ACTIN FILAMENT FORMATION IN PANCREATIC β-CELLS DURING GLUCOSE STIMULATION OF INSULIN SECRETION

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1. Introduction

Many of the motile processes performed by eucaryotic cells have been attributed to the functional properties of the actin-based microfilament system [1–3]. In addition to the presence of actin in microfilaments, this protein is also found as unpolymerized monomers in homogenates of various tissues and cells [3]. Monomeric actin can be isolated from a variety of sources in a 1:1 complex with profilin, a small basic protein affecting the polymerizability of actin [4–7].

A rapid biochemical assay for the quantification of actin has been described [8] which can discriminate unpolymerized and filamentous actin in mixtures of purified components and cell homogenates. Using this technique, it was shown that the thrombin-induced transformation of human platelets from smooth discs to spiky spheres, is concomitant with a reorganization of actin from an unpolymerized to a filamentous form [9,10].

The motile behaviour of insulin secreting β-cells from the pancreas is similar in several respects to that observed with other cells [11–18]. The β-cells have also been shown to contain actin [19] and myosin [20], and insulin-storage granules interact in vitro with purified filamentous actin [21].

Here we present evidence to support the idea that microfilaments are directly involved in the secretion of insulin since exposure of the β-cells to glucose, the major physiological stimulator, was accompanied by an increase in the fraction of filamentous actin.

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2. Materials and methods

Adult ob/ob mice taken from a local non-inbred colony were used as the source of pancreatic islets containing >90% β-cells [22]. In each experiment, the pancreatic glands were removed from 4 animals starved overnight. Each pancreas was cut into pieces and placed in 2 ml incubation medium supplemented with 3 mg collagenase (type IV, 170 U/mg, Worthington Biochemical Corp., Freehold, NJ, EC 3.4.24.3). The basal solution used for isolation and incubation of the islets was a modified Krebs-Ringer medium containing 1 mM glucose, 1 mg/ml bovine serum albumin and 2.56 mM $CaCl_2$, with phosphate and sulphate replaced by equimolar amounts of chloride, and the buffering capacity (pH 7.4) provided by 25 mM Hepes instead of bicarbonate [23]. After vigorous shaking for 18 min at 37°C, the digests were rinsed several times with incubation medium after which ~600 islets were selected under a stereo microscope.

The islets were pre-incubated in 6 ml incubation medium containing 1 mM glucose for 45 min with shaking at 37°C. At the end of this period, the islets were transferred to 6 ml fresh test medium containing either 1 mM or 20 mM glucose. After a further 60 min incubation, samples of the medium were taken for the measurement of insulin [24]. The islets were subsequently lysed and homogenized at 4°C in 50 μl 10 mM potassium phosphate buffer (pH 7.6) supplemented with 150 mM NaCl, 2 mM $MgCl_2$, 0.2 mM ATP, 0.2 mM dithioerythritol, 0.01 mM phenylmethylsulfonylfluoride and 0.5% Triton X-100. Samples of the lysed islets were retained for measurement of protein content [25].

Aliquots of the islet homogenates were taken for

the determination of unpolymerized and total actin by the DNase 1 inhibition assay [8]. The DNase 1 (DN-100, Sigma) batch used in these experiments was calibrated with known amounts of purified spleen profilactin [4]. Only 3.3 μl DNase stock solution (0.27 μg) was used in the assay together with 1 ml of the DNA substrate [8]. This modification in the assay procedure was introduced because the number of intact islets that could be obtained in each experiment was limited to ~600. For determination of inhibitor activity the DNase was mixed with 1–5 μl samples of the islet homogenate to inhibit the DNase activity to ~50%. In the determination of the total actin content, 10 μl of the homogenate was diluted with 40 μl lysis buffer to decrease the viscosity of the sample once it had been mixed at 4°C with 50 μl of the guanidine–HCl solution described in [8]. Of this mixture 10–20 μl samples were taken to the DNase inhibition assay.

The concentration of purified proteins was determined spectrophotometrically, using the following values for $\epsilon^{1\%}_{280}$: DNase 1 = 12.3 [26], and profilactin = 10.4 [4].

3. Results

One unit of inhibitor activity is defined as the amount of actin which gives 1% inhibition of the standard amount of DNase 1 (in this case 0.27 μg), and the batch of DNase used in these experiments was calibrated with known concentrations of purified profilactin (fig.1). Under these conditions, an inhibitor activity of 10 000 units/ml corresponds to ~40 μg/ml actin. With the miniaturization of the assay, the highest concentration of actin that could

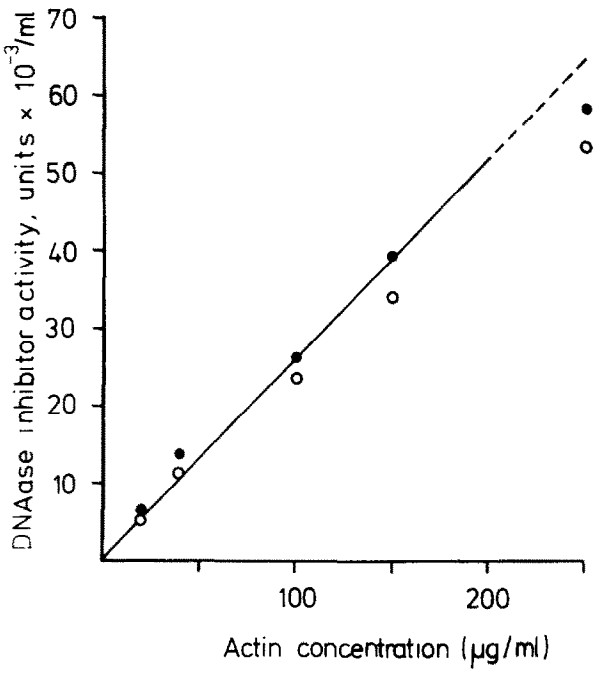

Fig.1. Standard curve for the estimation of actin using the DNase inhibition assay. Profilactin from calf spleen was prepared as in [10], and dissolved in 10 mM potassium phosphate buffer (pH 7.6), 150 mM NaCl, 2 mM $MgCl_2$, 0.2 mM ATP, 0.2 mM dithioerythritol and 0.5% Triton X-100. DNase inhibitor activity was measured as in section 2, both before (○) and after (●) treatment with guanidine–HCl over the range of actin concentrations indicated. All measurements were made with the same batch of DNase.

Table 1
Determination of actin pools and insulin release

Glucose (mM)	Unpolymerized actin as % total actin	Insulin release (μg/mg protein)	Unpolymerized actin in homogenates				Total actin	
			2 min after lysis		60 min after lysis		(μg/ml)	(μg/mg protein)
			(μg/ml)	(μg/mg protein)	(μg/ml)	(μg/mg protein)		
1	81.7 ± 2.8 (n = 5)	2.2 ± 0.2 (n = 11)	88 ± 12 (n = 9)	5.8 ± 0.4 (n = 9)	37 ± 3 (n = 8)	3.0 ± 0.2 (n = 8)	127 ± 15 (n = 9)	7.3 ± 0.7 (n = 9)
20	68.8 ± 2.9[a] (n = 7)	4.2 ± 0.7[b] (n = 9)	94 ± 13 (n = 10)	6.0 ± 0.8 (n = 9)	39 ± 5 (n = 8)	2.5 ± 0.3 (n = 7)	152 ± 22 (n = 8)	8.7 ± 1.3 (n = 8)

[a] $p < 0.02$; [b] $p < 0.005$

After pre-incubation for 45 min in medium containing 1 mM glucose, 600 islets were transferred to 6 ml medium containing either 1 or 20 mM glucose and incubated for 60 min, before insulin and actin pool measurements. The results represent mean values ± SEM for the no. expt given in parenthesis. Statistical significances were calculated by Student's t-test

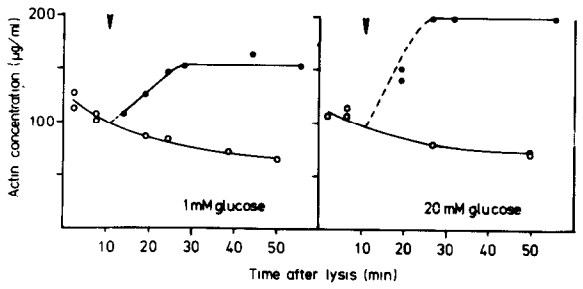

Fig.2. Determination of actin pools in homogenates of pancreatic β-cells before and after glucose stimulation. Islets were incubated for 60 min in the presence of either 1 mM or 20 mM glucose. Thereafter the cells were lysed as in section 2 and DNase inhibitor activity in the homogenates were determined at different times after lysis (○). At times indicated by the arrows, an aliquot of the homogenate was diluted with lysis buffer and treated with guanidine–HCl before the total amount of actin was determined (●). The results were expressed as μg actin/ml, calculated from the curve in fig.1.

be accurately measured was 250 μg/ml. This restriction is probably due to difficulties in adequate mixing of such small sample volumes. In all experiments the results are expressed as μg actin calculated from the curve in fig.1.

Analyses of the stability of actin pools in several types of cell homogenates have revealed that rapid changes in the polymerization state of actin occur after cell lysis [8,10]. It is therefore necessary to measure the actin pool sizes consecutively in each experiment. The results presented in fig.2 illustrate such measurements using pancreatic β-cell homogenates prepared from islets pre-incubated in medium containing either low (1 mM) or high (20 mM) glucose. In these particular experiments, the unpolymerized actin present 1–2 min after lysis represented ~77% of the total actin when the islets had been incubated at the low glucose concentration, and 54% when incubated at the high concentration of the sugar (fig.2). In expressing the unpolymerized actin as percentage of the total actin it became clear in a series of repeated experiments, that exposure of the islets to glucose was associated with a significant reduction in the proportion of unpolymerized actin (table 1). Accordingly, glucose stimulation of insulin secretion was associated with an ~2-fold increase in the pool of filamentous actin.

A considerable decrease in the pool of unpolymerized actin was observed during 30–50 min incubation at 4°C. After 1 h incubation, the concentration of unpolymerized actin had dropped to a constant level which corresponded to a mean of ~40 μg/ml actin (table 1). This final concentration was obtained irrespective of the total concentration of actin in the homogenates which ranged from 50–200 μg/ml.

The total amount of actin can be determined after treatment of the homogenates with guanidine–HCl at a concentration known to depolymerize filamentous actin [8]. In these homogenates, the release (depolymerization) of actin in the presence of guanidine–HCl takes up to 15 min to reach a plateau value (fig.2). As can be seen in table 1, the total actin concentration varied extensively between different preparations of islets, but nevertheless represented 0.7–0.9% of the islet protein irrespective of the glucose concentration during the incubation.

4. Discussion

The pancreatic β-cell responds rapidly and with great sensitivity by secreting insulin when exposed to glucose. The recognition of the secretagogic signal is believed to be followed by the accumulation of Ca^{2+} in the submembrane space and the subsequent triggering of exocytosis [27]. These data indicate that the pool of filamentous actin in β-cells increases after glucose stimulation of insulin release. Glucose control of β-granule discharge might therefore not only follow from Ca^{2+} regulation of the interaction between actin and myosin [28,29], but also from an increase in the polymerization of actin. The 1 h incubation of the islets in the presence of glucose may not reveal maximal changes in the actin pools. It is possible that the early phase of insulin release, which occurs at 5–10 min, is associated with an extensive polymerization of actin, while late phase is accompanied by stimulation of the synthesis of actin, predominantly the monomeric form. Whether a transient decrease in the fraction of unpolymerized actin exists or not will have to await further experimentation. However, in accordance with our results, the number of microvilli on the surface of β-cells in [30] were doubled after exposure to glucose.

The actin pools in homogenates of β-cells exhibited two unusual features.

(1) The pool of unpolymerized actin decreased to a level comparable to the critical concentration needed for filament formation with purified actin [31,32].

(2) The release of inhibitor activity in the presence of guanidine–HCl was ~10-times slower than that of purified filamentous actin [8,10].

When taken together with [8,10], this study indicates that a subtle balance between polymerizing and filament stabilizing activities on one hand and depolymerizing activities on the other hand, determine the stability of actin pools in cell lysates.

These observations show that a marked increase in the proportion of filamentous actin accompanies the glucose stimulation of insulin secretion. This suggests that microfilaments play an active role in the final stages of the secretory process. During the course of this work a short report was presented [33] where that the filamentous actin pool in pancreatic β-cells is proposed to be increased after glucose stimulation.

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