Biochemical and Biophysical Research Communications 445 (2014) 629–632

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Activation of alpha adrenergic and muscarinic receptors modifies early glucose suppression of cytoplasmic Ca^{2+} in pancreatic β-cells

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ARTICLE INFO

Article history:
Received 4 February 2014
Available online 21 February 2014

Keywords:
Acetylcholine
β-cell
Cytoplasmic Ca^{2+}
Epinephrine
Glucose
Ouabain

ABSTRACT

Elevation of glucose induces transient inhibition of insulin release by lowering cytoplasmic Ca^{2+} ($[Ca^{2+}]_i$) below baseline in pancreatic β-cells. The period of $[Ca^{2+}]_i$ decrease (phase 0) coincides with increased glucagon release and is therefore the starting point for antisynchronous pulses of insulin and glucagon. We now examine if activation of adrenergic α_{2A} and muscarinic M_3 receptors affects the initial $[Ca^{2+}]_i$ response to increase of glucose from 3 to 20 mM in β-cells situated in mouse islets. In the absence of receptor stimulation the elevation of glucose lowered $[Ca^{2+}]_i$ during 90–120 s followed by rise due to opening of voltage-dependent Ca^{2+} channels. The period of $[Ca^{2+}]_i$ decrease was prolonged by activation of the α_{2A} adrenergic receptors (1 μM epinephrine or 100 nM clonidine) and shortened by stimulation of the muscarinic M_3 receptors (0.1 μM acetylcholine). The latter effect was mimicked by the Na/K pump inhibitor ouabain (10–100 μM). The results indicate that prolonged initial decrease (phase 0) is followed by slow $[Ca^{2+}]_i$ rise and shorter decrease followed by fast rise. It is concluded that the period of initial decrease of $[Ca^{2+}]_i$ regulates the subsequent β-cell response to glucose.

1. Introduction

The insulin secretory response to glucose is traditionally divided into a first-phase with a prominent peak of insulin and a second-phase with repetitive pulses of the hormone. The first-phase release is supposed to be essential for glucose homeostasis by allowing prompt inhibition of the hepatic glucose production [1–3]. A low or absent first-phase peak is strongly predictive of type-1 diabetes in subjects with islet autoantibodies and a family history of the disease [4,5]. Attempts to re-establish the early rise of plasma insulin has been reported to improve the glucose control in patients with type-2 diabetes [3,6].

Glucose stimulation of β-cell release of insulin is mediated by increase of the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$). It was early observed that the voltage-dependent entry of Ca^{2+} is preceded by decrease of $[Ca^{2+}]_i$ with resulting inhibition of insulin release [7]. The initial suppression of $[Ca^{2+}]_i$, also referred to as phase 0 [8,9], is the starting point for 4–5 min pulses of insulin appearing in antisynchrony with glucagon [10–12]. So far the standardized intravenous glucose tolerance test is the technique of choice to register low or absent first-phase peak of insulin release [13].

Glucose-induced lowering of $[Ca^{2+}]_i$ is critically dependent on stimulated uptake of Ca^{2+} in ER. The importance of ER is evident from the absence of $[Ca^{2+}]_i$ decrease when the SERCA pump is inhibited by thapsigargin [8,14]. Glucose is equally effective in promptly inhibiting release of insulin from mouse [15] and human [11] islets. Initial suppression of $[Ca^{2+}]_i$ is a physiological phenomenon characteristic of healthy β-cells [12]. Absence of initial lowering of $[Ca^{2+}]_i$ has been reported in islets from spontaneously diabetic mice and rats [16,17] as well as in islets from rats made diabetic by neonatal injections of streptozotocin [17]. Other studies have shown decreased expression of SERCA2 in islets from patients with type-2 diabetes [18].

The observation that glucose generates prompt decrease of $[Ca^{2+}]_i$ makes it necessary to re-examine existing ideas why first-phase secretion is essential for glucose homeostasis. An important question is how the introductory lowering of $[Ca^{2+}]_i$ modifies the β-cell response to glucose. We now report that activation of the α_{2A} adrenergic receptors prolongs the glucose-induced decrease followed by slower rise of $[Ca^{2+}]_i$ and that exposure to acetylcholine or ouabain has the opposite effect.

2. Material and methods

2.1. Isolation and culture of islets

Pancreatic islets were obtained from 3 to 4 months old female C57Bl mice. The animals were killed by decapitation and the islets were isolated from pieces of pancreas by collagenase digestion. The

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http://dx.doi.org/10.1016/j.bbrc.2014.02.056
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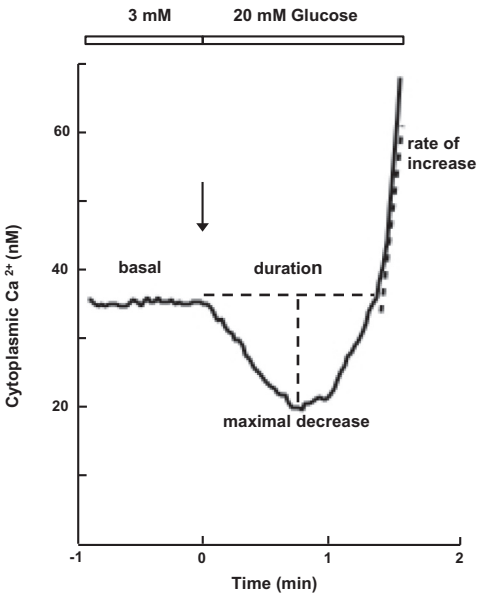

Fig. 1. Design of experiments for studying the alterations of $[Ca^{2+}]_i$ obtained by raising the glucose concentration from 3 to 20 mM. The following parameters are recorded: basal level (average $[Ca^{2+}]_i$ in nM during the 2 min period preceding the increase of glucose), duration (sec) and maximal decrease (nM) during phase 0 and rate of subsequent increase (nM/s).

islets were cultured free floating for 1 or 2 days at 37 °C in RPMI 1640 medium containing 11 mM glucose, 10% fetal calf serum, 100 IU/ml penicillin and 100 μg/ml streptomycin in an atmosphere of 5% CO_2 in humidified air. The local ethics committee approved the experimental procedures for the animal handling.

2.2. Measurements of cytoplasmic Ca^{2+}

The experiments were performed with a basal medium containing 3 mM glucose, 0.5 mg/ml bovine serum albumin and (in mM) 125 NaCl, 4.8 KCl, 1.3 $CaCl_2$, 1.2 $MgCl_2$, 3 glucose and 25 HEPES with pH adjusted to 7.40 using NaOH. The islets were loaded during 60–80 min with 0.6–1.0 μM of the acetoxymethyl ester of Fura-2 LeakRes (Teflab, Austin, TX) in the presence of 0.01% Pluronic acid. The islets were then allowed to attach to poly-L-lysine-coated coverslips and subsequently used for $[Ca^{2+}]_i$ measurements with similar medium lacking indicator. The coverslips with the attached islets were used as exchangeable bottoms of open chambers superfused at a rate set to obtain 40 s rise time for added glucose. A six channel multi-valve system (Warner Instruments, Hamden, CT) was used for exchange of medium.

2.3. Experimental design

An introductory period of 15 min with superfusion of the islets in absence or presence of test substances was followed by increase of the glucose concentration from 3 to 20 mM. The design of the experiments is shown in Fig. 1. Data are provided for basal $[Ca^{2+}]_i$ (nM) during a 2 min period preceding the glucose elevation, the duration (sec) and maximal decrease (nM) of $[Ca^{2+}]_i$ and the steepest rise of $[Ca^{2+}]_i$ (nM/s) obtained by depolarization related to the closure of the K_{ATP} channels. Results are presented as mean values ± SEM.

3. Results

The effects of raising glucose from 3 to 20 mM on $[Ca^{2+}]_i$ in mouse islets kept in culture for 1–2 days are summarized in Table 1. In the absence of additives the glucose increase rapidly suppressed basal $[Ca^{2+}]_i$ with 36 ± 2%. After 90–120 s of $[Ca^{2+}]_i$ decrease (phase 0) there was a marked rise due to entry of Ca^{2+} (Fig. 1).

The period of glucose-induced suppression was markedly prolonged when α_{2A}-adrenergic receptors were activated with epinephrine (Fig. 2A). Similar effects were obtained with the more specific inhibitor clonidine (Fig. 2 B). Both inhibitors diminished the rate of the following $[Ca^{2+}]_i$ increase ($P < 0.001$). The glucose-induced suppression of $[Ca^{2+}]_i$ was affected also by acetylcholine (Fig. 3). This compound did not prolong but shortened the period of decrease. In the presence of 0.1 μM acetylcholine the duration of the $[Ca^{2+}]_i$ decrease was reduced with about 50% ($P < 0.001$) and the rate of the subsequent rise more than doubled ($P < 0.001$). At high concentrations of acetylcholine (>1 μM) the initial $[Ca^{2+}]_i$ decrease was reversed into stimulation and the subsequent rise slower than in controls ($P < 0.001$).

The presence of ouabain (10 or 100 μM) modified the glucose-induced $[Ca^{2+}]_i$ response in a similar way as seen with 0.1 μM acetylcholine (Fig. 2C). The period of the initial $[Ca^{2+}]_i$ decrease was reduced with almost 50% and subsequent rise faster than the control value ($P < 0.001$).

4. Discussion

Elevation of glucose promptly lowers $[Ca^{2+}]_i$ in isolated β- and δ-cells but not in α-cells [19]. Within the islets most of the endocrine cells are β-cells and the various types of cells known to interact via electrical coupling and paracrine signaling. The importance of performing the studies with intact islets is emphasized from the observation that the glucose-induced inhibition of insulin release coincides with release of glucagon [10]. We now observe that glucose elevation from 3 to 20 mM lowers $[Ca^{2+}]_i$ during

Table 1
Early effects of glucose on cytoplasmic Ca^{2+} in islets exposed to various compounds.

Compound	Basal (nM)	Maximal decrease during phase 0 (nM)	Duration of phase 0 (sec)	Subsequent rate of increase (nM/s)
Control (30)	41.9 ± 1.6	14.8 ± 0.6	103.4 ± 5.0	3.8 ± 0.3
1 μM epinephrine (14)	42.6 ± 2.6	17.6 ± 0.8	229.2 ± 25.6	2.0 ± 0.2
100 nM clonidine (19)	38.5 ± 2.4	11.7 ± 1.0	236.0 ± 19.0	1.4 ± 0.2
10 nM acetylcholine (23)	46.0 ± 3.0	13.1 ± 0.7	97.7 ± 4.4	3.7 ± 0.3
0.1 μM acetylcholine (33)	42.2 ± 1.4	13.5 ± 0.8	58.6 ± 5.3	8.9 ± 0.8
1 μM acetylcholine (18)	54.4 ± 4.4	(increase 3.7 ± 1.4)	42.3 ± 7.8	1.7 ± 0.3
10 μM acetylcholine (19)	53.8 ± 2.5	(increase 12.0 ± 0.7)	63.4 ± 2.6	1.8 ± 0.2
100 μM acetylcholine (15)	64.3 ± 4.1	–	–	0.7 ± 0.1
10 μM ouabain (10)	45.3 ± 2.9	15.7 ± 1.0	65.1 ± 2.6	7.0 ± 1.0
100 μM ouabain (16)	50.7 ± 5.1	9.7 ± 0.7	41.4 ± 3.2	4.4 ± 0.5

The decrease of $[Ca^{2+}]_i$ is presented as the maximal lowering from the basal level during phase 0. There was no lowering but an increase of $[Ca^{2+}]_i$ in the presence of 1 and 10 μM acetylcholine. The number of islets studied is given within parenthesis.

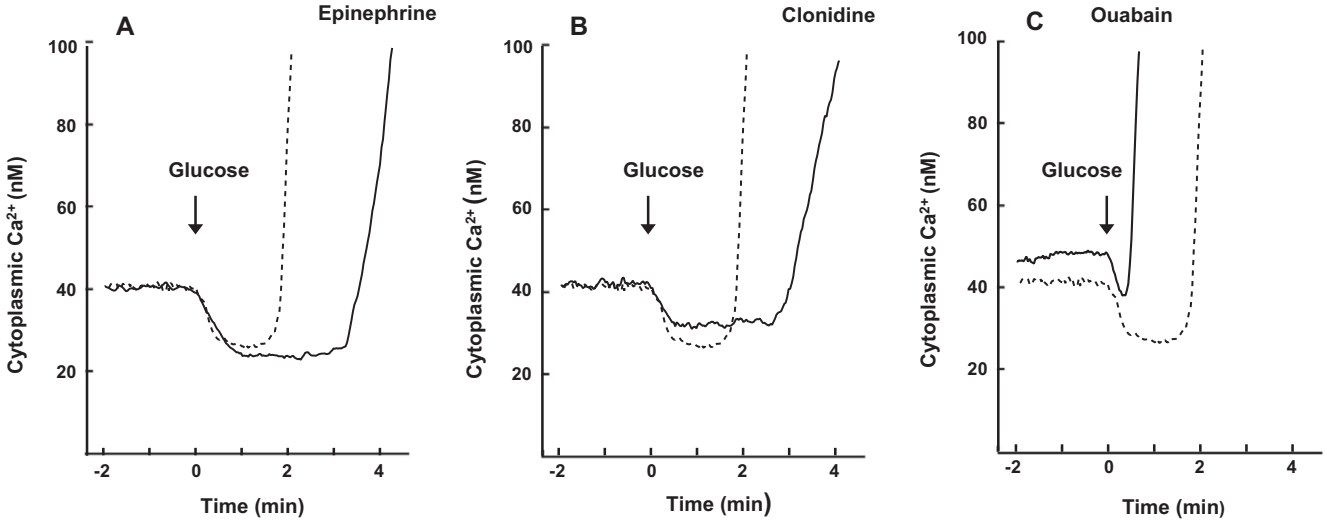

Fig. 2. Effects of raising the glucose concentration from 3 to 20 mM in the presence of 1 μM epinephrine (A), 100 nM clonidine (B) and 100 μM ouabain (C). The result from a control experiment (dotted line) is included.

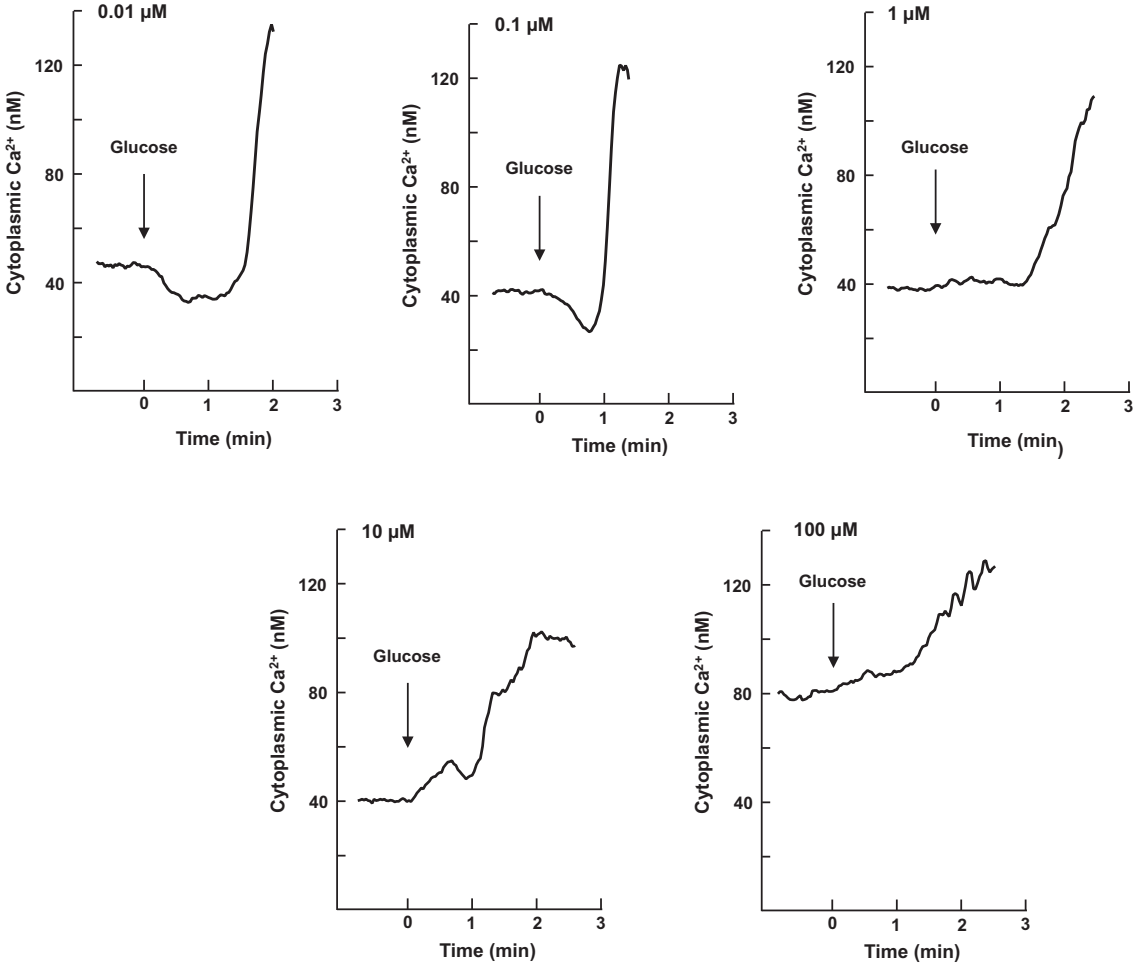

Fig. 3. Effects of raising the glucose concentration from 3 to 20 mM in the presence of various concentrations of acetylcholine.

90–120 s in β-cells situated in islets. After this decrease there is a rise due to depolarization with accompanying entry of Ca^{2+}.

During exposure to epinephrine, or the more specific α_{2A} adrenergic agonist clonidine, the glucose-induced $[Ca^{2+}]_i$ decrease was markedly prolonged and the following rise slower. Various mechanism may contribute to the observed prolongation of the $[Ca^{2+}]_i$ decrease: (1) hyperpolarization by activation of K^+ channels, (2) lowering of cyclic AMP and (3) inhibition of Ca^{2+} channels. More-

over, heterotrimeric G-proteins coupled to α_{2A} adrenergic receptors have effects of their own. The $\beta\gamma$ subunits are reported to interfere with the release of secretory granules by inhibition of synaptotagmin binding and blockade of the SNARE protein function [20]. It was recently shown that the ATP released during exocytosis activates purinergic $P2Y_1$ receptors with resulting generation of diacylglycerol [21]. Acting as a stimulator of protein kinase C, diacylglycerol both counteracts α_{2A} adrenergic inhibition of insulin secretion [22,23] and increases the number of secretory granules ready for release [24].

Like external ATP, exposure to acetylcholine results in generation of diacylglycerol. In this case activation of muscarinic M_3 receptors generates diacylglycerol together with IP_3 via G-protein-dependent phospholipase C [25,26]. We now observe that 0.1 μM acetylcholine shortens the period of glucose-induced suppression of $[Ca^{2+}]_i$. This action can be attributed to suppression of net uptake of Ca^{2+} in ER, a conclusion consistent with the report of markedly enhanced efflux of the ion from this organelle during cholinergic stimulation of the IP_3 receptors [27]. Activation of the M_3 receptor has effects on β-cells additional to those mediated by coupling to G-protein. The phosphorylated form of the receptor activates protein kinase D1, which is important for biogenesis of insulin secretory granules [28,29]. Another G-protein-independent effect is activation of Na^+ channels via the Src family of tyrosine kinases [30,31]. Exposure to carbachol raises the sodium content with 25–40% in mouse islets exposed to 20 mM glucose [32]. The presence of a prominent entry of Na^+ may well explain why high concentrations (>1 μM) of acetylcholine reverse glucose-induced suppression of $[Ca^{2+}]_i$ to increase.

Ouabain has a depolarizing action on β-cells by inhibiting the Na/K-ATPase activity. Besides to function as an electrogenic ion pump, Na/K-ATPase relays messages from the plasma membrane to intracellular organelles through several signal pathways [33]. It is possible that β-cells, like other types of cells [34,35], have microdomains of Na/K-ATPase tethered to the IP_3 receptors. In this case, ouabain promotes interaction between Na/K-ATPase and the IP_3 receptors with resulting $[Ca^{2+}]_i$ increase. Such microdomains are stabilized by ankyrin B, a scaffolding protein found in β- but absent in α- and δ-cells [36].

Comparing compounds with different effects on insulin release it was found that activation of adrenergic α_{2A} receptors (epinephrine and clonidine) prolongs the glucose-induced suppression of $[Ca^{2+}]_i$ and that acetylcholine stimulation of muscarinic M_3 receptors has the opposite effect. In the latter case the period of inhibition was reduced with 50% and the rate of subsequent $[Ca^{2+}]_i$ rise more than doubled. It will be a matter for future studies to decide if a certain period of $[Ca^{2+}]_i$ decrease is a prerequisite for the anti-phase relation between insulin and glucagon pulses.

Acknowledgment

The authors acknowledge the support from the Swedish Research Council (55X-06240).

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