distance (Zeiss Fluar 10×, 0.5 N.A.). Calculation of mean values and normalization of the data were performed by Prism 4 software (GraphPad, San Diego, CA, USA).