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Regulation of tubulin synthesis in islets of Langerhans

(microtubules/insulin secretion/adenosine 3′:5′-cyclic monophosphate)

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ABSTRACT **Tubulin represents a major protein in rat pancreatic islets, which averages 0.5% of the total protein content and 6% of the noninsulin protein synthesized under basal metabolic conditions. Glucose increases the synthesis of tubulin twofold to threefold. Tubulin synthesis is also stimulated by adenosine 3′:5′-cyclic monophosphate in both the absence and presence of glucose; this effect of adenosine 3′:5′-cyclic monophosphate occurs preferential to noninsulin protein synthesis at physiological glucose concentrations. Tubulin synthesis was decreased more than 75% by fasting, an effect prevented by maintaining animals on glucose exclusively. The fasting-induced reduction in tubulin synthesis is corrected *in vitro* by increasing adenosine 3′:5′-cyclic monophosphate levels. These findings parallel changes previously reported in insulin release and suggest that physiological agents can exert their insulin secretory action through an effect upon the rate of tubulin synthesis.**

Microtubules have been implicated in the mechanism of glucose-induced insulin release, based on the inhibiting effects of microtubule-disruptive agents such as colchicine and vinblastine (1, 2). Recent studies in our laboratory have demonstrated that rat islets contain both polymerized and depolymerized tubulin (3). It was further shown that the polymerization of tubulin was stimulated by insulinotropic agents such as glucose and theophylline. Total tubulin, as well as the proportion of polymerized tubulin, decreased in islets of fasted rats and was restored to normal by glucose feeding. In view of the marked changes in tubulin content associated with fasting and glucose feeding, the present study was undertaken to examine the regulation of tubulin synthesis in rat islets.

MATERIALS AND METHODS

Preparation of Islet Samples. Islets of Langerhans were isolated from male Sprague–Dawley rats (300–350 g) by the method of Lacy and Kostianovsky (4). Food was withheld for 60–72 hr from the fasted rats. Glucose-fed rats were maintained exclusively on 30% glucose drinking water. These animals ingest less than 10 g of glucose per day and consequently are in negative caloric balance since their basal requirement is, on the average, about 65 calories/day. Control animals had free access to regular Purina rat chow. Thirty islets were incubated at 37° under 5% CO_2–95% O_2 in siliconized tubes containing 0.5 ml of Krebs–Ringer bicarbonate medium and 50 μCi of L-[4,5-^{3}H]leucine (New England Nuclear Corp.; 41 Ci/mmol). After incubation, the islets were washed four times with Krebs–Ringer bicarbonate medium, sonicated in 300 μl of 2 M acetic acid, and centrifuged at 3000 × *g* for 15 min. Albumin (0.3%) was added before sonication to those extracts subsequently used for immunoprecipitation studies. Aliquots of the acetic acid extracts were lyophilized and stored at −20° until subjected to precipitation with 20% trichloroacetic acid (to measure total protein synthesis), immunoprecipitation (i.e., tubulin and insulin synthesis), and gel electrophoresis (5). Samples (equivalent to 60 or 120 islets) were boiled for 2 min in 2% sodium dodecyl sulfate ($NaDodSO_4$), 5% 2-mercaptoethanol before gel electrophoresis.

Gel Electrophoresis. Islet proteins were analyzed using continuous polyacrylamide gels consisting of 10% acrylamide, 0.2% *N,N′*-methylenebisacrylamide, 0.05% *N,N,N′,N′*-tetramethylethylene diamide, 0.2% $NaDodSO_4$, 0.05% ammonium persulfate, and 0.375 M Tris at pH 8.8; the gels were overlayed with a 5% polyacrylamide stacking gel. Electrophoresis was performed at 40 mA in 25 mM Tris-glycine buffer at pH 8.3 containing 0.1% $NaDodSO_4$. The gels were stained in 0.25% Coomassie brilliant blue, dissolved in methanol-acetic acid, destained in methanol-acetic acid, and scanned in a Gilford 2400-S spectrophotometer with model 2410 linear transport attachment. The distribution of [^{3}H]leucine labeled proteins was determined by slicing the gels, dissolving each slice in 1.0 ml NCS (diluted 4:1 in water) at 60° for 120 min, and counting the samples in 10 ml PCS (Amersham). Rat brain tubulin and rabbit skeletal muscle actin were run as standards on each gel slab.

Preparation of Rabbit Antitubulin Serum. Rat brain tubulin was purified by one polymerization–depolymerization cycle followed by elution on a 5 m column containing Biogel A (6, 7). The tubulin peak was collected, and concentrated in a minicon B-15 concentrater (Amicon); it exhibited >98% purity when analyzed by electrophoresis on a 10% acrylamide gel. The rabbits were injected at 3-week intervals for a total of three injections with 1 mg of purified tubulin suspended in Freunds adjuvant. Immunoreactivity of the rabbit serum was demonstrated by agar diffusion (8) in which a single precipitin line was formed between the antiserum and purified rat brain tubulin. No reaction was noted with purified skeletal muscle actin. Rat brain ^{125}I-labeled tubulin, as prepared by Kowit and Fulton (9), was precipitated quantitatively and displaced by the addition of unlabeled tubulin.

Tubulin Synthesis in Isolated Islets. Tubulin synthesis was measured by determining the incorporation of [^{3}H]leucine into islet protein immunoprecipitated specifically with rabbit antitubulin serum. Aliquots of the acetic acid extracts (10 μl) were lyophilized and incubated in triplicate with 50 μl of tubulin antiserum diluted 1:20 with tubulin depolymerizing solution‡ for 90 min at 37°; control samples were incubated with diluted normal rabbit serum. Goat anti-rabbit gamma globulin (50 μl, Gateway Immunosera) was then added without dilution, and the incubation was continued for 120 min at 4°. After addition of 100 μl of tubulin depolymerizing solution, the mixture was

Abbreviations: cAMP, adenosine 3′:5′-cyclic monophosphate; cGMP, guanosine 3′:5′-cyclic monophosphate; $NaDodSO_4$, sodium dodecyl sulfate.

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‡ The tubulin depolymerizing solution contained 0.25 M sucrose, 0.5 mM GTP, 0.5 mM $MgCl_2$, and 10 mM phosphate buffer at pH 6.95.

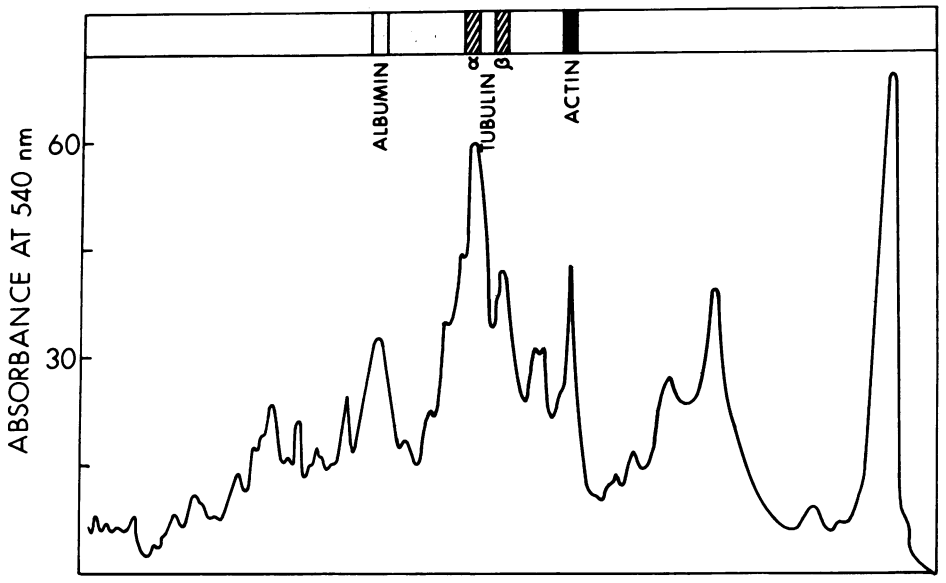

FIG. 1. A homogenate of 120 rat islets was eluted on 10% $NaDodSO_4$-polyacrylamide gels (see *Materials and Methods*). Bovine albumin, rat brain tubulin, and rabbit skeletal muscle actin were used as standard proteins in adjacent runs.

centrifuged at 3000 × *g* for 25 min. The pellet was washed with 200 μl tubulin solution, centrifuged, and dissolved in 200 μl of 0.1 M NaOH. Tubulin synthesis was calculated as the difference between control and immune serum precipitation.

Total protein synthesis was measured as the amount of [^{3}H]leucine incorporated into protein precipitated by 20% trichloroacetic acid. [^{3}H]Leucine incorporation into (pro)insulin was measured as described by Permutt and Kipnis (10).

RESULTS

Electrophoresis of acetic acid extracts of rat islets on 10% polyacrylamide gels at pH 6.4 indicated that a major peak of the noninsulin proteins comigrated with purified rat brain tubulin. When the pH was adjusted to 8.3, which is known to dissociate rat brain tubulin into its two subunits (11), two peaks were noted in the tissue extract that correspond to the alpha and beta components of tubulin (Fig. 1). These α and β peaks were specifically removed by the immunoprecipitation with tubulin antisera. Islets also contained a distinctive band with the same electrophoretic mobility as muscle actin. The profile of islet proteins, synthesized during a 180 min incubation, demonstrated the quantitative importance of tubulin synthesis relative to the noninsulin proteins synthesized (Fig. 2). Glucose (300 mg %) increased total noninsulin protein and tubulin synthesis twofold to threefold. However, as reported previously (10, 12–14), glucose preferentially stimulated (pro)insulin synthesis. The stimulatory effect of glucose on (pro)insulin synthesis was also evident in islets isolated from fasting animals, although the effect was somewhat less than observed in islets from fed rats (Fig. 3). In contrast, the synthesis of tubulin and other noninsulin proteins was markedly depressed in fasted islets exposed to 16 mM glucose (Fig. 3). The reduction in noninsulin protein synthesis in fasted islets was completely prevented when the rats were maintained exclusively on glucose, despite a significant weight loss of about 8 g/day. The addition of 1 mM

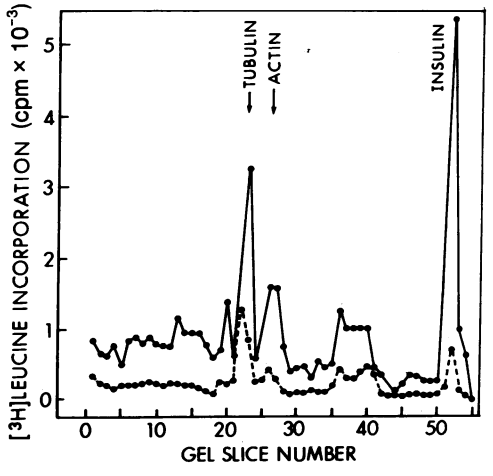

FIG. 2. Elution profile of newly synthesized islet proteins on 10% $NaDodSO_4$-polyacrylamide gels. Groups of 30 rats islets were incubated for 180 min in the presence of L-[^{3}H]leucine either in the absence (- - -) or in the presence (—) of 16 mM glucose.

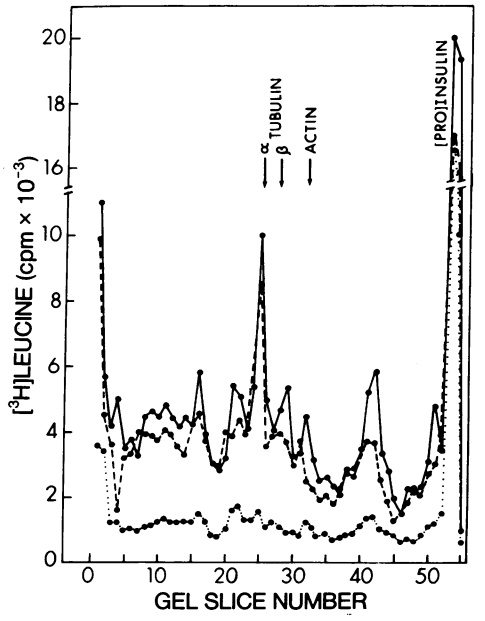

FIG. 3. Distribution of newly synthesized proteins after elution on 10% $NaDodSO_4$-polyacrylamide gels. Islets, isolated from fed (—), fasted (. . .), and glucose-fed (- - -) rats, were incubated for 45 min in the presence of L-[^{3}H]leucine and 16 mM glucose.

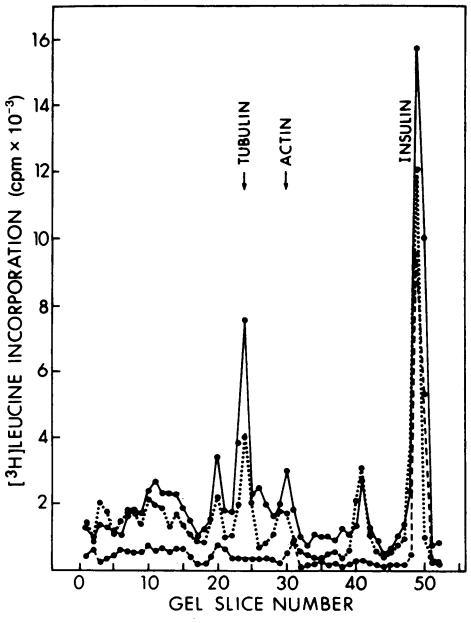

FIG. 4. The effect of 5 mM theophylline (—) and 1 mM dibutyryl cAMP (. . .) upon protein synthesis in fasted islets (- - -), incubated for 45 min in the presence of L-[³H]leucine and 16 mM glucose.

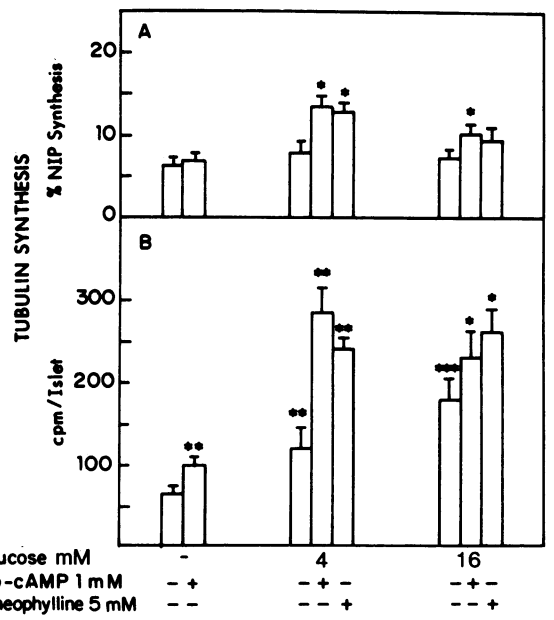

FIG. 5. The effect of glucose and cAMP upon tubulin synthesis in fed islets incubated for 45 min. The amount of newly synthesized tubulin is expressed (A) as a percent of total noninsulin protein (NIP) synthesis, and (B) as cpm per islet. All values represent mean ± SEM of five or more experiments. The statistical significance of the differences is calculated according to the Student's t test—* $P < 0.02$, * *$P < 0.01$, and * * *$P < 0.001$. N^6,O^2-dibutyryl-adenosine 3′:5′-cyclic monophosphate is abbreviated db-cAMP.

N^6,O^2-adenosine 3′:5′-cyclic monophosphate dibutyryl or 1.4 mM theophylline to the islets isolated from fasted animals restored the stimulatory effect of glucose on noninsulin protein synthesis and upon tubulin synthesis in particular (Fig. 4). To examine the extent and the specificity of the glucose and adenosine 3′:5′-cyclic monophosphate (cAMP) induced increases in tubulin synthesis, the amount of newly synthesized tubulin was measured in islets, obtained from fed rats, incubated for 45 min under varying conditions. Immunoprecipitation studies indicated that tubulin synthesis in the absence of glucose represents about 6.5% of the total noninsulin protein synthesized. Although glucose stimulated tubulin synthesis twofold to threefold, the relative proportion of tubulin synthesized remained constant even at glucose levels of 300 mg % (Fig. 5). cAMP stimulated tubulin synthesis in the absence and presence of glucose; this effect was most marked at physiological glucose concentrations, where tubulin synthesis was stimulated preferentially to the noninsulin protein synthesis (Fig. 5). Maximal rates of tubulin synthesis, which represented a fourfold increase over baseline levels in the absence of glucose were obtained with either N^6,O^2-adenosine 3′:5′-cyclic monophosphate dibutyryl or theophylline in the presence of glucose. Equimolar concentrations of N^6,O^2-guanosine 3′:5′-cyclic monophosphate dibutyryl exerted no stimulant action upon tubulin synthesis under similar experimental conditions. Omission of calcium from the incubation medium did not modify the glucose induced increase in tubulin synthesis.

DISCUSSION

Tubulin is a major protein in pancreatic islets being present at a concentration of about 4 ng per islet (3) equivalent to 0.4–0.6% of total islet protein. As the present study indicates, it is also one of the most actively synthesized proteins, and averages about 6% of the [³H]leucine incorporated into protein in the absence of glucose, a rate comparable to that of proinsulin synthesis. Glucose stimulates tubulin synthesis twofold to threefold, but this effect does not appear to be the specific type of response noted for proinsulin synthesis (which is stimulated greater than tenfold under similar conditions), since the ratio of tubulin to total noninsulin protein synthesis remains constant at all glucose levels tested. Fasting results in a 25% decrease in the total tubulin content of islets (3) and correspondingly, tubulin synthesis in fasted islets is decreased about 75% even in the presence of 300 mg % glucose. Under similar conditions, insulin content and its rate of synthesis are only modestly depressed (15). Total caloric restriction does not account for the effect of fasting, because glucose-stimulated tubulin synthesis was normal in animals maintained exclusively on glucose but who lost weight over a 3-day period. The inability of glucose to stimulate tubulin synthesis in fasted islets and the effectiveness of glucose-feeding in overcoming this defect parallel similar variations in the secretory response of the pancreatic beta cell to glucose (15, 16). In this context, it is also of interest that glucose has been shown to stimulate DNA synthesis and mitosis, a process known to require the participation of microtubules (17, 18).

If the insulin release mechanism is a microtubule-dependent process (1), the secretory defect observed in the fasted beta cell (15, 16, 19) might be accounted for, at least in part, by the decreased level of polymerized tubulin, which is a result not only of a decrease in tubulin polymerization (3) but also from a marked impairment of tubulin synthesis. These quantitative and qualitative changes in the microtubular system would be anticipated if the cAMP-adenylate cyclase (ATP pyrophosphate-lyase, EC 4.6.1.1) system is depressed by fasting, since both the degree of tubulin polymerization and the rate of tubulin synthesis are normalized when the intracellular cAMP levels are raised in fasting islet cells. Consistent with this view are recent reports demonstrating that fasting islets are characterized by a lower cAMP content (20) and by depressed adenylate cyclase and cAMP dependent protein kinase activities (21), all of which can be prevented by glucose feeding.

The effect of glucose on tubulin synthesis differs from that of the nucleotide in that glucose increased both tubulin and noninsulin protein synthesis equivalently, whereas the nucle-

otide, in the presence of glucose, stimulated preferentially tubulin synthesis. Furthermore, the stimulation of tubulin synthesis by cAMP can be demonstrated in the absence of glucose.

Although there is ample evidence for the participation of the cAMP-adenylate cyclase system in the process of insulin secretion (22, 23), the specific molecular events involved remain undefined. The demonstration that cAMP affects both microtubule assembly (3) and tubulin synthesis provides at least one mechanism by which the adenylate cyclase system can be involved in hormone release.

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