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Regulation of actin polymerization in rat islets of Langerhans

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A DNAase-inhibition assay was used to determine the proportions of globular (G-) and filamentous (F-) actin in islets of Langerhans after incubation in various conditions, or after subcellular fractionation. Stimulation of insulin secretion resulted in an ATP-dependent increase in the proportion of F-actin present; fractionation showed 80–90% of the actin to be present in the final supernatant.

Microfilaments composed of actin have been implicated in at least two ways in the mechanism of insulin secretion by exocytosis; Lacy *et al.* (1968) suggested that microtubules might act together with microfilaments to transport insulin storage granules from a cytoplasmic pool to the plasma membrane. Subsequently, Orci *et al.* (1972) indicated a role of microfilaments in forming a 'cell web' beneath the B-cell plasma membrane that acts as a physical barrier to the access of granules to the plasma membrane. We describe here attempts to explore the role of actin in insulin secretion in more detail, by estimation of the degree of polymerization of actin in extracts of islets of Langerhans after incubation of the intact islets *in vitro* in a variety of conditions that are known to alter rates of insulin secretion.

Experimental

Estimation of actin in islet extracts

Islets were isolated from rat pancreas as described previously (Howell & Taylor, 1966) with a bicarbonate-buffered medium gassed with O_2/CO_2 (19:1) (Gey & Gey, 1936), and were homogenized gently with a Tenbroek all-glass homogenizer (Kontes, Vineland, NJ, U.S.A.) in a buffer composed of 5mM-potassium phosphate/150mM-NaCl/2mM-$MgCl_2$/0.2mM-dithiothreitol/0.2mM-ATP/0.5% Triton X-100/0.01mM-phenylmethanesulphonyl fluoride, pH 7.5. Aliquots (20μl) of these homogenates were used directly for estimation of the G-actin content of the homogenates. Total actin was estimated after incubation of 20μl of homogenate with 20μl of a buffer containing 1.5M-guanidine hydrochloride/1M-sodium acetate/1mM-$CaCl_2$/1mM-ATP/20mM-Tris, pH 7.5 for 5min at 0°C as described by Blikstad *et al.* (1978).

Abbreviations used: G-actin, globular actin; F-actin, filamentous actin.

Assay for actin

DNA substrate [3ml of 0.1M-Tris/4mM-$MgSO_4$/1.8mM-$CaCl_2$ containing 40μg of DNA (Sigma type I)/ml] was incubated with 10μl of islet extract or with homogenizing buffer alone that had been mixed a few s before use with DNAase solution [50mM-Tris/HCl/0.01mM-phenylmethanesulphonyl fluoride/0.2mM-$CaCl_2$/0.1mg of DNAase I (DN100; Sigma)/ml]. The change of absorbance at 260nm was recorded over a period of 2min and the concentration of DNAase inhibitor (G-actin) present was calculated as units/mg of extract (Blikstad *et al.*, 1978), by comparison with a standard preparation of rabbit muscle actin (Sigma) shown by polyacrylamide-gel electrophoresis to contain more than 90% G-actin. Total actin was determined in a similar way, utilizing islet extracts that had been pretreated with guanidine hydrochloride for 5min at 0°C.

Subcellular fractionation

Batches of 600 islets were incubated for 15min in bicarbonate-buffered medium containing 5 or 20mM-glucose before homogenization in 150mM-NaCl/2mM-$MgCl_2$/0.2mM-$MgCl_2$/0.2mM dithiothreitol/0.2mM-ATP/0.01mM-phenylmethanesulphonyl fluoride, pH 7.0. The actin content of aliquots of the homogenates was assayed with that of the nuclei and debris (600*g* for 5min) mitochondrial plus granule (24000*g* for 10min), microsomal (105000*g* for 60min) and supernatant fractions after resuspension of the pellets in the same buffer. This differential centrifugation procedure has been described previously (Howell *et al.*, 1969).

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Results and discussion

As reported previously (Howell & Tyhurst, 1980) the total actin content of isolated islets of Langerhans amounts to 1–2% of the total protein. This total actin content, comprising both depolymerized and polymerized components, does not significantly change during incubation of the intact islets for 15 min at 37°C in bicarbonate-buffered salt solution (Howell & Tyhurst, 1980). Therefore, estimation of the content of depolymerized (G-) actin in islet homogenates after incubation of islets under various conditions should give an accurate estimate of the degree of polymerization of the overall actin pool.

It is clear from Table 1 that stimulation of rates of insulin secretion by 20 mM-glucose resulted in a significant decrease of the G-actin content of the islets, implying an increase in its polymerization to F-actin. Isobutylmethylxanthine (0.5 mM), a phosphodiesterase inhibitor that potentiates glucose-induced insulin secretion by raising intracellular cyclic AMP concentrations, similarly increased the degree of polymerization that was observed. Omission of calcium, which abolishes the insulin secretory reponse to glucose, could not prevent the polymerization of actin by these agents. Conversely, addition of 2,4-dinitrophenol (0.25 mM) an uncoupling agent that decreases cellular ATP contents, thereby inhibiting exocytotic secretion, appeared to cause disaggregation of the actin into the G-form. These results imply that ATP, but not calcium, may be essential for the polymerization of actin in B cells *in vivo*, and renders it unlikely that increasing cytosolic calcium concentrations leads to stimulation of insulin secretion solely by an effect in increasing microfilament polymerization.

Estimates of the actin content of subcellular fractions of rat islets were made after differential centrifugation in conditions expected to maintain the actin in its filamentous form during subcellular fractionation. These experiments indicated that the great majority of the actin was present in the supernatant during fractionation of this type, regardless of whether the cells had been stimulated to rapid secretion by glucose in the period immediately preceding homogenization. The distribution of actin in the various fractions from islets incubated in 5 mM-glucose was: nuclei and debris (600 *g* for 5 min) 5 ± 3%; mitochondria and granules (24000 *g* for 10 min) 7 ± 2%; microsomes (105000 *g* for 60 min) 3 ± 2%; supernatant 87 ± 7% (means ± S.E.M. for four observations). It is uncertain whether the residue that was present in the particulate fractions resulted from contamination of the fractions with F-actin, which was subsequently depolymerized, or whether G-actin was actually present in association with the membranes of one or more organelles. Unfortunately, the more sophisticated fractionation procedures required to settle this point decrease the concentration of actin present below that required for detection by this procedure.

There have been some indications of an association between secretory-granule membranes and actin in adrenal medullary cells (Burridge & Phillips, 1975), and of an ability of isolated granules from rat anterior pituitary (Ostlund *et al.*, 1977) and pancreatic B cells (Howell & Tyhurst, 1979) to interact with F-actin in a system *in vitro*. In the case of the B cells this interaction was enhanced by ATP but diminished in the presence of high concentrations of calcium (1–2 mM). The present studies indicate that stimulation of insulin secretion is accompanied by an increase in F-actin in the islets (and presumably in the B cells, which comprise 70% of the cell population). This is consistent with an involvement of F-actin in the intracellular movement of granules via the microtubular–microfilamentous system (Lacy *et al.*, 1968). Alternatively, polymerization might allow the formation of 'islands' of F-actin elsewhere in the cytoplasm, allowing more ready freedom of movement of the granules to the plasma membrane; ultrastructural localization of actin within the B cells would be required to resolve this point. The previously observed reorientation of the cell web to allow access of granules to the plasma membrane (Orci *et al.*, 1972) is thus assumed to result from redistribution of the microfilaments rather than any major alteration in their state of polymerization, unless such changes have been masked in these experiments by relatively much

Table 1. *Proportion of total actin present in polymerized forms after incubation of islets under various conditions*
Islets were incubated at 37°C for 15 min under the conditions shown before homogenization and assay of G-actin and total actin by the methods described in the text. Results shown are means ± S.E.M. for ten observations. *$P < 0.05$ for value observed after incubation in 5 mM-glucose alone, as indicated by Student's *t* test.

Additions to incubation medium	F-actin (% of total actin)
5 mM-Glucose	37 ± 3
20 mM-Glucose	52 ± 4*
20 mM-Glucose + 0.1 mM-isobutylmethylxanthine	71 ± 6*
20 mM-Glucose + 0.25 mM-dinitrophenol	39 ± 3
20 mM-Glucose, calcium omitted	48 ± 5*

greater changes in a distinct actin pool elsewhere in the cytoplasm.

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