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Effect of Calcium on Histamine Release From Pleural and Peritoneal Mast Cells Induced by Catechol

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Histamine release from rat pleural and peritoneal mast cells induced by catechol (1, 10, 50, 250 μM and 1 mM) has been studied. The dose-response induced by catechol is non-cytotoxic, is not modified by purification of mast cells and is calcium independent. The sensitivity and maximum response to catechol is the same irrespective of the presence or absence of Ca^{++}, except on purified pleural mast cells, that showed a plateau response at 250 μM catechol in the absence of Ca^{++}, and on unpurified peritoneal mast cells which exhibited a lower maximum response equally in the absence of Ca^{++}.

The release is induced by catechol at concentrations as low as 50 μM in all cases, and the maximum response is reached at 1 mM.

Key words: Pleural mast cells, Peritoneal mast cells, Histamine release, Catechol, Calcium.

It is widely accepted that calcium plays a role in the secretory process (3) and that mast cell degranulation depends on calcium (4). There are a great number of substances which degranulate mast cells, and they can be classified into two groups: selective and non-selective (7). The former, to which catechol belongs, do not induce cell damage, and the latter are cytotoxic. In the present study the effect of calcium on the non-cytotoxic histamine-releasing activity of catechol on rat pleural and peritoneal mast cells, cell populations with different response patterns to the same histamine-releasing compounds (2) is reported.

Materials and Methods

Male and female Wistar rats weighing 250-350 g were used in each experiment.

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Cell incubation. Isolation, purification and incubation of mast cells with catechol were carried out as described elsewhere (2). During incubation, catechol oxidation was prevented by adding ascorbate (10^{-4} M). The incubations were achieved at 37°C and stopped by addition of cold physiological saline (5 ml, pH 7). In order to remove the catechol, which greatly interferes with the histamine assay, cells were centrifuged at 1,000 *g* for 5 min and the supernatant was discarded. The washing process was repeated twice.

Cells were placed in each tube and suspended in 1 ml of medium with the following composition (mM): 142.3 Na^+, 5.94 K^+, 1 Ca^{++}, 1.2 Mg^{++}, 126.1 Cl^-, 22.85 CO_3H^-, 1.2 $PO_4H_2^-$, 1.2 $SO_4^=$, and 1 mg/ml of glucose and bovine serum albumin. The suspension was incubated at 37°C for 15 min. Appropriate controls for total and spontaneous histamine release were included in each experiment.

Determination of histamine. The histamine content was determined according to the method described by SHORE (8), omitting the extraction procedure (9). Spectrofluorometric determinations were carried out in a Shimadzu digital spectrofluorophotometer, model RF-510. The percentage of histamine release was calculated according to the following equation: $\% R = [(C - S)/C] \times 100$, where C and S = control and sample, respectively (histamine content of the pellet in the absence or presence of catechol respectively). Spontaneous histamine release was calculated according to the equation: $\% R_s = [(T - C)/T] \times 100$, where T = total histamine content in each tube (pellet plus supernatant).

Statistical analysis. Student's t-test for non-paired data was used for the statistical evaluation of the results. Probability values (P) less than 0.05 were considered significant. The results were expressed as percent of response (mean ± S.E.M.). Spontaneous histamine release was deducted and never greater than 6 %.

Cell viability. It was tested with the trypan-blue exclusion method as described elsewhere (1).

Chemicals and drugs. Catechol, bovine serum albumin (BSA, fraction V) and ortho-phthalaldehyde (OPT) were purchased from Sigma. Trypan blue was obtained from Flow Laboratories and Percoll from Pharmacia. Other reagents were purchased from usual commercial sources.

Results

Catechol-induced histamine release in the presence of 1 mM Ca^{++}. Histamine release was triggered with 50 µM catechol. The release percentages from purified and unpurified peritoneal mast cells were 35.4 ± 1 and 32.7 ± 4 respectively. Those from purified and unpurified pleural mast cells were 30.8 ± 2 and 34.5 ± 1.3, respectively. The maximum release from purified and unpurified peritoneal mast cells was reached at 1 mM catechol (80.4 ± 2.3 and 87.9 ± 4, respectively). The maximum release from purified and unpurified pleural mast cells was 86.2 ± 3.4 and 88 ± 0.9 %, respectively (table I).

Catechol-induced histamine release in the absence of extracellular Ca^{++}. The lack of extracellular calcium did not change the pattern of catechol-induced histamine release, and the percentages of release from purified and unpurified peritoneal mast cells at 50 µM catechol were 29.9 ± 2 and 25.2 ± 1.2 respectively, and those of purified and unpurified pleural mast cells were 36.9 ± 2.6 and 35 ± 1.2 %, respectively. With purified pleural mast cells, a plateau of response was reached around a catechol concentration of 250 µM. The maximum responses were reached at 1 mM catechol and were

Table I. *Dose-response profile in rat pleural and peritoneal mast cells stimulated with catechol (1 μM to 1 mM) in the presence of 1 mM Ca^{++} or in the absence of Ca^{++}.*
Experiments were carried out in purified mast cells (with Percoll®) and in unpurified mast cells. Values are expressed as percent of histamine release (mean ± SEM). Spontaneous histamine release was corrected for each tube. Significant differences are denoted by •.

	Unpurified cells		Purified cells	
Catechol	Peritoneal	Pleural	Peritoneal	Pleural
Ca^{++} 1 mM				
1 μM	5.0 ± 2.5	3.0 ± 3.0	8.0 ± 2.5	7.0 ± 2.0
10 μM	18.4 ± 2.0	21.0 ± 3.5	21.7 ± 4.0	18.4 ± 2.5
50 μM	32.7 ± 4.0	34.5 ± 1.3	34.5 ± 1.0	30.8 ± 2.0
250 μM	50.0 ± 4.4	52.6 ± 4.0	52.5 ± 3.2	55.3 ± 2.1
1 mM	87.9 ± 4.0	88.0 ± 0.9	84.0 ± 2.3	86.2 ± 3.4
Ca^{++} FREE				
1 μM	5.0 ± 2.2	3.0 ± 2.0	3.0 ± 2.5	2.0 ± 1.0
10 μM	17.8 ± 1.7	21.0 ± 2.5	30.4 ± 3.2	16.4 ± 3.0
50 μM	25.2 ± 1.2 •	35.0 ± 1.0 •	29.9 ± 2.0 •	36.9 ± 2.6 •
250 μM	53.9 ± 4.0 •	76.0 ± 2.5 •	58.5 ± 5.2 •	79.6 ± 2.5 •
1 mM	75.1 ± 1.9 •	88.4 ± 4.0 •	87.3 ± 2.0	84.5 ± 0.7

87.3 ± 2 and 75.1 ± 1.9 % in purified and unpurified peritoneal cells respectively, and 84.5 ± 0.7 and 88.4 ± 4 % in purified and unpurified pleural mast cells respectively (table I).

Cell viability. The viability of cells after incubation with 250 μM and 1 mM catechol was 95 % in both.

Discussion

In the present work, the response profile of rat pleural and peritoneal mast cells to the effect of calcium on the histamine-releasing action of catechol (pyrocatechol) is studied. It has been firmly established that metabolic energy and increased calcium concentrations in the cytosol are required for mast cell degranulation (4, 6). Ennis *et al.* (5) proposed the existence of three calcium pools in mast cells: the one loosely bound to the plasma membrane, the one more closely connected to the membrane at the regulatory sites, and the one in the intracellular reservoirs. Some compounds release histamine in the absence of extracellular calcium, probably from intracellular calcium resevoirs (7). The present results show a dose-response relationship for the catechol effect, and independence of the catechol effect from extracellular calcium, as indicated by the fact that, except for unpurified peritoneal cells, almost the same maximal was obtained irrespective of the presence or absence of extracellular calcium. Sensitivity of pleural mast cells was not affected by the absence of extracellular calcium, but peritoneal cells showed a slightly but significantly lower response. Unexpectedly, the response of pleural mast cells reached a plateau in the absence of extracellular calcium at lower catechol concentrations than in the presence of calcium. The reason for this result is not clear at present.

It has been previously demonstrated that purification with Percoll diminishes the maximum response of pleural and peri-

toneal mast cells to certain polyamines (2). However, such an effect of purification was not observed in the mast cell response to catechol.

Catechol-induced histamine release is unlikely to be due to a lytic process even at the highest concentrations. The action of catechol seems to be non-receptor mediated, since it is commonly used to diminish non-specific binding of radiolabelled adrenergic tracers (10).

In summary, histamine release from pleural and peritoneal rat mast cells by catechol is a non-specific, non-cytotoxic response, which does not require extracellular calcium.

Resumen

Se estudia en mastocitos de pleura y de peritoneo de rata la liberación de histamina inducida por el catecol (1, 10, 50, 250 µM y 1 mM). La respuesta al catecol no es citotóxica, ni se modifica por la purificación de los mastocitos y es independiente del calcio. La sensibilidad y la respuesta máxima es independiente de la presencia o ausencia de Ca^{++}, excepto en mastocitos puros de pleura, que muestran una meseta en el perfil de respuesta con catecol 250 µM, y en mastocitos de peritoneo no purificados, que muestran una respuesta máxima menor, también en ausencia de Ca^{++}. En todos los casos la liberación de histamina se consigue con catecol 50 µM, y la respuesta máxima se alcanza con catecol 1 mM.

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