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INCREASED FILAMENTOUS ACTIN IN ISLETS OF LANGERHANS FROM FASTED HAMSTERS

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SUMMARY: We measured pools of soluble and sedimentable actin in hamster islets using a new DNase I binding/immunoprecipitation assay. Islets from fed and fasted hamsters contained the same amount of actin when expressed per microgram of protein. In three experiments the sedimentable (filamentous) actin increased significantly in islets from fasted hamsters. Recovery determinations demonstrate that during fasting the sedimentable actin pool (F) is increased as the soluble (G) actin pool decreases. These results suggest that the microfilament system is affected by the metabolic state of hamsters and may be responsible, in part, for inhibiting insulin secretion during fasting.

INTRODUCTION

Microfilaments are known to be involved in many cellular processes including cell movement, cytokinesis, receptor capping, phagocytosis, and exocytosis. (1). Since Lacy first proposed that the microfilament-microtubule system must be intact for glucose-induced insulin secretion, this hypothesis has been studied extensively (2). Orci et al (3) proposed that the cell web may play a role in controlling access of secretory granules to the cell membrane. The cell web is part of an extensive microfilament network in non-muscle cells composed of actin and various actin-associated proteins. In non-muscle cells actin is distributed in two major pools: G-actin is a soluble, globulin-shaped single polypeptide of 42,000 daltons; the polymerized form of actin is called filamentous, or F-actin (4). Recently, we have developed a highly sensitive actin assay which employs the competition of G-actin and ^{125}I-G-actin in binding DNase I. Furthermore, we can measure G- and F-actin by differential sedimentation of actin pools followed by depolymerization of F-actin using guanidine-HCl (4). We have examined the possible involvement of actin in insulin secretion by measuring G- and F-actin in islets of fed and fasted hamsters, conditions of vastly different insulin secretion. Our results demonstrate that

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in fasted hamsters, sedimentable (F-actin) increased significantly in three separate experiments.

MATERIALS AND METHODS: Adult male hamsters were fed ad libitum or fasted 72 hr prior to decapitation the morning of the experiments. Pancreases were removed and the islets isolated by the collagenase digestion method of Lacy and Kistianovsky (5). Islets were placed into Hank's medium on ice without glucose and then placed into microfuge tubes in groups of 10 islets. The media were drawn off after centrifugation at 500 x g for 3 min and 1.0 ml of lysis buffer was added (90mM KCl, 10mM PIPES, 2.0mM $MgCl_2$, 1.0mM EGTA, 0.2mM ATP, 0.05% NaN_3, 0.1% Triton X-100, pH 6.9). Islets were lysed by vortexing 10 sec in lysis buffer. Aliquots were taken for total, G, and F-actin. For the measurement of total actin, a 100ul aliquot of lysate was removed and 100ul of 3.0 M GdnHCl added to the sample to depolymerize filamentous actin (6). Two hundred microliters of lysate were placed into an Eppendorf microfuge tube and centrifuged for five min to sediment the filamentous actin. We have shown previously that the procedure sediments F-actin (4,7). The supernatant was removed and 100ul of 1.5 M Gdn-HCl was added to 100ul of the supernatant. A mixture of 100ul of lysis buffer and 100ul of 1.5 M Gdn-HCl was added to the pelleted material. The Gdn-HCl containing lysates were vortexed briefly and placed on ice for 30 min. At 30 min, all samples were diluted to 1.0 ml in the actin assay buffer which consists of 0.2 M NaCl, 5mM phosphate, 0.2mM ATP, 0.2mM $CaCl_2$, 1.0mM $MgCl_2$, 0.05% NaN_3, pH 7.4 (3). At 4°C actin in this buffer will retain its ability to bind DNase I for several weeks.

Actin measurements were made using the DNase I binding/immunoprecipitation assay described previously (4). Actin from hamster islets has been shown previously to compete in parallel with the rabbit skeletal muscle actin used as standard (4). Each actin value was determined using three dose levels in duplicate and potency estimates determined using the radioimmunoassay program of Duddleson et al (8). Proteins were determined by the method of Bradford (9) using bovine serum albumin as standard. Statistical analysis utilized an unpaired Student's t-test.

RESULTS

The table summarizes our results of comparing actin pools in islets isolated from fed and fasted hamsters in three separate experiments. Actin represents 2 to 4% of cell protein in hamster islets. The total amount of actin is decreased in islets from fasted hamsters as is the amount of soluble and filamentous actin. However, the amount of protein in ten islets from the fasted hamsters is also decreased. When actin is expressed as nanograms per microgram of protein, the total amount of actin does not change in islets after three days of fasting. In all experiments, a "recovery" was calculated as the sum of G actin + F-actin was divided by the total actin. When the ratio was expressed as a percentage, all recoveries were between 83 and 115% in the three experiments. The actin recoveries in each experiment were not different between the two experimental groups. The percent of sedimentable actin in fasted hamsters

TABLE: EFFECT OF A 72 HOUR FAST ON ACTIN POOLS IN HAMSTER ISLETS OF LANGERHANS

EXPT.		[1]TOTAL ACTIN ug	G-ACTIN ug	F-ACTIN ug	[2] TOTAL PROTEIN	[3] % RECOVERY	ng ACTIN/ ug PROTEIN	[4] % F-ACTIN
No. 1	Fed N=10	1.56 ± 0.08	1.37 ± 0.11	0.32 ± 0.07	46.4 ± 2.4	110.4 ± 9.3	34.1 ± 2.29	16.2 ± 2.5
	Fasted N=10	0.90 ± 0.11	0.76 ± 0.07	0.21 ± 0.02	32.5 ± 6.3	112.9 ± 9.3	31.6 ± 6.6	24.9 ± 4.0
No. 2	Fed N=8	1.43 ± 0.09	1.33 ± 0.14	0.20 ± 0.03	82.0 ± 13.0	109.0 ± 12.1	19.8 ± 3.8	13.3 ± 1.9
	Fasted N=8	0.64 ± 0.08	0.55 ± 0.05	0.12 ± 0.02	48.2 ± 10.5	115.0 ± 15.5	17.4 ± 4.2	22.0 ± 1.7
No. 3	Fed N=10	1.58 ± 0.24	1.07 ± 0.13	0.13 ± 0.04	41.2 ± 3.3	83.1 ± 9.2	39.7 ± 7.8	10.4 ± 2.5
	Fasted N=10	1.09 ± 0.13	0.81 ± 0.08	0.21 ± 0.05	28.1 ± 3.1	99.9 ± 10.3	38.8 ± 6.1	19.4 ± 3.4

[1] Actin was measured by the DNase I binding/immunoprecipitation assay
[2] Protein was measured by the method of Bradford
[3] % recovery = (G + F-actin) / total actin X 100
[4] % F-actin = F actin / (G-actin + F-actin) X 100

inreased significantly in all three experiments (each $P < 0.05$). When the fraction of sedimentable actin was averaged for the three experiments, F-actin in islets of fasted hamsters was 22% of the total actin, a 70% increase over that found in fed animals.

DISCUSSION

These data demonstrate that in the islets of fasted hamsters there is an increase in the fraction of actin that is sedimentable and presumably is in the filamentous form. This increase occurs without any change in the amount of actin per microgram of islet protein. The secretory response of the pancreas to glucose has been shown to be impaired with fasting in rodents and man (11). Thus, in a physiological state in which insulin secretion is inhibited, we have found more filamentous actin in islets of Langerhans. This result is consistent with an earlier finding of Orci et al (2) who observed an increased insulin secretion in islets treated with cytochalasin B, a treatment known to depolymerize actin filaments and to destroy the cell web (3). Our data are consistent with the hypothesis that an increase in filamentous actin, possibly in the cell web, acts as a barrier to glucose-induced insulin secretion.

One advantage of our DNase I binding/immunoprecipitation assay is that six determinations were easily made to estimate the actin in each sample. In addition to G- and F-actin measurements, independent total actin determinations were made and compared with the sum of the soluble and sedimentable pools of actin. The "recovery" calculation is a further check on validity of results of individual experiments. The recoveries were near 100% and were the same for the two groups in each experiment.

Swaston-Flatt et al (10) have measured actin pools in mice islets using the DNase I inhibition assay of Blikstad et al (6) which determines actin pools based on the differential interaction of G-actin and F-actin with DNase I. These investigators found twice as much filamentous actin as we are reporting here. The difference may be due to species differences, experimental design, or the method of actin measurement. However, in a previous report using the

Blikstad et al assay for actin (12) we observed a fraction of sedimentable actin very similar to the percent F-actin reported in the present study.

The increased amount of F-actin in fasted hamsters is consistent with the theory of Orci et al (3) that the cell web may represent a barrier for access of secretory granules to the cell membrane. Our data are in accord with the hypothesis that the microfilament system is a dynamic component which regulates insulin secretion.

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