Resistance to apamin of the Ca^{2+}-activated K^+ permeability in pancreatic B-cells

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The bee venom neurotoxin apamin failed to affect ^{86}Rb outflow and insulin release from rat pancreatic islets stimulated by D-glucose or the Ca^{2+}-ionophore A23187. Apamin, in contrast to quinine or A23187, also failed to affect bioelectrical activity in mouse islet cells. These findings suggest that, like in erythrocytes, and at variance with the situation found in smooth muscle, liver or neuroblastoma cells, the Ca^{2+}-activated K^+ permeability in the pancreatic B-cell is resistant to apamin.

Pancreatic islet cell *Ca^{2+}-activated permeability* *Apamin* *Insulin release* *Electrical activity* *A23187 ionophore*

1. INTRODUCTION

In several cell types, including the pancreatic B-cell, the cytosolic accumulation of Ca^{2+} provokes a rise in K^+ permeability [1–10]. In the pancreatic B-cell, such a phenomenon may play a role in the rhythmic pattern of bioelectrical and secretory activity; e.g., by participating in the repolarization which terminates each burst of spikes [6,11,12]. The neurotoxin apamin, which is a bee venom polypeptide composed of 18 amino acids, was recently found to block selectively the Ca^{2+}-dependent K^+ channel in smooth muscle, hepatocyte and neuroblastoma cell [13–16]. In these experiments, the concentration of apamin required to affect K^+ permeability ranged from 1–100 nM; i.e., at least two orders of magnitude lower than the concentration of quinine generally used for the same purpose [9]. We here investigated the effect of apamin upon K^+ permeability, bioelectrical activity and insulin release in pancreatic islets, with emphasis on situations characterized by activation of the Ca^{2+}-responsive modality of K^+ extrusion.

2. MATERIALS AND METHODS

The methods used to measure ^{86}Rb fractional outflow rate (FOR) and insulin release from perifused pancreatic islets removed from fed rats [17,18] and to follow bioelectrical activity in microdissected mouse islets [19] were described in detail in prior publications. The ionophore A23187 (Calbiochem, La Jolla CA) was dissolved in dimethylsulfoxide, which was added to both control and test media at final concentrations not exceeding 0.1% (v/v). Apamin, purified as in [20] was kindly provided by Dr M. Lazdunski (Centre de Biochimie, CNRS, Nice). All results are expressed as mean value (± SEM) together with the number of individual determinations (n). The statistical significance of differences between control and experimental values was assessed by use of Student's t-test.

3. RESULTS

When A23187 (10 μM) was administered to rat islets exposed to 5.6 mM D-glucose, it provoked a

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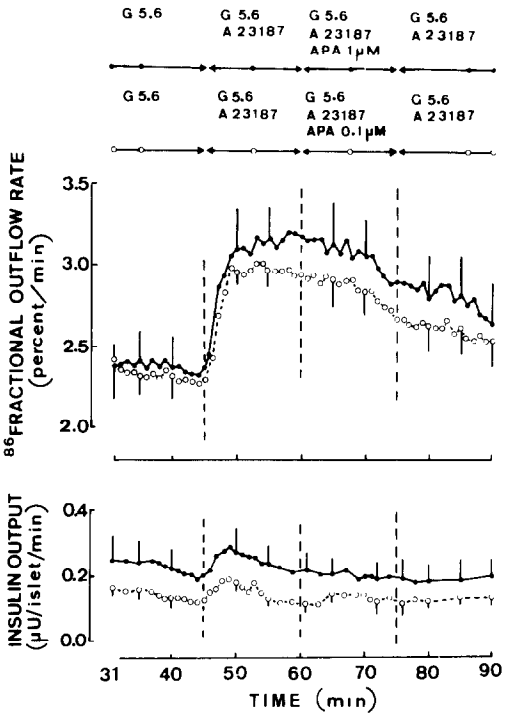

Fig.1. Effect of apamin (0.1 μM, open circles; 1.0 μM, closed circles) administered from min 61–75 upon ^{86}Rb fractional outflow rate (upper panel) and insulin release (lower panel) from islets exposed from min 45–90 to A23187 (10 μM) and throughout the perifusion period to D-glucose (5.6 mM). Mean values (± SEM) refer to 4 individual experiments.

rapid and sustained increase in ^{86}Rb FOR and a modest and transient stimulation of insulin release. Apamin (0.1 or 1.0 μM) failed to cause any obvious decrease in A23187-stimulated ^{86}Rb FOR (fig.1). When apamin (0.1 μM) was administered throughout the perifusion period, it failed to affect significantly the steady-state value for ^{86}Rb FOR found after 40–44 min of exposure to either 5.6 or 8.3 mM D-glucose (table 1). In these experiments, apamin also failed to alter the increment in ^{86}Rb FOR evoked by either A23187 in the presence of 5.6 mM D-glucose or by a rise in D-glucose concentration for 8.3 to 16.7 mM (table 1). The release of insulin evoked by D-glucose or A23187 was also unaffected by apamin (fig.1, table 1). When the glucose concentration of the perifusate was suddenly raised at the 45th min of perifusion from zero to 16.7 mM, the glucose-induced decrement in ^{86}Rb FOR, as judged from the mean values recorded in each experiment between min 40–44 and 56–60, respectively, averaged 2.25 ± 0.38 and 1.99 ± 0.34%/min in the absence and presence of apamin (0.1 μM), respectively ($p > 0.5$).

In microdissected mouse islets, apamin (0.01 μM, 0.1 μM or 1.0 μM) failed to affect either the resting membrane potential in the absence of glucose or the burst pattern of bioelectrical activity found in the presence of 11.1 mM D-glucose (fig.2). This lack of effect contrasts with the tran-

Table 1

Effect of apamin (0.1 μM) on ^{86}Rb outflow and insulin release

Experimental conditions	^{86}Rb FOR (%/min)		Insulin output (μunits.min^{-1}.islet^{-1})	
Secretagogue (mM)	Control	Apamin	Control	Apamin
Steady-state values (min 40–44)				
D-glucose (5.6)	1.95 ± 0.15	2.00 ± 0.11	0.23 ± 0.03	0.18 ± 0.04
D-glucose (8.3)	1.38 ± 0.03	1.44 ± 0.04	0.59 ± 0.07	0.71 ± 0.07
Short-term changes (maximal increment at min 47–50)				
D-glucose (8.3 ⟶ 16.7)	0.40 ± 0.03	0.46 ± 0.02	1.26 ± 0.11	1.55 ± 0.21
A23187 (0.01, at glucose 5.6)	0.65 ± 0.06	0.79 ± 0.05	0.18 ± 0.08	0.14 ± 0.03

n = 4 in each case

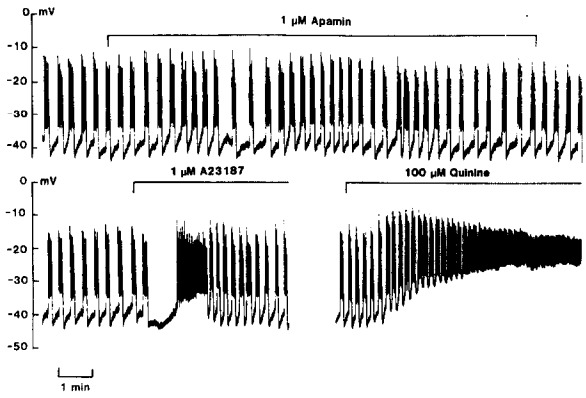

Fig.2. The upper trace illustrates the burst pattern of electrical activity in a mouse pancreatic B-cell exposed to 11.1 mM D-glucose. Apamin (1.0 μM) was added for 13 min as indicated by the horizontal line. The lower left trace is a continuation of the upper trace and shows the effect of A23187 (1.0 μM) added in the presence of 11.1 mM D-glucose. The lower right trace was recorded from the same cell, 56 min later, and documents the response to quinine (100 μM). The time calibration applies to all 3 traces.

sient hyperpolarization evoked by A23187 and the depolarization induced by quinine in the same cells. Thus, apamin was ineffective in a cell which, otherwise, displayed activation and inhibition of K^+ permeability in response to A23187 and quinine, respectively.

4. DISCUSSION

The present results suggest that, in the pancreatic B-cell of normal rats or mice, the Ca^{2+}-responsive K^+ permeability is resistant to apamin. This is not a unique situation. Apamin also fails to affect Ca^{2+}-dependent K^+ permeability in human erythrocytes [13,14]. The electrical data collected in microdissected mouse islets indicate that the resistance to apamin of the pancreatic B-cell does not result from a damaging influence of collagenase, used for the isolation of rat islets, on the apamin receptor site [15,21,22]. Our results rather suggest that the pancreatic B-cell could be distinguished from certain other cell types by a low number or poor affinity of the postulated apamin receptor sites or apamin-sensitive K^+ channel. In this respect, the pancreatic B-cell would resemble erythrocytes, both cell types being equipped with Ca^{2+}-sensitive K^+ channels appropriately responsive to quinine and yet resistant to apamin [13,14]. The functional implication, if any, of such a tissue specificity remains to be assessed.

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