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PHLORIZIN: ITS EFFECT ON GLUCOSE-INDUCED INSULIN SECRETION AND PROTECTION AGAINST THE ALLOXAN EFFECT IN ISOLATED ISLETS

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1. Introduction

By using isolated rat islets in a perifusion system, 5 min exposure to alloxan (20 mg%) in a 1 mg/ml glucose medium is shown to produce complete inhibition of glucose (5 mg/ml)-induced insulin secretion and this alloxan effect is prevented by simultaneous presence of 5 mg/ml glucose in alloxan medium [1]. The order of potencies of hexoses to protect against alloxan toxicity in vitro [glucose(100)>3-*O*-methyl-glucose⩾mannose>2-deoxyglucose galactose>>L-glucose(0)] does not parallel the established order of their insulin-releasing action [glucose(100)>mannose>fructose>>galactose = 2-deoxyglucose = 3-*O*-methyl-glucose = L-glucose(0)] [1]. To further understand the effect of alloxan, phlorizin, an inhibitor of glucose transport, is used in the present experiment. Alloxan was shown to induce penetration of extracellular marker ([^{14}C] Mannitol) into toadfish islet tissue and this effect was protected by simultaneous presence of phlorizin (0.5 mM) [2]. The interaction of alloxan and phlorizin has not been reported so far in isolated islets in terms of insulin secretion.

2. Materials and methods

More than two hundred islets of Langerhans were isolated from one fed male Sprague-Dawley rat (200–300 g) by the standard collagenase technique [3]. The perifusion system utilized was the same as reported previously except for the three chambers utilized simultaneously: one used for the control and the others as experimental chambers. The chambers were maintained at 37°C and perifused at a flow rate of 0.7–0.8 ml/min. The perifusate was collected at one- or five-minute intervals and the insulin content of aliquots was determined by the radioimmunoassay and expressed as μU/islet/min [4].

Exposure to alloxan: Alloxan monohydrate (Sigma Chemical, St. Louis) was dissolved at a concentration of 20 mg% in previously warmed (37°C) and gassed (95% O_2, 5% CO_2) Krebs-Ringer bicarbonate medium immediately prior to perifusion. Phlorizin solution: Phlorizin (K & K Lab., Hollywood, Calif.) was dissolved in 1–2 ml of distilled water at 58°C, then was made to final concentrations of phlorizin (0.5–10 mM) and glucose (1 mg/ml) by adding warmed (37°C) bicarbonate medium.

3. Results

Glucose-stimulated insulin secretion was tested using a 2 mg/ml, 3 mg/ml and 5 mg/ml glucose medium. Baseline secretion was established by first perifusing the islets with a 1 mg/ml glucose medium for 50 min. The amount of insulin secretion was parallel to the concentrations of glucose with maximal insulin secretion occurring in a 5 mg/ml glucose medium. The biphasic secretory profile was evident after perifusing with a 5 mg/ml glucose medium. The first phase occurred after 5–7 min, and the second phase 20–30 min after stimulation (fig.1). Accordingly, the following experiments were performed using a 5 mg/ml glucose medium, which induced the maximal and the most prominent biphasic secretory profile.

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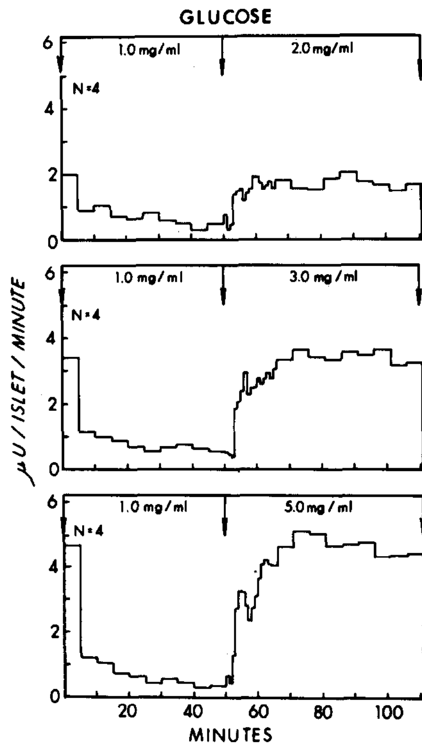

Fig.1. Glucose-induced insulin secretion was tested by simultaneous three chamber perifusion to a 2 mg/ml, 3 mg/ml and 5 mg/ml glucose medium after obtaining baseline secretion by 50 min perifusion with a 1 mg/ml glucose medium. The first phase secretion was observed by all of the stimulating glucose levels at 5–6 min and the second phase was most prominent at 20-30 min perifusion by a 5 mg/ml glucose medium. The total insulin secretion by a 2 mg/ml, 3 mg/ml and 5 mg/ml glucose medium was: 9.0 ± 2.7, 19.2 ± 4.7, and 24.4 ± 2.4 mU/100 islets/60 min respectively. Upper: 2 mg/ml, Middle: 3 mg/ml and Lower: 5 mg/ml glucose stimulation.

Fig.2 shows that a five minute exposure to 10 mM phlorizin inhibited both the first and second phases of insulin secretion. The biphasic pattern was still maintained with a total insulin output of 64% of the control (table 1). The presence of alloxan in the phlorizin solution yielded exactly the same secretory profile with complete abolishment of the alloxan effect (20 mg%). 4 mM phlorizin produced similar effects on glucose-induced insulin secretion and reversed the alloxan toxicity as the same degree as 10 mM (table 1). Less than 2 mM concentrations produced less inhibition of insulin secretion although there was no direct

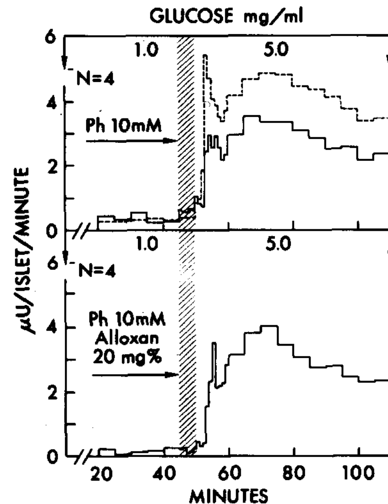

Fig.2. A 5 min exposure to 10 mM phlorizin in a 1 mg/ml glucose medium inhibited the subsequent glucose-induced insulin secretion with decreased but retained biphasic profiles. The concomitant presence of alloxan in a mixture of phlorizin and glucose yielded exactly the same secretory pattern with no effect of alloxan. Upper: Control (broken line) and 5 min exposure to 10 mM phlorizin (solid line), Lower: 5 min exposure to a mixture of phlorizin and alloxan.

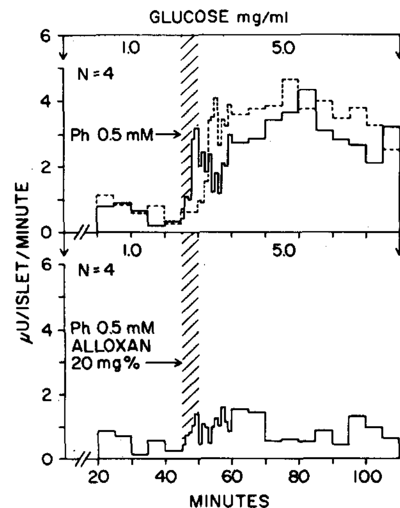

Fig.3. A 5 min exposure to 0.5 mM phlorizin in a 1 mg/ml glucose medium produced less inhibition of biphasic insulin secretory profile. The first phase had already started during perifusion with the phlorizin medium. The reversal of the alloxan effect was least effective by the 0.5 mM phlorizin medium with less prominent secretory phases. Upper: Control (broken line) and 5 min exposure to 0.5 mM phlorizin (solid line), Lower: 5 min exposure to a mixture of phlorizin and alloxan.

Table 1
Effect of different current ratios of phlorizin on glucose-induced insulin secretion

Group of exp.	5 Min exposure Phlorizin (mM)	Alloxan (mg%)	Glucose 5 mg/ml Insulin secretion mU/100 islets/60 min mean ± SEM		[a]Percent secretion	[b]Percent inhibition
I	0	0	24.23 ± 1.12	(4)	100	0
	10	0	15.47 ± 1.88	(4)	63.8	36.2
	10	20	15.76 ± 1.20	(4)	64.9	35.1
II	0	0	22.78 ± 1.40	(4)		
	4	0	15.74 ± 1.66	(4)	69.1	30.9
	4	20	15.59 ± 1.56	(4)	68.4	31.6
III	0	0	21.69 ± 0.92	(3)		
	2	0	18.79 ± 2.18	(3)	86.6	13.4
	2	20	15.26 ± 1.75	(3)	70.4	29.6
IV	0	0	23.16 ± 1.48	(3)		
	1	0	18.81 ± 1.39	(3)	81.2	18.8
	1	20	11.63 ± 0.55	(3)	50.2	49.8
V	0	0	21.22 ± 0.64	(4)		
	0.5	0	18.41 ± 1.13	(4)	86.8	13.2
	0.5	20	5.50 ± 0.32	(4)	25.9	74.1

[a]The total insulin secreted with a 5 mg/ml glucose was divided by the control insulin secretion in each group of experiments.
[b]Percent inhibition = 100% – percent secretion.

dose-dependent fashion of inhibition in the range between 2 mM and 0.5 mM (table 1). The reversal of alloxan toxicity by phlorizin showed declining tendency of protection from 2 mM to 0.5 mM (table 1).

Fig.3 demonstrates that a 5 min exposure to 0.5 mM phlorizin produced less inhibition of biphasic insulin secretory profile. The first phase had already started during perifusion with the phlorizin medium (fig.3). The reversal of the alloxan effect declined to 25.9% by the 0.5 mM phlorizin medium (table 1).

4. Discussion

Phlorizin has been widely used to study hexose transport by renal tubules [5], yet its action in islet tissue is still obscure. Lambert et al. reported that the concomitant presence of phlorizin (1 and 2 mM) inhibited the glucose (0.25 mg/ml)-stimulated insulin release to less than 68% of the control value seen in cultured pancreatic explants of fetal rats [6]. Using microdissected islets of obese-hyperglycemic mice, Hellman et al. disclosed that the uptake of phlorizin occurs rapidly within a 7 min exposure and is concentration-dependent up to 5 mM [7]. Phlorizin at concentrations 1–5 mM did not modify the insulin release stimulated by a 1.8 mg/ml glucose medium, but a 10 mM concentration of phlorizin depressed the insulin secretion to 56% of the controls [8]. 10 mM phlorizin also provided the islets with inhibition to glucose transport (100%) and glucose oxidation (37%) [8].

Thus the rat islets used in this study showed different insulin secretion from the diabetic mouse islets [8]. The rat islets were more sensitive to low concentrations (0.5–2 mM) of phlorizin, yielding more than 13% inhibition of insulin secretion with no dose-dependent relationship to the increasing concentrations of phlorizin (table 1). Whereas the hyperglycemic mouse islets were reported to produce less than 8% of inhibition of insulin secretion by concomitant presence of 1 and 5 mM phlorizin in a

1.8 mg/ml glucose medium [8]. This difference is probably due to the difference of sensitivity to phlorizin between normoglycemic rat islets and hyperglycemic mouse islets.

As for the reversal of alloxan effect in rat islets, 10, 4 and 2 mM phlorizin retained insulin secretion above 65% of the control while 1 and 0.5 mM produced 50% and 25% of protection respectively with dose-dependent declining protection against alloxan (table 1).

Phlorizin (5–15 mM) alone was shown to stimulate insulin secretion in the absence of glucose, and its stimulatory effect was inhibited by mannoheptulose, suggesting that phlorizin exerts its effect by the same system which recognizes glucose as an insulin secretagogue [7].

The results in this paper showed the two different degrees of inhibition of glucose-induced insulin secretion between 5–10 mM and 0.5–2 mM of phlorizin yet both the first and second phases of secretory profile were distinctively preserved. This effect of phlorizin is in contrast to that of mannoheptulose, the latter inhibits completely and specifically the first phase secretion alone [1].

The glucose-stimulated insulin secretion is accompanied by electrical activity [9]. The different effects of phlorizin and mannoheptulose may be explained by this respective effect on the electrical activity. Phlorizin (10 mM) causes a partial inhibition whereas mannoheptulose (20 mM) causes complete inhibition of electrical activity [10].

Phlorizin appears to have at least two different effects. High concentrations of phlorizin inhibit metabolism and hence depress energy-yielding reaction, whereas an action on glucose transport is demonstrable at far lower concentrations [5]. Thus, phlorizin at 0.5 mM concentration has little effect on glucose metabolism but it interacts with the glucose transport site to competitively inhibit glucose transport [2]. This low concentration inhibits glucose-induced insulin secretion more potently than its effect to interact against the alloxan toxicity, probably acting at the common site of transport for glucose, alloxan and phlorizin (table 1). It is also in accord with the fact that protection against alloxan is provided by non-metabolizable hexoses, which share the same transport site as glucose [1].

More recently, McDaniel et al. reported that alloxan did not exert an effect on permeability to extracellular markers (sucrose, D-mannitol or L-glucose) and that alloxan did not alter the rate of D-glucose or 3-*O*-methyl-glucose transport into rat islets [11]. If this is true, the primary site of action of alloxan is likely a glucoreceptor site, which is also shared by phlorizin at least in part. It is still not known how a glucoreceptor site separate from a transport site on the beta-cell membrane and how two sites interact each other.

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