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EVIDENCE FOR A ROLE OF MICROFILAMENTS IN INSULIN RELEASE FROM PURIFIED β-CELLS

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Summary: To determine if the failure of purified β-cells to secrete insulin in response to a glucose stimulus results from the absence of a cytoskeletal response, the effects of cytochalasins D and B on glucose-induced insulin release were investigated. Glucose alone failed to stimulate insulin release whereas glucose in the presence of glucagon, theophylline, cytochalasin D or B markedly potentiated insulin release. Cytochalasin D potentiated insulin secretion in a dose-dependent manner, and the combination of theophylline and cytochalasin D resulted in an insulin secretory response no greater than that produced by either agent alone. Both glucagon and theophylline are believed to mediate their effects via cAMP, however, cytochalasin D did not affect β-cell cAMP levels. These results suggest that the inability of purified β-cells to release insulin may result from the absence of the necessary modulation of the state of the microfilaments.

Early events associated with glucose-induced insulin release from the β-cell are depolarization of the plasma membrane, opening of voltage-dependent Ca^{2+} channels and the triggering of the release of insulin by an influx of Ca^{2+}. Our previous study has demonstrated that glucose induces an increase in intracellular Ca^{2+} concentration in rat pancreatic β-cells purified by autofluorescence-activated cell sorting (1). However, it has been reported by Pipeleers et. al. that glucose-induced insulin release is significantly impaired from purified β-cells (2). These observations suggest that an increase in intracellular Ca^{2+} concentration per se, although necessary, is not sufficient to result in insulin secretion. The defect in glucose-induced insulin release from purified β-cells is believed to be due to decreased cAMP levels because the insulin secretory response to glucose stimulation is markedly enhanced by agents which elevate cAMP (3).

Although it is well established that cAMP potentiates insulin release from the β-cell, the mechanism mediating this effect is not known (4). A possible cellular mechanism to explain the effects produced by cAMP in stimulus-secretion coupling is that elevated cAMP results in the alteration of cytoskeleton associated proteins. Because a substantial amount of indirect evidence implicates a regulatory role for the β-cell cytoskeleton, i.e. microfilaments and microtubules, in the insulin secretory process, it is possible that the absence of a cytoskeletal response may be responsible for the inability of purified β-cells

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to release insulin in response to a glucose stimulus (5). The cytochalasins D and B disrupt the microfilamentous component of the cytoskeleton and also potentiate glucose-induced insulin release by islets similar to the effect of agents which modulate cAMP levels (6). Therefore, we have evaluated the ability of cytochalasins D and B to mimic the ability of cAMP to facilitate glucose-induced insulin release from purified β-cells.

MATERIALS AND METHODS

Islets of Langerhans were isolated from male Sprague-Dawley rats by the collagenase digestion procedure (7). After 24 h in culture, the islets were dispersed into individual islet cells by incubation with dispase (0.25 mg/ ml) in a Ca^{2+} and Mg^{2+} free Hank's solution at 31°C for 15 min (1,8). The dispersed islet cells were then incubated for 45-60 min in CMRL-1066 culture medium at 37°C before purification by autofluorescence-activated cell sorting (FACS) as previously described (9).

After FACS separation, purified β-cells were cultured overnight in CMRL-1066 medium in Petri dishs (35x10 mm) at 37°C under an atmosphere of 95% air and 5% CO_2. Prior to experimentation, suspended β-cells were collected from the Petri dishes and cell viability was determined by Trypan blue staining. Purified β-cells (3.5-5.0 x 10^4) were aliquoted into separate microfuge tubes (1.5 ml) and washed with Krebs-Ringer Bicarbonate (KRB) medium (139 mM Na^+, 5 mM K^+, 2.5 mM Ca^{2+}, 1 mM Mg^{2+}, 127 mM Cl^-, 24 mM HCO_3^-, and 25 mM Hepes, pH 7.4) containing 3 mM glucose and 0.1% BSA. The β-cells were gently sedimented (I.E.C, Model HN-S) at 250 x g for 2 min and then resuspended in 1 ml KRB medium containing theophylline, glucagon, or cytochalasins D or B. The β-cell suspensions were then mixed and transfered to Petri dishes and incubated at 37°C under an atmosphere of 95% air/5% CO_2 for 3 h. At the end of the incubation period, the β-cell suspensions were collected and sedimented as described above. A portion of the supernatant (100 µl) was removed for insulin determination by radioimmunoassay.

cAMP content was measured both in purified β-cells and in islets. Either 100 islets or $1x10^5$ β-cells were preincubated in 0.5 ml KRB medium contained in microfuge tubes and placed in a shaking incubator at 37°C for 30 min, and then centrifuged at 250 x g for 2 min. The supernatant was removed and the pellet was resuspended in 0.5 ml of KRB medium supplemented as described in the results. The islets or purified β-cells were then incubated for 30 min at 37°C under an atmosphere of 95% air/5% CO_2. The cAMP content of the cells was subsequently measured by radioimmunoassay (Rianen Assay System- Dupont Corp) after trichloroacetic acid extraction and acetylation (10). Extraction of cAMP by this procedure was greater than 90% efficient as assessed by inclusion of a ^{3}H-cAMP standard. The data are expressed as the Mean ± SEM.

RESULTS

1. Effect of theophylline and glucagon on insulin release from purified β-cells

The effects of theophylline and glucagon, agents which elevate intracellular cAMP levels, were evaluated on insulin secretion from purified β-cells at different glucose concentrations (Fig.1). Basal insulin release from purified β-cells incubated in the presence of 3 mM glucose was 274 ± 34 pg/1000 cells/h, and no significant stimulatory effect of 20 mM glucose on insulin release could be detected under these same conditions (358 ± 17 pg/1000 cells/h). Theophylline, a phosphodiesterase inhibitor, markedly increased the rate of insulin secretion in the presence of 20 mM glucose, but did not alter the basal release of insulin at a nonstimulating glucose concentration (Fig. 1). A marked enhancing effect of glucagon, an activator of adenylate cyclase activity, on

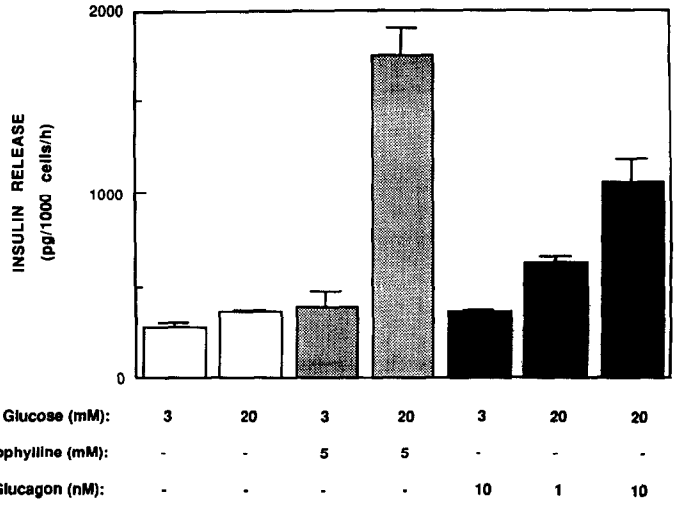

Figure 1. Effect of theophylline and glucagon on glucose-induced insulin release from purified β-cells. Islet β-cells were purified by autofluorescence-activated cell sorting and cultured overnight in CMRL-1066 medium at 37°C. Insulin release was measured after 3 h incubation in KRB medium in the presence of the indicated agents. Results are presented as the Mean±SEM from 5 separate experiments.

glucose-induced insulin release was also observed only when purified β-cells were exposed to 1 or 10 nM glucagon in the presence of 20 mM glucose.

2. Effects of cytochalasin D and B on insulin release from purified β-cells

To investigate the possibility that the microfilamentous component of the cytoskeleton is actively involved in the insulin secretory response and may be responsible for the

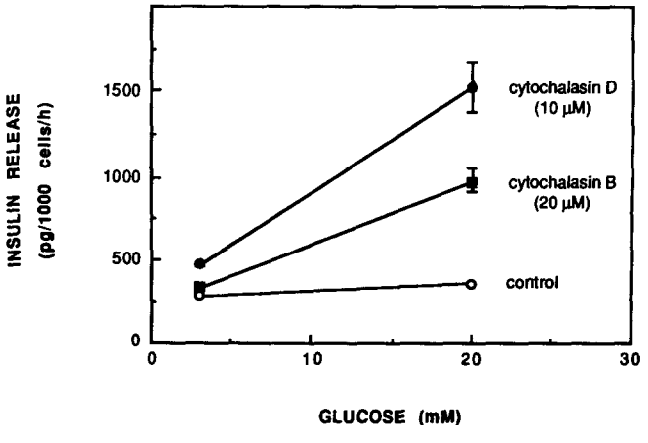

Figure 2. Effect of cytochalasin D and B on glucose-induced insulin release from purified β-cells. Purified β-cells were cultured overnight in CMRL-1066 medium at 37°C. Insulin release was measured after 3 h incubation in KRB medium in the presence of glucose (3, 20 mM) + vehicle (0.2% DMSO) o; cytochalasin D 10 μM, ●; and cytochalasin B 20 μM, ■. Results are presented as the Mean±SEM from 6 separate experiments.

impaired insulin release exhibited by purified β-cells, the effects of cytochalasin D and B on glucose-induced insulin release were examined (Fig. 2). Both cytochalasin D (10 μM) and B (20 μM) failed to affect insulin release in the presence of 3 mM glucose. However, the insulin secretory response of purified β-cells to 20 mM glucose was markedly enhanced in the presence of either cytochalasin D or cytochalasin B.

3. The concentration dependency of cytochalasin D on glucose-induced insulin release

To examine in more detail the influence of cytochalasin D on glucose-induced insulin release from purified β-cells, insulin secretion was measured in KRB medium containing 3 or 20 mM glucose alone and with increasing concentrations (0.1-60 μM) of cytochalasin D (Fig 3). Cytochalasin D potentiated insulin secretion in a dose dependent manner in the presence of 20 mM glucose but not in the presence of 3 mM glucose. The rate of insulin release averaged 340 ± 25 pg/1000 cells per h in the absence of cytochalasin D, and maximum enhancement of insulin release i.e., 5-8 fold higher than that induced by 20 mM glucose alone, was obtained at cytochalasin D concentrations of 5 μM or greater.

4. Combination of theophylline and cytochalasin D on glucose-induced insulin release from purified β-cells

The effect of maximum concentrations of theophylline and cytochalasin D on the insulin secretory activity of purified β-cells was evaluated in the presence of 20 mM glucose. As illustrated in Fig. 4, the combined presence of maximally effective concentrations of theophylline (10 mM) and cytochalasin D (60 μM) resulted in the same increase in insulin release as observed in the presence of each agent separately. These results suggest

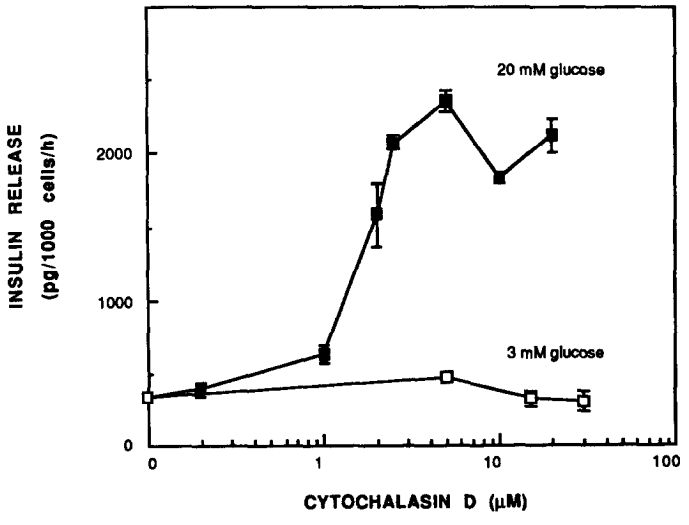

Figure 3. Dose-dependency of the effect of cytochalasin D on glucose-induced insulin secretion from purified β-cells. Purified β-cells were exposed to 3 or 20 mM glucose and different cytochalasin D concentrations in KRB medium at 37°C as indicated. Results are presented as the Mean±SEM from 3 separate experiments.

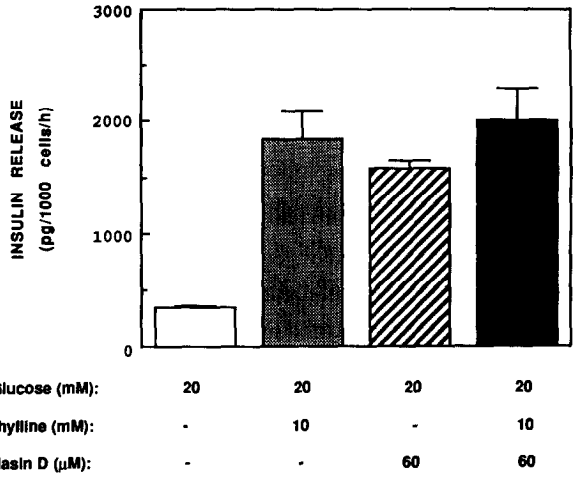

Figure 4. Effect of a combination of theophylline and cytochalasin D on the glucose-induced insulin release from purified β-cells. Insulin release from purified β-cells was measured after 3 h incubation in KRB medium at 37°C in the presence of the indicated agents. Results are presented as the Mean±SEM of 3 separate experiments.

that the insulinotropic action of these agents may be mediated by a similar cellular mechanism.

5. Effect of cytochalasin D on cAMP levels in purified β-cells and islets

The effect of cytochalasin D on cAMP formation and insulin release was determined with purified β-cells (Table 1). Exposure of purified β-cells to glucose (20 mM) and theophylline (5 mM) for 30 min resulted in a significant increase in cAMP formation which correlated with increased insulin release when compared to purified β-cells exposed to glucose (20 mM) alone. In contrast, exposure of purified β-cells to cytochalasin D (10 μM) and glucose (20 mM) for 30 min resulted in similar increase in insulin release while exerting no effect on cAMP formation. Under similar conditions cytochalasin D was also found to significantly enhance glucose-induced insulin release without altering cAMP levels from isolated islets (data not shown).

Table 1. Effect of cytochalasin D on cAMP content of purified β-cells

Experimental conditions	cAMP content (fmol/10^3 cells/30 min)	Insulin Release (pg/10^3 cells/30 min)
20 mM glucose	22.7±13.8 (7)	470.3± 28.1 (6)
20 mM glucose + 5 mM theophylline	50.0± 8.4 (8)	1576.5± 207.5 (8)
20 mM glucose + 10 μM cytochalasin D	25.1± 5.0 (8)	1573.2± 212.2 (8)

DISCUSSION

In the present study the effects of cytochalasins D and B on glucose-induced insulin release from purifed β-cells were investigated. Glucose alone, even at an elevated concentration (20 mM) did not induce a significant increase in insulin secretion from FACS purified β-cells. This defect in insulin release is not caused by cellular damage resulting from the FACS procedure since the insulin secretory activity of β-cells is significantly increased by theophylline or glucagon in the presence of elevated glucose concentrations. The enhancing effect of theophylline or glucagon on glucose-induced insulin release is believed to be mediated by an increase in intracellular cAMP formation by purified β-cells. Previous studies have indicated that the poor insulin secretory response of purified β-cells to glucose is markedly increased after the addition of Bt_2-cAMP, glucagon or glucagon-secreting α-cells (3). We have observed in the present study that cytochalasin D or B produces a significant enhancement of glucose-induced insulin release from purified β-cells similar to those agents which modulate cAMP concentration. The enhancing effect of cytochalasin D was observed only in the presence of elevated glucose concentrations, while exerting no effect at basal glucose concentrations. These results suggest that cytochalasin D mimics the ability of cAMP to facilitate insulin secretion induced by glucose from purified β-cells.

An important observation in this study was that the effects of maximum concentrations of either theophylline alone or cytochalasin D alone were no more effective than the combination of these two agents in enhancing glucose-induced insulin release from purified β-cells. These results suggest that both agents may be facilitating the release of insulin by a similar mechanism. In the present study, cytochalasin D unlike theophylline did not affect intracellular cAMP formation from purified β-cells. These results suggest that the modulation of insulin release from purified β-cells by cytochalasin D does not appear to be mediated through the production of cAMP.

Cytochalasin D has been shown both to disrupt microfilaments and to potentiate glucose-induced insulin release from islets. Therefore, the enhancement of glucose-induced insulin release from the purified β-cell by cytochalasin D may be a result of a primary effect on the microfilaments of the cytoskeleton (11). Previous studies have clearly demonstrated that cAMP-dependent processes also produce dramatic effects on elements of the cytoskeleton (12,13). Overall, these results suggest that the inability of purified β-cells to release insulin in response to glucose stimulation may result from the absence of a necessary modulation of the state of the microfilaments. Thus, the mechanism of action of these agents, i.e. cAMP, cytochalasin D and B, which facilitate glucose-induced insulin release from purified β-cells may be directly mediated via an alteration of the β-cell cytoskeleton and may be explained possibly by their ability to sensitize elements of the cytoskeleton to increases in intracellular Ca^{2+}.

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