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SHORT COMMUNICATIONS

Cytochalasin B: Inhibition of Glucose-Induced Insulin Release from Isolated Rat Pancreatic Islets

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Summary. Cytochalasin B (200 μg/ml) completely inhibited the glucose-induced insulin release from isolated rat islets. Basal release was unaffected. The cytochalasin-induced inhibition was rapidly reversible. Pretreatment with cytochalasin B seemed to increase the sensitivity of islets to a subsequent glucose stimulation.

Key words: Cytochalasin B, microfilamentous system, isolated pancreatic islets, insulin release.

Involvement of microfilaments in the release of insulin has been suggested since cytochalasin B (CCB), a drug known to interfere with the organization of microfilaments [1, 2, 3], was shown to stimulate glucose-induced insulin (IRI) release from rat pancreas [4,5,6,7]. However, several reports on the inhibitory effect of CCB upon hormone release (growth hormone, norepinephrine, thyroxine) [8,9,10], prompted us to restudy its effect on glucose-induced IRI release. A concentration-dependent stimulatory and inhibitory action was found.

Materials and Methods

Fed, male Wistar rats (200—250 g) were used throughout the study. CCB was purchased from Imperial Chemical Industries Ltd, Macclesfield, England; bovine serum albumin (lot ORHD 20) from Behringwerke A.G. Marburg; iodinated insulin (specific activity 150—200 mC/mg) from Farbwerke Hoechst A.G. Frankfurt; human insulin (lot 41-4866) from Novo Industri A.S. Copenhagen, Denmark; dimethylsulphoxide (DMSO) and all other reagents from E. Merck A.G., Darmstadt. Islets, isolated from rat pancreas by collagenase digestion [11], were incubated at 37° C in a metabolic shaker (72 cycles/min). All experiments were preceded by a preincubation of 15 min. Incubations were in 500 μl Krebs-Ringer bicarbonate buffer (95% O_2: 5% CO_2) containing bovine serum albumin (1 mg/ml). Concentration of glucose and CCB is indicated in the tables. CCB was dissolved in DMSO; final concentration of DMSO 10 μl/ml incubation medium. Groups of 10 islets were incubated for one or two periods of 45 min. Between incubations islets were washed once with 1 ml Krebs-Ringer buffer. Insulin content of the media was determined by radioimmunoassay [12] with human insulin as reference standard. By appropriate dilutions of media aliquots all readings off the standard curve were done in that section (1—10 μU/tube) that closely paralleled a standard curve established with rat insulin. IRI release was expressed as μU/islet/45 min.

Results

As indicated in Table 1, DMSO (10 μl/ml) did not affect the glucose-induced IRI release from isolated rat islets. CCB had a dual effect. Depending on its concentration in the incubation medium IRI release was stimulated (10 μg/ml) or inhibited (200 μg/ml). "Basal insulin release" was unaffected since insulin output from islets incubated at a non stimulatory glucose concentration (2 mM) with or without cytochalasin B (200 μg/ml) was identical.

Table 1. *Inhibition or stimulation of glucose-induced insulin release from isolated rat pancreatic islets by cytochalasin B. Mean values ± SEM are shown with the number of individual observations in parentheses. An asterisk indicates a significant difference from the respective control value ($p < 0.01$ or less). Values for "P" were calculated by the "t" test based on nonpaired comparisons*

Glucose (mM)	DMSO (μl/ml)	Cytochalasin B (μg/ml)	Insulin secretion (μU/islet/45min)
16.6	—	—	44.3±3.0 (32)
16.6	10	—	41.6±3.3 (16)
16.6	10	10	55.0±3.7 (16)*
16.6	10	200	9.5±0.7 (46)*
2.0	10	—	9.3±0.8 (32)
2.0	10	200	8.3±1.0 (16)

Results demonstrating the reversibility of the CCB-induced inhibition of glucose-mediated IRI release are summarized in Table 2. There was not only a complete recovery in the secretory capacity of CCB-pretreated

islets but even an increased sensitivity to a subsequent glucose stimulation. Islets exposed in a first incubation (0—45 min) to CCB (200 μg/ml) did release more insulin during a second incubation (46—90 min) in a CCB-free medium compared with controls. This was seen regardless of whether the glucose concentration during the first incubation (with CCB) was stimulatory (16.6 mM) or non stimulatory (2 mM).

Table 2. *Reversibility of cytochalasin B-induced inhibition of insulin release from isolated rat pancreatic islets. Mean values ± SEM are shown with the number of observations in parentheses. The % change refers to insulin release during the second incubation (46—90 min) compared with the first incubation (0—45 min). In addition, IRI release from cytochalasin B-pretreated islets may be compared with IRI release from untreated controls. An asterisk indicates a significant difference from the respective control value ($p < 0.02$ or less). Values for "P" were calculated by the "t" test based on paired comparisons*

Incubation period (min)	Glucose (mM)	Cytochalasin B (μg/ml)	Insulin secretion (μU/islet/45 min)	% Change
0—45	16.6	200	8.9±0.9 (16)	+585
46—90	16.6	—	52.1±2.8 (16)*	
0—45	16.6	—	48.9±3.7 (16)	— 25
46—90	16.6	—	36.4±3.3 (16)	
0—45	2.0	200	8.3±1.0 (16)	+406
46—90	16.6	—	33.7±3.6 (16)*	
0—45	2.0	—	8.6±1.2 (16)	+269
46—90	16.6	—	23.1±2.3 (16)	

Discussion

CCB appears to interact with microfilaments, a contractile system often found beneath cell membranes [1, 2, 3]. Since, following the administration of CCB, changes in the organization of the microfilamentous system of B cell membranes did coincide with an increased IRI release, involvement of microfilaments into the release mechanism was postulated [4, 5, 6, 7]. The stimulatory effect of low concentrations of CCB (10 μg/ml) on glucose-induced IRI release is confirmed. However, IRI release was abolished by high concentrations of CCB (200 μg/ml) (Table 1). Although information on effects of CCB on the pancreatic B cell metabolism is scanty, permanent toxic effects can be ruled out since inhibition of IRI release was readily reversible (Table 2). Both the stimulatory and the inhibitory action of CCB are apparent in the presence of high glucose concentrations only. Insulin release under "basal conditions", i.e. in the presence of low non-stimulating glucose concentrations, was neither stimulated nor impaired, as shown in this report and by previous investigators [4]. CCB may therefore be characterized as a modifier of glucose-induced IRI release.

The glucose-induced secretory response in control islets was larger after preincubation with 16.6 mM glucose than after preincubation with 2.0 mM glucose (Table 2). An analogous observation has been made with microdissected islets from obese-hyperglycemic mice [18]. Since exposure to glucose is known to increase the glycogen level in islets of obese mice [19], it was proposed that the elevated IRI release after pretreatment with glucose could be due to an increased supply of glycolytic intermediates from glycogen stores [18].

At present, the mode of action of CCB is unknown. A specific action upon the microfilamentous cell web from islet tissue has been suggested [4, 5, 6, 7], but this seems unlikely in view of the fact that CCB influences the uptake of various substances (D-glucose, 2-deoxy-D-glucose, D-glucosamine, orotic acid, uridine, thymidine) into several lines of mammalian cells, thus indicating an effect upon cell membranes [13, 14, 15, 16, 17]. The rapidity with which CCB affects the transport of these substances is also compatible with a membrane-directed action [16, 17]. The rapid induction and reversion of the stimulatory and inhibitory effects of CCB on IRI release supports this assumption, as well as the finding that pretreatment with CCB seems to increase the sensitivity of islets to glucose (Table 2).

As long as a specific action of CCB upon the microfilamentous cell web from islet tissue is doubtful, it remains questionable to what extent the observed effects of CCB on IRI release can be used as an argument for the involvement of the microfilamentous system into the process of insulin release.

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