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Somatostatin promotes glucose generation of Ca^{2+} oscillations in pancreatic islets both in the absence and presence of tolbutamide

Bo Hellman*, Heléne Dansk, Eva Grapengiesser

Department of Medical Cell Biology, Uppsala University, Biomedicum Box 571, SE-75123 Uppsala, Sweden

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ABSTRACT

Many cellular processes, including pulsatile release of insulin, are triggered by increase of cytoplasmic Ca^{2+}. This study examines how somatostatin affects glucose generation of cytoplasmic Ca^{2+} oscillations in mouse islets in absence and presence of tolbutamide blockade of the K_{ATP} channels. Ca^{2+} was measured with dual wavelength microflurometry in isolated islets loaded with the indicator Fura-2. Rise of glucose from 3 to 20 mM evoked introductory lowering of Ca^{2+} prolonged by activation of somatostatin receptors. During continued superfusion exposure to somatostatin triggered oscillations mediated by periodic increase from the basal level (absence of tolbutamide) or by periodic interruption of an elevated level (presence of tolbutamide). In the latter situation the oscillations were transformed into sustained elevation by activation of muscarinic receptors (acetylcholine) or increase of cyclic AMP (IBMX, 8-bromo-cyclic AMP, forskolin). The observed effect of cyclic AMP raises the question whether high proportions of the glucagon-producing α-cells promote steady-state elevation of Ca^{2+}. In support for this idea somatostatin was found to trigger glucose-induced Ca^{2+} oscillations essentially in small islets that contain very few α-cells. The results indicate that somatostatin promotes glucose generation of Ca^{2+} oscillations with similar characteristics both in the absence and presence of functional K_{ATP} channels.

1. Introduction

Insulin-producing β-cells are oscillators entrained into a common rhythm by gap junctions and diffusible factors [1]. Typically, increase of glucose generates periodic variations of the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) resulting in pulsatile release of insulin. It is well established that increased metabolism of glucose depolarises β-cells by rhythmic closure of the K_{ATP} channels accompanied by entry of Ca^{2+} via voltage-activated channels. However, glucose generates rhythmic variations of the membrane potential and $[Ca^{2+}]_i$ also after knockout of the K_{ATP} channels [2–5] and when these channels are closed with sulfonylurea [6]. Absence of functional K_{ATP} channels is accompanied by increased membrane resistance, which makes islet cells sensitive to opening of low conductance ion channels [7].

Rapid elevation of glucose results in brief (60–90 s) initial decrease of $[Ca^{2+}]_i$ from the basal level by activation of the SERCA pump [8–10]. This decrease is the starting point for the antiphase relation between insulin and glucagon pulses [6,11]. After tolbutamide blockade of the K_{ATP} channels elevation of glucose lowers $[Ca^{2+}]_i$ from an elevated level during 3–5 min [6,12]. Both alternatives for $[Ca^{2+}]_i$ decrease are prolonged by activation of α_2 adrenergic receptors, an effect attributed to hyperpolarisation [11,12].

In attempts to understand how glucose regulates pulsatile release of the islet hormones the action of somatostatin deserves particular attention. In mouse islets subtypes of the somatostatin receptor are expressed not only in β- but also in α- and δ-cells [13,14]. It is well established that somatostatin release from δ-cells has a tonic inhibitory action on adjacent α-cells [15,16]. We now examine how somatostatin affects glucose generation of oscillatory Ca^{2+} signals also after tolbutamide blockade of the K_{ATP} channels. Both somatostatin and the analog octreotide promoted the appearance of oscillations mediated by periodic interruption of elevated $[Ca^{2+}]_i$. This process was reversibly antagonized by increase of cyclic AMP or addition of acetylcholine.

2. Methods

2.1. Isolation and handling of islets

Pancreatic islets were obtained from 3 to 4 months female C57BL/6 mice. The local ethics committee approved the experimental procedures for the animal handling. The mice were killed by decapitation and islets isolated from pieces of pancreas with collagenase. Most experiments

* Corresponding author.
E-mail address: bo.hellman@mcb.uu.se (B. Hellman).

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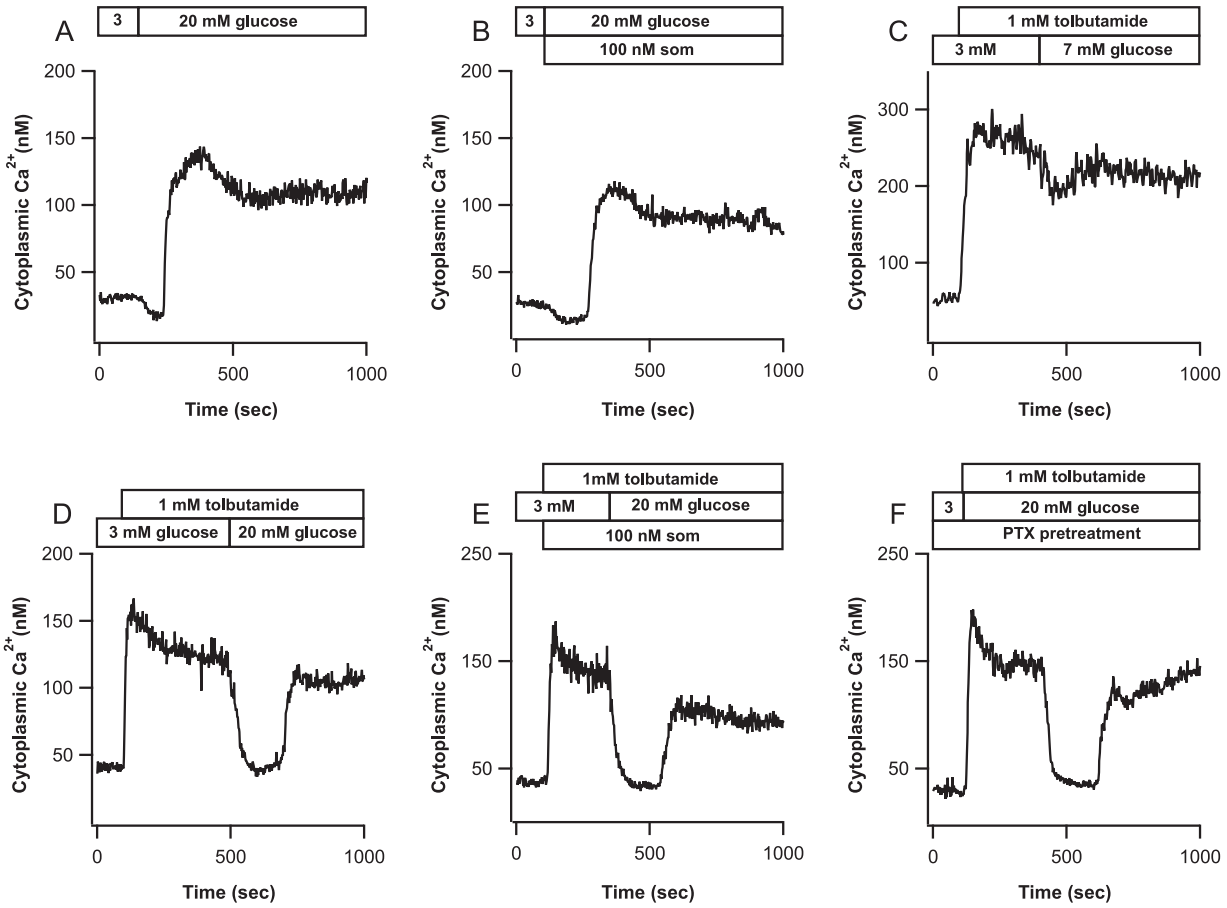

Fig. 1. Early alterations of $[Ca^{2+}]_i$ after raising glucose above 3 mM in the absence (A, B) and presence (C–F) of 1 mM tolbutamide. The data are representative for 45 (A), 17 (B), 34 (C), 42 (D), 37 (E) and 15 (F) islets.

were performed with medium-sized islets (diameter 55–85 µm). In some studies the responses of small (diameter < 55 µm) and big (diameter 120–150 µm) islets were compared. 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 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 $MgCl_2$ 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 LR (TEFLABS, Austin TX) in the presence of 0.01% Pluronic acid. After washing with basal medium the islets were attached to cover slips coated with poly-L-lysine. The cover slips with the attached islets were used as exchangeable bottoms of open chambers and superfused at a rate set to obtain 40 s rise time (10–90 %). Some batches of islets were pretreated for 20 h with 200 ng/ ml pertussis toxin to block the activation of somatostatin receptors by ADP ribosylation of the $G_{i/0}$ protein α-subunit. The experiments were performed with somatostatin-14 supplied by Sigma.

2.2. Measurements of cytoplasmic Ca^{2+}

A six channel multi-valve system (Warner Instruments, Hamden, Ct 06514, USA), was used for exchange of medium. The studies were performed at 37 °C using an inverted microscope (Nikon Diaphot) with a 40 X Fluor oil immersion objective (NA 1.3). The microscope was equipped with an epifluorescence illuminator connected through a 5 mm diameter liquid guide to an Optoscan monochromator (Cairn Research Ltd. Faversham, UK) with rapid grating and slit-width adjustment and a 150 W xenon arc lamp. The monochromator provided excitation light at 340 and 380 nm. Emission was measured at 510 nm (30-nm half-bandwidth) using a 400 nm dichroic beam splitter and a CCD camera (iXonEM, Andor Technology, Belfast, UK). The Metafluor software (Molecular Devices Corp. Downington, Pa) controlled the monochromator and the camera, acquiring pairs of 340 and 380 nm images from whole islets every two sec. Ratio frames were calculated after background subtraction and $[Ca^{2+}]_i$ was estimated assuming a K_D for the Ca^{2+}- Fura-2 LR complex of 250 nM.

2.3. Design of experiments

The experiments were designed essentially as described in previous studies of glucose generation of $[Ca^{2+}]_i$ oscillations in presence and absence of tolbutamide [6,12]. Somatostatin-14 was added to the medium together with or after exposure to tolbutamide. Results are presented as mean values ± SEM and the differences evaluated with *t*-test and chi-square test.

3. Results

3.1. Somatostatin regulation of introductory $[Ca^{2+}]_i$ decrease in presence and absence of tolbutamide

Early effects of glucose rise above 3 mM on the initial $[Ca^{2+}]_i$ decrease are shown in Fig.1. In absence of tolbutamide increase of glucose to 20 mM lowered $[Ca^{2+}]_i$ below the basal level during 121 ± 6 s (panel A). During exposure to 100 nM somatostatin the decrease was extended to 197 ± 6 s ($P < 0.01$; panel B). Rise of glucose from 3 to 7 mM in the presence of tolbutamide resulted in $[Ca^{2+}]_i$ decrease during 255 ± 7 s (panel C). Similar rise to 20 mM glucose lowered