The effect of phalloidin on insulin secretion from islets of Langerhans isolated from rat pancreas

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Received 16 July 1984

Phalloidin, which stabilizes F-actin, has no effect on insulin secretion from intact islets, but penetrates and increases secretion from islets previously made permeable using a high voltage discharge technique. Use of this highly specific drug strongly suggests a role for microfilaments composed of F-actin in the insulin secretory process.

Phalloidin *Microfilament* *Insulin secretion*

1. INTRODUCTION

The postulated role of the microtubule-microfilamentous system in the mechanism of insulin secretion [1] rests in part on the effects of drugs which interfere with microtubule and microfilament function. Thus colchicine [1], vinblastine, deuterium oxide [2,3], taxol or nocodazole [4] will affect microtubule polymerization and inhibit insulin secretion. Conversely, a role for microfilaments was suggested from the effects of cytochalasin B of insulin secretion [5], although doubts have been expressed about the specificity of this agent.

Phalloidin is a filamentous-actin specific drug which reacts stoichiometrically with muscle F-actin in vitro [6]. It stabilizes polymerized actin against depolymerization, proteolytic digestion and heat denaturation without influencing its physiological functions [7] and hence shifts the equilibrium between globular and filamentous actin in favour of actin polymerization.

We report here that in B cells of islets of Langerhans which have been permeabilized by high voltage electric discharge, phalloidin penetrates readily and induces significant stimulation of calcium-evoked insulin secretion. This suggests an important role for F-actin in the insulin secretion mechanism.

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2. EXPERIMENTAL

2.1. *Media*

Islet isolation and static secretion experiments using intact islets were carried out in a bicarbonate buffered medium (pH 7.4) [8] equilibrated with O_2/CO_2 (19:1, v/v).

The pulsation medium used for high voltage discharge [9] and perifusion of the permeabilized islets, had the following composition (mM): Na^+, 72; K^+, 70; Mg^{2+}, 1; Cl^-, 72; isethionate, 70; EGTA, 1; ATP, 1; Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], 16; glucose, 5; and bovine serum albumin (BSA) (0.5 mg/ml). $CaCl_2$ was added to give final concentrations of 10^{-6} M or 10^{-8} M Ca^{2+} and the pH was adjusted to 7.0 with NaOH.

2.2. *Isolation of islets*

Islets were isolated from the pancreata of male Wistar rats (100–150 g) by a collagenase digestion technique [10]. After isolation the islets were preincubated for 30 min at 37°C in the bicarbonate buffered medium containing 5 mM glucose.

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00145793/84/$3.00

2.3. *Static incubations*

After the preincubation period the islets were washed several times in the bicarbonated buffered medium. Groups of 3 islets were incubated at 37°C in the medium supplemented with 0.5 mg/ml BSA and 5 mM or 20 mM glucose, with and without addition of phalloidin at concentrations ranging from 10^{-7} M to 10^{-2} M.

2.4. *Perifusion*

After the preincubation period the islets were washed several times in the pulsation medium containing 10^{-8} M Ca^{2+}. Then groups of 50 islets were transferred to pulsation cells containing 1 ml of the medium and subjected to 3 discharges of 3.6 kV cm^{-1} for 18 μs (at 15 s intervals) and resuspended between discharges [9]. The islets were then placed on a 10 μm-mesh filter (Plastok, Birkenhead) in a Swinnex filter holder (Millipore) and perifused using a Gilson peristaltic pump (Anachem, Luton). The islets were washed with pulsation medium at 37°C at a flow rate of 1 ml/min with pulsation medium containing 10^{-6} M or 10^{-8} M Ca^{2+} with and without addition of 10^{-4} M phalloidin for a further 10 min. Concentrations of Ca^{2+} were controlled by EGTA buffers [11] and calculated using a modification of the ligand metal-binding programme of [12].

Insulin concentrations in media were determined by radioimmunoassay, using guinea pig anti-insulin serum (donated by Dr W. Montague, University of Leicester), purified rat insulin standards (Novo, Denmark) and ^{125}I-labelled bovine insulin iodinated in our laboratory by a chloramine-T method [13]. The antibody bound fraction was separated from the unbound fraction by precipitation in 12% polyethylene glycol 6000 [14].

2.5. *Histology*

Some of the intact islets and some of the islets subjected to the high voltage exposure were incubated with trypan blue for 5 min [15] or rhodaminylphalloin (fig.1) for 30 min and then washed well before examination under a Leitz dialux 200 microscope.

3. RESULTS AND DISCUSSION

Concentrations of phalloidin ranging from

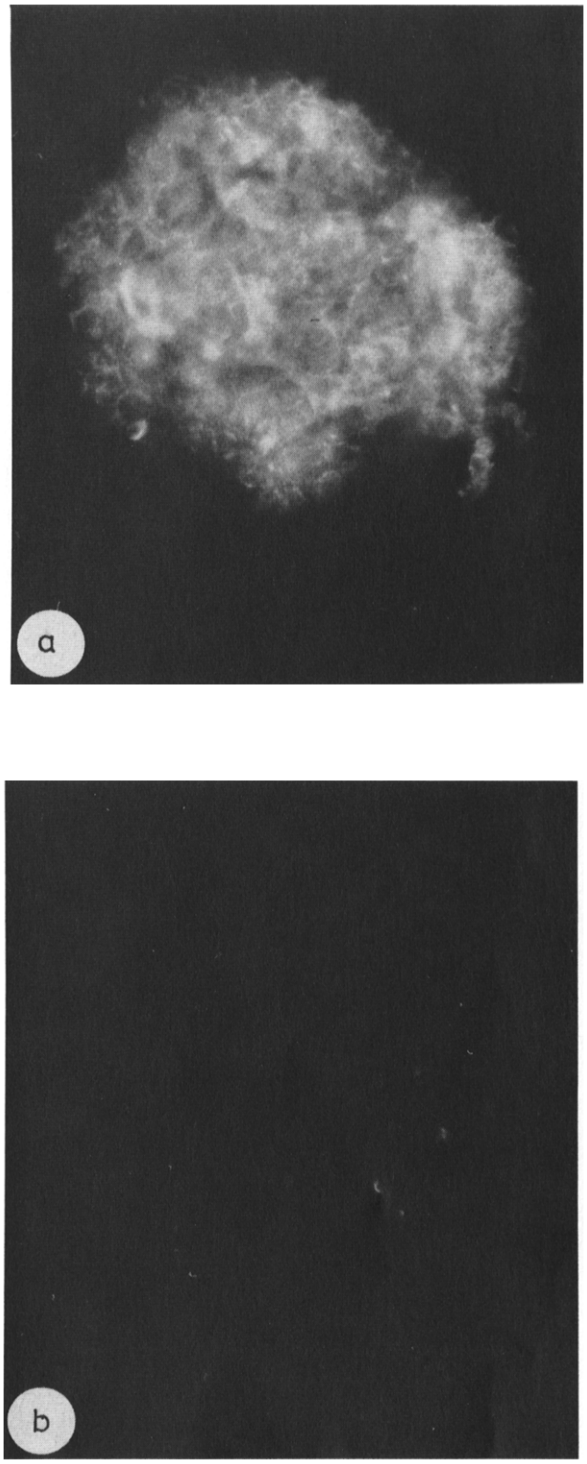

Fig.1. Shocked (a) and non-shocked (b) islets incubated with rhodaminylphalloin. Magnification = 140 ×.

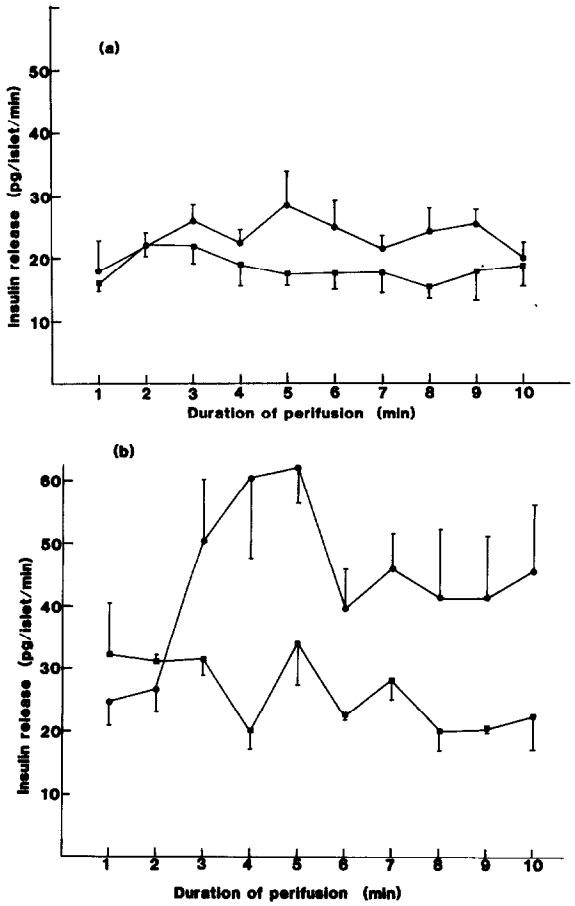

Fig.2. Insulin secretion from permeabilized islets perifused with phalloidin. (a) (■) Islets discharged and perifused in 10^{-8} M Ca^{2+} (control channel) and (●) islets discharged and perifused in 10^{-8} M Ca^{2+} supplemented with 10^{-4} M phalloidin. (b) (■) Islets discharged and perifused in 10^{-6} M Ca^{2+} (control channel) and (●) islets discharged and perifused in 10^{-8} M Ca^{2+} supplemented with 10^{-4} M phalloidin. Points on the curve represent mean and the bars the SEM of 7 perifusions. Integrated areas under the curve were compared with the control and p was <0.005 in each case, calculated by Student's t-test.

10^{-7} M to 10^{-2} M did not affect insulin secretion in static incubations of intact islets in the presence of 5.5 or 20 mM glucose (not shown). Islet cells were therefore rendered accessible to normally impermeable molecules by exposure to a high voltage discharge [9]. A trypan blue test confirmed the cells had been made permeable. Using the fluorescent rhodaminylphalloin it was shown that B cells of intact islets are normally impermeable to rhodaminylphalloin and that the high voltage discharge allowed entry of this molecule (fig.1).

Insulin secretion from B cells in the permeabilized islets was stimulated in perifusion medium containing 10^{-6} M Ca^{2+} (fig.2) in comparison to 10^{-8} M Ca^{2+}. Addition of 10^{-4} M phalloidin to the perifusion medium significantly increased insulin secretion at both concentrations of calcium over a 10-min period (fig.2 and table 1).

These results clearly show that entry of phalloidin into B cells is essential for its action and an increase in microfilament polymerization by this apparently specific drug will increase insulin secretion in permeabilized islets but not in intact islets. Possible ways in which F-actin, along with tubulin and myosin, might work together to facilitate the intracellular movement of insulin storage granules prior to their secretion have recently been discussed [16].

Table 1

Effect of phalloidin perifusing permeabilized islets

Calcium concentration of medium (M)	Integrated insulin secretion over a 10-min period (pg/islet per min)		n	p
	Control (mean ± SE)	With 10^{-4} M phalloidin (mean ± SE)		
10^{-8}	18.35 ± 0.75	23.50 ± 0.87	7	<0.005
10^{-6}	26.34 ± 1.78	43.93 ± 3.92	7	<0.005

ACKNOWLEDGEMENTS

Phalloidin and rhodaminylphalloin were generous gifts from Professor Theodor Wieland, Max-Planck Institut für Medizinische Forschung, Heidelberg, FRG. Financial assistance from the Medical Research Council through project grants and the MRC Secretory Mechanisms Research Group, and the British Diabetic Association is gratefully acknowledged.

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