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Effects of mastoparan on catecholamine release from chromaffin cells

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Release of catecholamines from bovine adrenal chromaffin cells exposed to mastoparan, a wasp venom peptide which activates GTP-binding proteins and phospholipase A_2, was evaluated. Release of catecholamines was dependent on mastoparan concentration and time of exposure. This release was, however, independent of extracellular calcium and accompanied by release of the cytoplasmic marker lactate dehydrogenase. Mastoparan also inhibited catecholamine secretion evoked by nicotine, but the peptide had little or no effect on release induced by other secretagogues. These findings suggest that in chromaffin cells mastoparan is not a secretagogue but rather causes cell lysis and blocks nicotinic receptor function.

Adrenal medulla; Chromaffin cell; Mastoparan; Secretion; Catecholamine; Peptide effect

1. INTRODUCTION

Mastoparan, a tetradecapeptide from wasp venom (Ile-Asn-Leu-Lys-Ala-Leu-Ala-Ala-Leu-Ala-Lys-Lys-Ile-LeuNH_2), is reportedly a secretagogue for a variety of cell types including peritoneal mast cells [1,2], anterior pituitary cells [3], and adrenal chromaffin cells [4]. The activities of mastoparan and structurally related peptides apparently result from activation of GTP-binding proteins (G-proteins) and subsequent stimulation of phosphoinositide-specific phospholipase C or from direct stimulation of phospholipase A_2 [2,5,6]. In mast cells, activation of a pertussis toxin-sensitive G-protein mediates mastoparan-induced histamine release [6].

Recent studies have indicated a role for G-proteins in exocytotic secretion from adrenal medullary chromaffin cells. Nonhydrolyzable guanine nucleotide analogs stimulate calcium-independent catecholamine secretion from permeabilized chromaffin cells [7,8]. Pertussis toxin, which ADP ribosylates the α-subunit of several G-proteins, potentiates catecholamine secretion from chromaffin cells [9,10] and itself stimulates a slow catecholamine release [11]. Because of the potential for elucidating the molecular mechanisms of exocytosis including the role of G-proteins, the present study was undertaken to examine in detail the effects of mastoparan on secretion from chromaffin cells. The results show that the chromaffin cell 'secretion' observed does not result from exocytosis, but rather from lysis of the cells.

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2. MATERIALS AND METHODS

2.1. *Materials*

Mastoparan was obtained from Bachem, Inc. (Torrance, CA). Pertussis toxin, atropine, hexamethonium, veratridine, tetrodotoxin, pertussis toxin, and nicotine were obtained from Sigma Chemical (St. Louis, MO). Ionomycin and d-tubocurarine were obtained from Calbiochem (San Diego, CA).

2.2. *Cell cultures*

Bovine adrenal chromaffin cells were isolated by collagenase digestion and density gradient centrifugation on Renografin [12]. The cells were further purified by differential plating [13],

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and finally plated in serum-containing medium at a density of 400000–500000 per cm^2 in 24-well tissue culture plates (Corning). After approx. 16 h, the serum-containing medium was removed and culture continued in serum-free medium [14]. Cultures were used between 2 and 10 days after plating.

2.3. *Catecholamine release and analysis*

Culture medium was removed and replaced with release medium (150 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 7.8 mM glucose, and 5 mM Hepes, pH 7.4) at 37°C with or without mastoparan or other additions. At the indicated times, aliquots of the medium were removed and acidified (0.1 M perchloric acid final concentration). After removal of release medium, cells were lysed by addition of cold 0.1 M perchloric acid. Norepinephrine, epinephrine, and dopamine were measured by reversed-phase high-performance liquid chromatography with electrochemical detection [12]. Because only minor differences in the secretion of the different catecholamines were observed, results are reported as the percentage of total cell catecholamines released. The catecholamine contents of the cultures averaged 140 nmol per 10^6 cells.

2.4. *Lactate dehydrogenase release and assay*

Cultures were incubated at 37°C in release medium with or without mastoparan. Aliquots of the medium were removed as indicated. Release medium and cell lysates (0.2% Triton X-100 in release medium) were kept on ice and assayed within 4 h. Lactate dehydrogenase activity was measured as described [15]. The lactate dehydrogenase activity of the cultures averaged 31 nmol per min per 10^6 cells.

3. RESULTS AND DISCUSSION

3.1. *Catecholamine release from mastoparan-stimulated chromaffin cells*

As reported [4], mastoparan induced release of catecholamines from cultures of bovine adrenal medullary chromaffin cells. Catecholamine release was dependent on the concentration of mastoparan (fig.1) and on the time of exposure to the peptide (fig.2). Mastoparan-induced catecholamine release was not blocked by cholinergic antagonists (1 μM atropine, 50 μM d-tubocurarine, 500 μM hexamethonium) or by the sodium channel blocker tetrodotoxin (1 μM). Catecholamine release elicited by exposure to mastoparan was unaffected by pretreatment (20 h) of the cultures with pertussis toxin (10 to 1000 ng/ml), conditions which enhanced nicotinic secretion by approx. 35%, as reported [13–15].

3.2. *Lactate dehydrogenase release from mastoparan-treated cells*

Further studies, however, revealed that mastoparan-induced catecholamine release was independent of extracellular calcium and that the cytoplasmic marker lactate dehydrogenase was released during exposure to the peptide (table 1). These results suggest that mastoparan is either directly lysing the cells or exerts a lytic effect by activation of phospholipase A_2 as reported for several cell types [2]. Alternatively, activation of G-proteins [5], leading to stimulation of other phospholipases, could account for these results. Preferential release of lactate dehydrogenase may indicate permeabilization of cells without escape of chromaffin vesicles, as seen with digitonin [15], although this was not observed consistently.

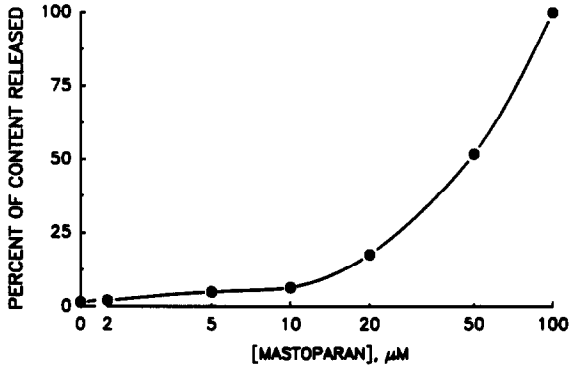

Fig.1. Concentration dependence of mastoparan on release of catecholamines from chromaffin cells. Incubation time was 15 min. Data are the means of 3 determinations, except for the 100 μM concentration where $n = 1$; the standard errors of the mean are smaller than the plotting symbols used.

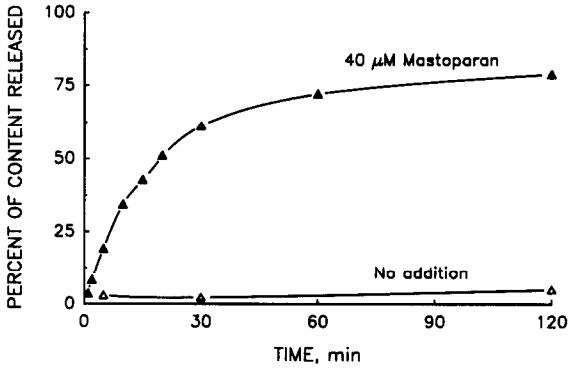

Fig.2. Time course for mastoparan-induced catecholamine release. Data represent the averages of duplicate determinations.

Table 1

Calcium dependence of lactate dehydrogenase and catecholamine release induced by mastoparan

Addition	Lactate dehydrogenase		Catecholamine	
	Calcium	EGTA	Calcium	EGTA
None	6.0 ± 0.2	7.1 ± 0.5	2.1 ± 0.1	7.3 ± 0.4
Mastoparan, 30 μM	34.9 ± 0.7[a]	51.5 ± 0.8[a]	21.1 ± 0.5[a,b]	26.2 ± 1.5[a,c]

[a] $p < 0.001$ compared to no mastoparan added
[b] $p < 0.05$ compared to lactate dehydrogenase release under the identical condition
[c] $p < 0.001$ compared to lactate dehydrogenase release under the identical condition

Chromaffin cell cultures were incubated for 10 min either in release medium containing 2.0 mM calcium or calcium-free medium containing 0.5 mM EGTA. Data are presented as mean ± SE, $n = 3$

3.3. *Effects of mastoparan on catecholamine secretion evoked by other agents*

The effects of low concentrations of mastoparan on catecholamine release evoked by other secretagogues were also examined (table 2). Mastoparan inhibited secretion induced by nicotine (table 2) and acetylcholine (not shown), but had little or no effect on secretion resulting from the other agents tested. Hence, low concentrations of mastoparan which produce little or no lysis of chromaffin cells do not synergistically activate release of catecholamines in the presence of secretagogues. Neither does mastoparan block the secretory mechanism, except by interaction with the nicotinic acetylcholine receptor.

Table 2

Effect of mastoparan on secretion induced by other agents

Secretagogue	Catecholamine release (%)	
	− mastoparan	+ mastoparan
None	4.2 ± 0.6	4.8 ± 0.2
Nicotine, 10 μM	17.7 ± 0.3	8.1 ± 0.2[a]
Veratridine, 40 μM	26.8 ± 0.4	24.9 ± 0.2[b]
Barium chloride, 250 μM	24.0 ± 0.8	24.7 ± 0.6
Potassium, 50 mM	13.7 ± 0.2	14.0 ± 0.02
Ionomycin, 20 μM	37.8 ± 0.5	37.2 ± 1.7

[a] $p < 0.001$ compared to cultures without mastoparan addition
[b] $p < 0.05$ compared to cultures without mastoparan addition

Chromaffin cells were incubated (15 min) as indicated ± 5 μM mastoparan. KCl was substituted for NaCl to achieve 50 mM K^+. Data are presented as mean ± SE, $n = 3$

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