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EFFECTS OF MAITOTOXIN ON IONIC AND SECRETORY EVENTS IN RAT PANCREATIC ISLETS

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Summary : Maitotoxin (MTX) provoked a dose-dependent increase in both ^{45}Ca efflux and insulin release from rat pancreatic islets perifused in the presence or absence of glucose, provided that Ca^{2+} was present in the perifusate. The stimulatory effect of MTX on ^{45}Ca outflow was enhanced by CGP 28392. The toxin did not reduce ^{86}Rb outflow and ^{86}Rb inflow. It is suggested that the secretory response to MTX is mediated by direct activation of voltage-dependent Ca^{2+} channels.

Numerous marine toxins have been used as tools to elucidate the function of ion channels. Maitotoxin (MTX), a highly potent marine toxin, has recently been extracted from dinoflagellate cells (1). The toxin appears to stimulate a broad spectrum of calcium-dependent physiological processes, including contraction of cardiac and smooth muscles, and hormone secretion (2-8). The stimulatory actions of MTX have been mainly attributed to an increase in Ca^{2+} permeability occuring through activation of voltage-sensitive Ca^{2+} channels (2-8). MTX does not exhibit any ionophoretic activities on rat mitochondrial or liposomal membranes, but it has been reported that the toxin inhibits Na^+-K^+ ATPase from microsomes of cat and human kidneys (7,9).

Since Ca^{2+} entry and the cytosolic accumulation of Ca^{2+} are thought to represent critical events in the insulin releasing process (10), we examined the effects of MTX on ionic and secretory events in rat pancreatic islets. The present study was also undertaken to define the mode of action of MTX in insulin secreting cells, and to gain further insight into the Ca^{2+} channels properties of these endocrine cells.

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Materials and Methods : The methods used to measure ^{86}Rb uptake, ^{86}Rb or ^{45}Ca fractional outflow rate (FOR) and insulin release from pancreatic islets removed from fed rats, were described in prior publications (11,12). Procedures for extraction and purification of Maitotoxin (MTX) were previously reported (1). The LD_{50} of the purified toxin was 0.16 ug/kg (i.p) in mice. MTX was dissolved in H_2O and stored at -20°C until use. The concentration of the toxin was expressed gravimetrically. CGP 28392 (Ciba-Geigy AG, Basle, Switzerland) was dissolved in dimethylsulfoxide (DMSO), which was added to both control and test media at final concentrations not exceeding 0.1 % (v/v). At this concentration, DMSO fails to affect islet function (13).
The media deprived of $CaCl_2$ were enriched with 0.5 mM EGTA [ethylene glycol-bis-(B-aminoethyl ether)-N,N' tetraacetic acid]. Ouabain was obtained from Sigma Chemical Company (St Louis, MO). All results are expressed as the mean (± SEM) together with the number of individual experiments (n). Peak ^{45}Ca efflux and peak insulin release were estimated from the differences in ^{45}Ca efflux or insulin release between the highest value recorded during stimulation and the mean basal value found within the same experiment between the 40th-44th min of perifusion. The statistical significance of differences between mean experimental and control data, was assessed by use of Student's t test.

Results : Maitotoxin (MTX; 10^{-10} g/ml) provoked a rapid and sustained increase in both ^{45}Ca efflux and insulin release from islets perifused in the presence of 5.6 mM glucose and 1 mM Ca^{2+} (Fig.1). These stimulatory effects of MTX were also observed in the absence of glucose or the presence of 2.8, 8.3, 11.1, 16.7, and 27.8 mM glucose (Fig.3 and data not shown). The effects of the toxin were completely suppressed in the absence of extracellular Ca^{2+} (Fig.1 and data not shown).

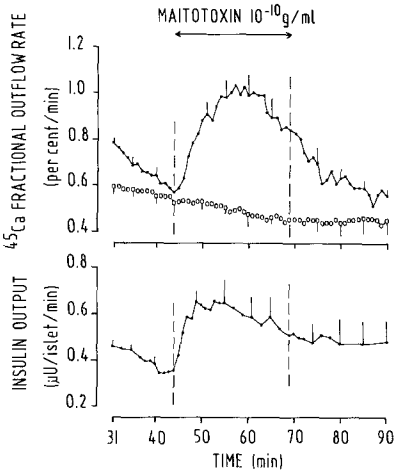
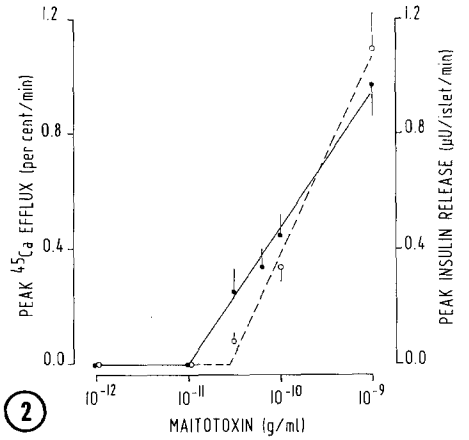


Figure 1. Effect of maitotoxin (10^{-10} g/ml) on ^{45}Ca efflux (upper panel) and insulin release (lower panel) from islets perifused in the absence (o-o) or presence of extracellular Ca^{2+} (1 mM, ●-●). Basal media contained 5.6 mM glucose. (n=4)



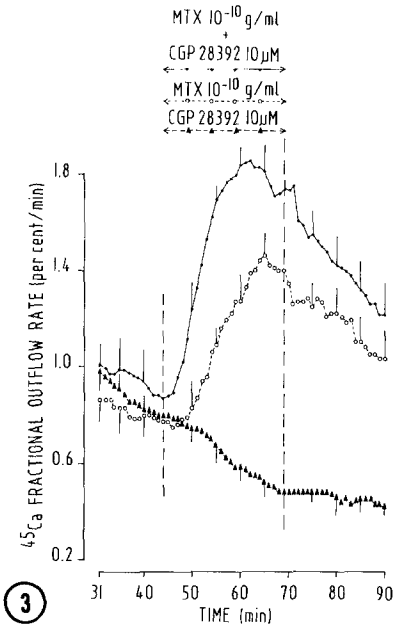


Figure 2. Effect of increasing concentrations of maitotoxin on peak ^{45}Ca efflux (●—●) and peak insulin release (o-o) from islets perifused in the presence of 1 mM Ca^{2+} and 5.6 mM glucose. (n=4-6)

Figure 3. Effect of maitotoxin (MTX) (10^{-10} g/ml, o-o); CGP 28392 (10 µM, ▲-▲); or MTX + CGP 28392 (●—●) on ^{45}Ca efflux from islets perifused in the presence of glucose (2.8 mM) and extracellular Ca^{2+} (1 mM). (n=3-4)

Fig.2 illustrates the dose-action relationships of MTX on peak ^{45}Ca efflux and insulin release from islets exposed to 5.6 mM glucose. From a threshold concentration of 10^{-11} g/ml, MTX induced a dose-dependent stimulation of both ^{45}Ca efflux and insulin release.

To better evaluate the effects of MTX on Ca^{2+} channels, experiments were performed in the presence of CGP 28392; a dihydropyridine derivative known to act as a "Ca^{2+} channel agonist" (14). At low glucose concentration (2.8 mM), CGP 28392 (10 µM) did not stimulate ^{45}Ca outflow when used alone, but greatly enhanced the ionic response of the islets to the toxin (Fig.3). Peak ^{45}Ca outflow induced by MTX (10^{-10} g/ml) averaged 0.69 ± 0.04 (n=3) and 1.02 ± 0.09 (n=4) % min in the absence and presence of CGP 28392, respectively ($P<0.05$).

Finally, in order to exclude any action of MTX on passive and active K^+ fluxes, the effects of MTX on ^{86}Rb outflow and inflow were investigated. The toxin (10^{-10} g/ml) did not decrease ^{86}Rb efflux from islets perifused in the absence or presence of gluco-

Table 1. EFFECT OF MAITOTOXIN AND OUABAIN ON ^{86}RB UPTAKE

Expt	Line	Preincubation	Incubation			^{86}Rb uptake pmol/islet/5 min	P
		glucose (mM)	glucose (mM)	maitotoxin (g/ml)	ouabain (mM)		
A	1	2.8	2.8	Nil	Nil	55.19 ± 4.87 (12)	
	2	2.8	2.8	10^{-10}	Nil	49.91 ± 2.12 (12)	2 vs.1 NS
	3	2.8	2.8	Nil	1.0	38.87 ± 2.26 (12)	3 vs.1 P<0.01
	4	2.8	2.8	10^{-10}	1.0	41.12 ± 1.84 (12)	4 vs.3 NS
B	5	8.3	8.3	Nil	Nil	57.46 ± 4.46 (12)	
	6	8.3	8.3	10^{-10}	Nil	51.31 ± 5.36 (12)	6 vs.5 NS
	7	8.3	8.3	Nil	1.0	23.28 ± 1.70 (12)	7 vs.5 P<0.001
	8	8.3	8.3	10^{-10}	1.0	24.84 ± 1.37 (12)	8 vs.7 NS

The islets were preincubated during 30 min and incubated during 5 min.

se and/or extracellular Ca^{2+} (data not shown). In the presence of 2.8 or 8.3 mM glucose, ^{86}Rb uptake measured over short incubations periods (5 min) was not affected by MTX (10^{-10} g/ml) (Table 1). Ouabain, however, markedly decreased ^{86}Rb inflow from islets incubated in the presence or absence of the toxin (Table).

Discussion : The present data confirm previous results, indicating that MTX stimulates insulin release by increasing Ca^{2+} entry into pancreatic B-cells (5). This view is supported by the finding that MTX induced a dose-dependent increase in both ^{45}Ca efflux and insulin release, provided that Ca^{2+} was present in the perifusate. In prelabelled islets, an increase in Ca^{2+} inflow is known to induce an increase in ^{45}Ca outflow as a result of a process of ^{40}Ca-^{45}Ca exchange (10-11). Incidentally, the absence of effect of MTX on ^{45}Ca outflow from islets perifused in the absence of extracellular Ca^{2+}, suggests that the toxin did not exert any primary effect upon either the intracellular handling or efflux of Ca^{2+}.

The threshold concentration for MTX effects on both ^{45}Ca efflux and insulin release was slightly higher than 10^{-11} g/ml. Such findings confirm that the threshold concentration for MTX effects is lower in pancreatic B-cells than in other endocrine cells (4,5,7), and underline the importance of Ca^{2+} influx for the control of insulin release.

Our data also suggest that MTX increased Ca^{2+} inflow by directly activating the voltage-dependent Ca^{2+} channels of B-cells. First, MTX did not reduce ^{86}Rb inflow. This implies that the secretory and ionic effects mediated by the toxin cannot be attri-

buted to any interference with the Na^+-K^+ pump (3,4,8,10). Second, MTX did not appear to reduce K^+ permeability, as indicated by the failure of the toxin to decrease ^{86}Rb outflow. Last, MTX increased ^{45}Ca outflow from islets perifused in the presence of depolarizing or non-depolarizing concentrations of glucose (e.g. 2.8 and 0 mM). Under the latter experimental condition, the B-cell membrane potential remains at a hyperpolarized level around -70 mV (15). Thus a membrane depolarization is not required for the toxin to manifest its stimulatory action on Ca^{2+} influx; a finding again compatible with the suggestion that MTX has a direct effect on B-cell Ca^{2+} channels. It is conceivable that MTX stimulates Ca^{2+} entry by shifting the voltage dependency of Ca^{2+} channel activation towards more negative values.
The finding that CGP 28392 enhanced the effects of MTX, reinforces our hypothesis. CGP 28392 is a dihydropyridine derivative which prolongs the mean open time of voltage-sensitive Ca^{2+} channels but fails to induce spontaneous openings when the membrane is held at negative potential (14). The absence of stimulatory effect of CGP 28392 on ^{45}Ca outflow from islets perifused in the presence of a non-depolarizing concentration of glucose, confirms previous observations and shows that the resting Ca^{2+} channels of the B-cells are unaffected by the dihydropyridine derivative (16,17). Hence, the enhancing action of CGP 28392 on the ionic response of the islets to MTX supports the view (i) that the two substances interact with the same target system; namely the voltage-dependent Ca^{2+} channels and, (ii) that MTX activates voltage-dependent Ca^{2+} channels.

In conclusion, this study shows that MTX stimulates insulin release by promoting Ca^{2+} entry into pancreatic B-cells. It is suggested that the toxin may directly activate the voltage-dependent Ca^{2+} channels.

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