FEBS Letters 586 (2012) 89–95

journal homepage: www.FEBSLetters.org

Disruption and stabilization of β-cell actin microfilaments differently influence insulin secretion triggered by intracellular Ca^{2+} mobilization or store-operated Ca^{2+} entry

Jean-Claude Henquin *, Nizar I. Mourad, Myriam Nenquin

Unit of Endocrinology and Metabolism, University of Louvain Faculty of Medicine, Brussels, Belgium

ARTICLE INFO

Article history:
Received 6 September 2011
Revised 8 November 2011
Accepted 26 November 2011
Available online 3 December 2011

Edited by Laszlo Nagy

Keywords:
Acetylcholine
Exocytosis
Insulin granule
Pancreatic islet
Store-operated Ca^{2+} entry

ABSTRACT

Latrunculin depolymerizes and jasplakinolide polymerizes β-cell actin microfilaments. Both increase insulin secretion when Ca^{2+} enters β-cells during depolarization by glucose, sulfonylureas or potassium. Mouse islets were held hyperpolarized with diazoxide, and stimulated with acetylcholine to test the role of microfilaments in insulin secretion triggered by intracellular Ca^{2+} mobilization and store-operated Ca^{2+} entry (SOCE).

Jasplakinolide slightly attenuated Ca^{2+} mobilization and did not affect SOCE, but consistently inhibited the attending insulin secretion. Latrunculin did not affect Ca^{2+} changes induced by acetylcholine, but consistently increased insulin secretion, its effect being larger in response to Ca^{2+} entry than to Ca^{2+} mobilization. Microfilaments have thus a distinct impact on exocytosis of insulin granules depending on the source of triggering Ca^{2+}.

1. Introduction

Pancreatic β-cells synthesize insulin and store it in large dense-core vesicles (the insulin granules) that are released at precisely controlled rates to ensure optimal glucose homeostasis. Glucose-induced insulin secretion is triggered by an increase in β-cell cytosolic free calcium concentration ($[Ca^{2+}]_c$) and amplified by incompletely elucidated mechanisms that augment the number of granules undergoing exocytosis in response to Ca^{2+} [review in 1]. This rise in $[Ca^{2+}]_c$ culminates a sequence of events involving glucose metabolism, closure of ATP-sensitive potassium (K_{ATP}) channels, depolarization, opening of voltage-gated calcium channels (VGCC) and Ca^{2+} influx [review in 2]. Non-metabolized agents that cause depolarization by directly closing K_{ATP} channels (sulfonylureas) or other mechanisms (high extracellular KCl) also increase $[Ca^{2+}]_c$ and trigger insulin secretion [3].

The rise of $[Ca^{2+}]_c$ in β-cells induces exocytosis of release-competent insulin granules. This pool of readily releasable granules is small (50–100 ie <1% of the ~10000 granules per β-cell) [4–6]. There is evidence that these granules are docked to the plasma membrane, associated with or at least in close proximity of VGCC [7–9] although recent studies using total internal reflection fluorescence microscopy indicate that slightly more distant (non-docked) granules are also releasable and even preferentially exocytosed when Ca^{2+} influx through VGCC is induced by glucose [10–12]. To sustain insulin secretion, the readily releasable pool must then be constantly refilled by mobilization of granules from other pools [4–6].

As in other endocrine cells [13], β-cell actin microfilaments form a web under the plasma membrane, which is thought to impede access of secretory vesicles to release sites. Thus, pharmacological destruction of this web increases the number of insulin granules close to the membrane [14,15] and augments rapid and sustained insulin secretion induced by glucose or sulfonylureas in islets [14–17], and by electrical depolarization in single cells [18]. Recently, we further established that not only depolymerization but also polymerization of actin microfilaments augments glucose and tolbutamide-induced insulin secretion, and that both do so without increasing the triggering $[Ca^{2+}]_c$ signal [19]. Collectively, these studies show that interference with actin microfilaments increases the number of granules that are releasable by Ca^{2+} influx via VGCC.

A rise in $[Ca^{2+}]_c$ can also occur independently of VGCC, by mobilization of Ca^{2+} from intracellular stores and subsequent

Abbreviations: ACh, acetylcholine; $[Ca^{2+}]_c$, cytosolic free calcium concentration; VGCC, voltage-gated calcium channel; SOCE, store-operated Ca^{2+} entry; K_{ATP} channels, ATP-sensitive potassium channels

* Corresponding author. Address: Unité d'Endocrinologie et Métabolisme, UCL 55.30, Avenue Hippocrate 55, B-1200 Brussels, Belgium.

E-mail address: jean-claude.henquin@uclouvain.be (J.-C. Henquin).

0014-5793/$36.00 © 2011 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2011.11.030

store-operated Ca^{2+} entry (SOCE) [20,21]. In β-cells, such effects are produced by acetylcholine (ACh) [22,23] that acts on M_3 muscarinic receptors to activate phospholipase C and produce inositol 1,4,5-trisphosphate and diacylglycerol [24]. Insulin secretion is also stimulated [25], but it is not known from which pools exocytosed granules originate. Here, we investigated whether interference with actin microfilaments influences ACh-induced Ca^{2+} mobilization and SOCE, as it variably does in some other systems [26–32], and impacts on insulin secretion induced simultaneously. The study was performed with mouse islets held hyperpolarized, by opening K_{ATP} channels with diazoxide [33], to avoid $[Ca^{2+}]_c$ changes mediated by Ca^{2+} entry through VGCC. Islets were treated with latrunculin B or jasplakinolide which we found to cause almost complete depolymerization and polymerization of actin, respectively [19].

2. Materials and methods

The study was approved by, and the experiments were conducted in accordance with the guidelines of, the Animal Research Committee of our institution.

2.1. Preparation, solutions and reagents

Islets isolated by collagenase digestion of the pancreas of female C57BL6 mice were selected by hand and cultured for ~44 h in RPMI 1640 medium kept at 37 °C in a 95% air–5% CO_2 atmosphere [19]. The control medium was a bicarbonate-buffered solution containing 120 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 24 mM $NaHCO_3$, 10 mM glucose, and 1 mg/ml bovine serum albumin. It was gassed with O_2/CO_2 (94/6) to maintain a pH of 7.4. In Ca-free solutions (Ca 0), $CaCl_2$ was omitted, $MgCl_2$ was increased to 3.7 mM and 100 μM EGTA was added. When the concentration of KCl was increased to 30 mM, that of NaCl was decreased accordingly. Diazoxide was added from fresh stock solutions in 0.1 N NaOH. Latrunculin B and jasplakinolide (Calbiochem, San Diego, CA) were added from stock solutions in dimethylsulfoxide, and acetylcholine from a stock solution in water.

2.2. Measurements of insulin secretion

Cultured islets were first preincubated for 90 min at 37 °C in 2 ml control medium containing 10 mM glucose. Batches of 25 preincubated islets were then perifused at 37 °C [34]. Forty minutes after the start of the perifusions, the islets were stimulated as indicated at the top of the figures. When tested, latrunculin (1 μM) was added from min −40 and remained present throughout. Because jasplakinolide is expensive and produces irreversible effects [19], the drug (1 μM) was only present during the preincubation period. Effluent fractions were collected at 2-min intervals and saved for insulin assay, using rat insulin as a standard [34]. At the end of the experiments, the islets were recovered, and their insulin content was determined after extraction in acid–ethanol [35]. Fractional insulin secretion rates were then calculated as the percentage of islet insulin content that was secreted per minute [34].

2.3. Measurements of islet $[Ca^{2+}]_c$

Cultured islets were loaded with the Ca^{2+} indicator fura-PE3/AM (2 μM) for 2 h at 37 °C, in 2 ml control medium containing 10 mM glucose. Stimulus-induced $[Ca^{2+}]_c$ changes were then measured in individual islets as described [19] and averaged for presentation as mean traces. Jasplakinolide was present during the preincubation only whereas latrunculin was added during the last 30 min of preincubation and throughout the experiments.

2.4. Measurements of polymerized and depolymerized actin

The procedure was similar to that previously described in detail [19].

2.5. Measurements of [^{3}H] inositol efflux

The technique evaluates phopholipase C activation by measuring inositol efflux from islets in which phosphoinositides pools have been labelled [25,36–38]. Details of the method have been reported elsewhere [25]. In brief, batches of 100 cultured islets were preincubated for 3 h at 37 °C, in 0.1 ml control medium supplemented with 20 μCi myo [2-^{3}H]inositol (sp. act., 20 Ci/mmol; Perkin–Elmer) to label phosphoinositides pools. When appropriate, the medium also contained jasplakinolide during the last 2 h. After loading, the islets were placed in the same perifusion chambers as those used for insulin secretion studies. Effluent fractions were collected at 2-min intervals and their content in ^{3}H was measured. From these values and the number of counts remaining in the islets at the end, the fractional efflux of [^{3}H] inositol was calculated [25].

2.6. Presentation of results

All experiments have been performed in a paired fashion (controls, latrunculin and jasplakinolide) with islets from 3 to 8 preparations. Results are presented as means ± SD or SE as indicated in legends. No correction was applied in the figures for the ~1 min dead space of the perifusion systems. The statistical significance of differences between means was assessed by ANOVA, followed by a Dunnett's test for comparisons of test groups with controls. A difference was considered significant for $P < 0.05$ at least.

3. Results

3.1. Effects of ACh on islet actin

The proportion of polymerized/depolymerized actin was 22%:78% in islets incubated in 10 mM glucose ($n = 5$). It was not affected by diazoxide alone (21%:79%) or in combination with ACh (25%:75%). Under these conditions more than 90% of actin is depolymerized by latrunculin and polymerized by jasplakinolide [19].

3.2. Stimulation by depolarization with KCl

All experiments of this study were done in the presence of 10 mM glucose and 250 μM diazoxide. Opening of K_{ATP} channels with the drug prevents glucose from depolarizing β-cells, promoting Ca^{2+} influx, raising $[Ca^{2+}]_c$ and inducing insulin secretion (Fig. 1). Depolarization with a high extracellular KCl concentration is the classic way to stimulate Ca^{2+} influx via VGCC under these conditions. The marked and reversible rise in $[Ca^{2+}]_c$ produced by 30 mM KCl was unaffected by latrunculin and jasplakinolide, except for a minor (10%, $P < 0.05$) inhibition by jasplakinolide during the first 5 min (Fig. 1A). However, the accompanying stimulation of insulin secretion was increased by both agents (Fig. 1B). The effect of latrunculin (>3-fold, $P < 0.01$ during both phases) was larger than that of jasplakinolide which was significant during second phase only (~1.5-fold, $P < 0.05$). Importantly, the effects of the two drugs on actin were not altered by 30 mM KCl: 90% depolymerization after latrunculin and 91% polymerization after jasplakinolide ($n = 2$). These results indicate that both disruption of β-cell microfilaments by latrunculin and their stabilization by jasplakinolide increase the