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Increased responsiveness of rat mast cells to compound 48/80 due to removal of extracellular magnesium

Effects of ouabain and EGTA

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A decreased secretory response of mast cells to compound 48/80 (12% of control value) after preincubation of the cells with magnesium but without calcium was partially restored by removal of magnesium. EGTA (10 μM) blocked the restoration and decreased the restored secretory activity again, while this was further increased by ouabain (1 mM). Furthermore, ouabain completely restored the decreased secretion (50% of control value) due to preincubation without the divalent cations. This may indicate that magnesium influences a pool of cellular calcium that is involved in the stimulus-secretion coupling and is available to EGTA, and ouabain did not counteract the inhibitory mechanism of magnesium.

Secretion; Histamine; Magnesium; EGTA; Mast cell; Ouabain

1. INTRODUCTION

Histamine secretion from rat peritoneal mast cells induced by compound 48/80 occurs by exocytosis [1], and activation of the secretory mechanism seems to involve a G protein [2–4]. The secretion can occur in the absence of extracellular calcium [5]. Then the secretory responsiveness of the mast cell is likely to be dependent on cellular calcium [6–9]. Magnesium is known to inhibit exocytotic processes in various tissues that are dependent on extracellular calcium [10–11]. However, there is evidence that compound 48/80 induced histamine secretion in the absence of extracellular calcium may also be inhibited by magnesium [12]. Recently, we have demonstrated that ouabain enhances the secretory response of mast cells to compound 48/80 after preincubation in a medium with magnesium but without calcium. This is likely to be related to an inhibition of the Na^+-K^+ pump activity, which may influence a cellular calcium pool that is utilized in compound 48/80-induced histamine secretion [13].

In the present investigation we have studied if the inhibitory effect of magnesium is reversible and if ouabain or EGTA may influence the response to compound 48/80 observed after removal of extracellular magnesium.

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2. MATERIALS AND METHODS

2.1. *Cell preparation*

Male Sprague-Dawley rats, 350–500 g, were used for the experiments. Suspensions of mixed peritoneal cells (Figs. 1–4) and suspensions of pure mast cells (Figs. 5,6) were prepared as described previously [14]. A Coulter counter (Model 134, Analys Instrument AB, Sweden) was used to count the number of cells, and inspection of stained smears (Toluidine blue) was used to determine the fractions of mast cells in the suspensions of mixed peritoneal cells (9.6% ± 0.5%, mean and S.E.M., $n = 18$) and populations of pure mast cells (97.9% ± 0.5%, mean and S.E.M., $n = 8$).

2.2. *Incubation procedures*

Cells pooled from 1–2 rats were divided into samples with the same cell density. The number of cells per sample was $59.4 \times 10^3 \pm 6.12 \times 10^3$ (populations of pure mast cells, mean and S.E.M., $n = 8$) and $7.68 \times 10^5 \pm 0.64 \times 10^5$ (mixed peritoneal cells, mean and S.E.M., $n = 18$). The cells were preincubated at 37°C two or three times and then incubated with compound 48/80 (1 μg/ml) for 10 min at 37°C in a final volume of 4 ml (Figs. 1–4) or 0.5 ml (Figs. 5,6) in order to induce the secretion of histamine. The first preincubation for 60 min was performed in a calcium-free medium with magnesium 1.2 mM (Figs. 1–4) or without magnesium (Figs. 5,6). Then magnesium was removed (Figs. 1–4) by centrifugation of the cells (10 min, 220 × *g*) at 4°C and resuspension of the pellet with a calcium- and magnesium-free buffered salt solution. The second and third preincubations were performed as described in legends to figures. Control experiments showed that the temperature at which the removal of magnesium was performed after the first preincubation did not influence the effect of the second preincubation of the cells with and without magnesium or EGTA on histamine secretion ($P > 0.1$) (Fig. 4).

2.3. *Histamine release*

After the incubation with compound 48/80 the samples were placed in an ice-chilled water bath (Figs. 1–4) or 1.75 ml ice-chilled buffered salt solution was added to the cell samples, which were then placed in the ice-chilled water bath (Figs. 5,6). The samples were then centrifuged (220 × *g* for 10 min) and the histamine content of the supernatant

and the pellet was determined by a modification of the fluorimetric method described by Shore et al. [15], in which the extraction procedure was omitted [16].

2.4. *Drugs and materials*

Percoll was supplied by Pharmacia Fine Chemicals (Sweden) and bovine serum albumin, compound 48/80, EGTA (ethyleneglycol-bis(betaaminoethyl ether)-*N*,*N*'-tetraacetic acid), HEPES (*N*-(2-hydroxyethyl)piperazine-*N*-(2-ethanesulfonic acid)) and Trizma base (Tris(Hydroxymethyl)aminomethan) were supplied by Sigma Chemical Company (St. Louis, MO, USA). Ecoscint was supplied by BN Plastic (Helsinge, Denmark), glucose by Fluka (Switzerland) and ouabain by Mecobenzon (Denmark). All other chemicals were of analytical grade.

2.5. *Solutions*

The buffered salt solution contained (Figs. 1-4) (mM): NaCl 144.5, KCl 4.75, HEPES 10.0, glucose 5.6 and bovine serum albumin 1 mg/ml (pH: 7.2-7.3), or it contained (Figs. 5-6) (mM): NaCl 136.8, KCl 4.75, Tris 12.5, glucose 5.6 and bovine serum albumin 1 mg/ml (pH: 6.8-7.1). The pH was determined at 37°C (PHM 64 Research pH meter, Radiometer, Denmark). The different combinations of calcium, magnesium and EGTA had no effect on pH.

2.6. *Presentation of data and statistics*

Histamine secretion was calculated as a percentage of the total histamine content of the cells. The values were corrected for the spontaneous secretion of histamine that occurred in the absence of compound 48/80 (range: 0.6% to 9.6 %). The various drug combinations had no effect on the spontaneous secretion of histamine.

Differences between groups were examined by the Mann-Whitney U-test. A value of $P<0.05$ was considered significant. The effects of ouabain and EGTA in Fig. 3 were evaluated by the Friedman two-way of variance [17].

3. RESULTS

Histamine secretion was less than 10% after the first preincubation period with magnesium in the absence of calcium, and the secretion remained at that level, if the cells were exposed to magnesium in the second preincubation period (Fig. 1). However, there was a time-dependent and partial restoration of the cellular responsiveness to compound 48/80 when the second preincubation occurred in a magnesium-free solution. At 90 min after magnesium was removed the histamine release was 49% (Fig. 1). Control samples were exposed to both calcium and magnesium during the second preincubation. After 10 min the histamine release was 74%, and this level was not changed by prolonging the second preincubation period up to 120 min. EGTA dose-dependently inhibited the restorative effect of the

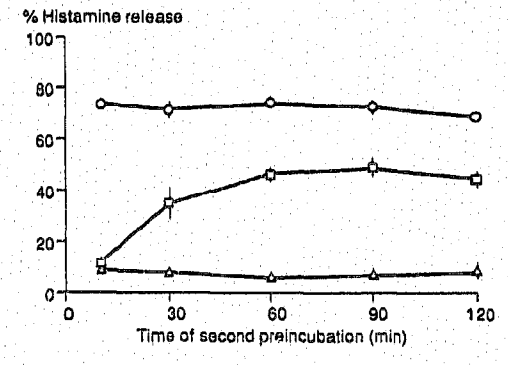

Fig. 1. Reversal of the decreased histamine secretion by removal of extracellular magnesium. The cells were preincubated twice. Magnesium 1.2 mM was present in the first preincubation. The second preincubation for 10-120 min (abscissa) was performed either in a calcium-free medium without (□) or with (△) magnesium, or in a medium with both calcium (1.0 mM) and magnesium (1.2 mM) (○). Mean value from five experiments are shown; vertical lines show S.E.M.

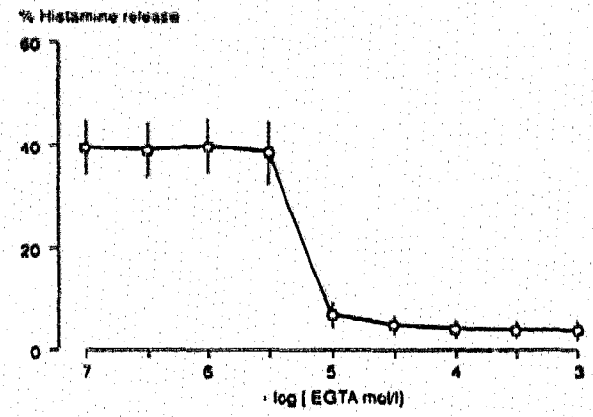

Fig. 2. Dose-dependent inhibition by EGTA of the effect of removal of magnesium on histamine secretion. The cells were preincubated twice. Magnesium 1.2 mM was present in the first preincubation. The second preincubation for 90 min was performed in the presence of various concentrations of EGTA (abscissa: 0.1 μM to 1 mM) in the absence of the divalent cations. The control values of the secretory response (67% ± 4.4%, mean value and S.E.M.) was obtained from samples that were exposed to calcium and magnesium during the second preincubation period. Mean values from four experiments are shown; vertical lines show S.E.M.

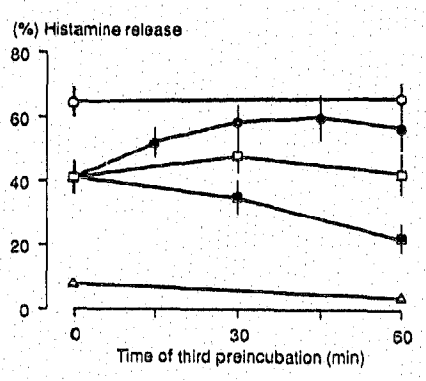

Fig. 3. Effects of ouabain and EGTA on histamine secretion after the partial restoration of the responsiveness caused by the removal of magnesium. The cells were preincubated three times. Magnesium (1.2 mM) was present in the first preincubation. The second preincubation of 60 min was performed in the absence of the divalent cations. The third preincubation lasted for 15 to 60 min (abscissa). This was initiated by addition of 1.0 mM ouabain (●) or 10 μM EGTA (■) to the cell suspension. (□) denotes no drug addition. Control samples were run in parallel containing either magnesium (△) or magnesium and calcium (○) during the second and third preincubation periods. Mean values from five experiments are shown; vertical lines show S.E.M.

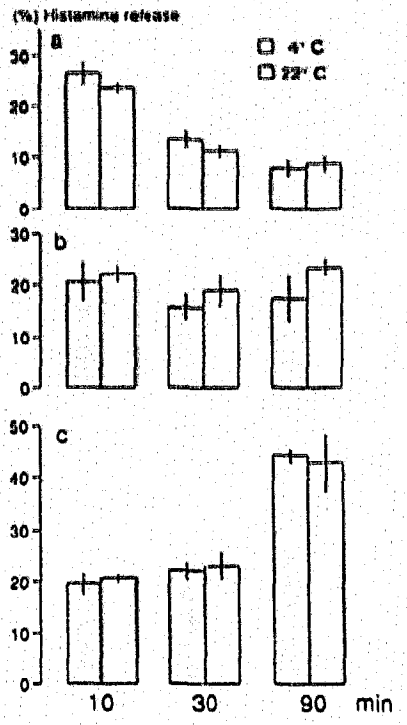

Fig. 4. Effect of centrifugation at either low (4°C) or high (22°C) temperature for the removal of magnesium. The cells were preincubated twice. Magnesium (1.2 mM) but not calcium was present in the first preincubation. The cells were then centrifuged either at 4°C or 22°C, and the second preincubation at 37°C for 10-90 min (abscissa) was performed in a calcium-free medium without (c) or with magnesium, 1.2 mM (a) or with EGTA, 10 μM (b). Control values of histamine secretion from samples preincubated in parallel with calcium and magnesium present during the second preincubation of 90 min were: 72.1% ± 2.8% (4°C) and 69.3% ± 0.8% (22°C) (mean values and S.E.M.). Mean values from four experiments are shown; vertical lines show S.E.M.

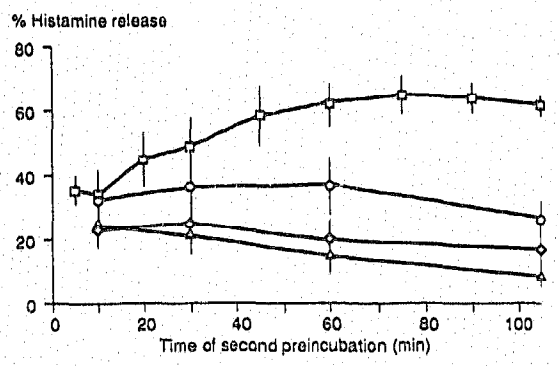

Fig. 5. Enhancement by ouabain of the cellular responsiveness to compound 48/80. The cells were preincubated twice in a calcium- and magnesium-free medium without centrifugation and resuspension in between. Ouabain 1 mM (□), ouabain 1 mM and EGTA 0.1 mM (◇) or EGTA 0.1 mM (△) were added to the cells in the second preincubation that lasted for 5-105 min (abscissa). (○) denotes samples with no drug addition. The control values of histamine secretion were obtained from mast cells exposed to calcium for 120 min (60 min on the abscissa: 68.3% ± 2.8%, mean and S.E.M.) and 165 min (105 min on the abscissa: 68.0% ± 3.7%, mean and S.E.M.). Mean values from four experiments are shown; vertical lines show S.E.M.

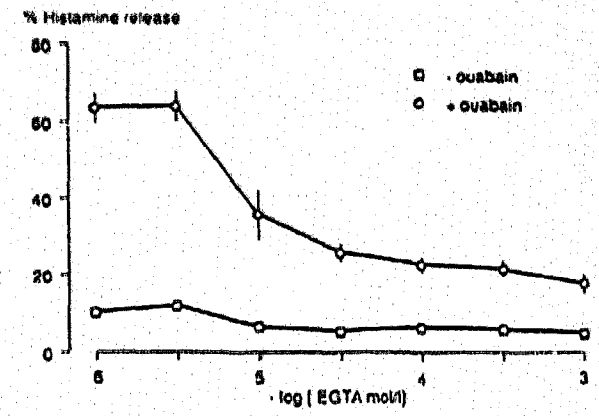

Fig. 6. Dose-dependent inhibition by EGTA of the effect of ouabain on the cellular responsiveness to compound 48/80. The cells were preincubated twice in a calcium- and magnesium-free medium without centrifugation and resuspension in between. EGTA (abscissa: 1 μM to 1 mM) with ouabain (1 mM) (○) or without ouabain (□) was added to the cells in the second preincubation that lasted for 105 min. The control values of histamine secretion obtained from mast cells exposed to calcium for 165 min were 71.6% ± 4.9% in the absence of ouabain and 62.5% ± 2.6% in the presence of 1 mM ouabain (mean values and S.E.M.). Mean values from four experiments are shown; vertical lines show S.E.M.

removal of extracellular magnesium. While 3 μM EGTA had no effect, maximal inhibition was observed in the presence of 10 μM EGTA (Fig. 2). After the secretory responsiveness was partially restored by the removal of extracellular magnesium, the addition of 10 μM EGTA gradually decreased the response to compound 48/80 again (Fig. 3). The histamine secretion was 41% before the addition of EGTA and 60 min after the addition of EGTA it was 23%. In contrast, if 1 mM ouabain was added to the cells after the secretory responsiveness was partially restored, there was a further, time-dependent increase in the secretion of histamine up to 59% (Fig. 3) ($P<0.001$).

By performing the first preincubation in the absence of both magnesium and calcium the secretory responsiveness was decreased to about 50% of the control value from cells exposed to calcium (Fig. 5). Addition of ouabain in the second preincubation caused a time-dependent restoration of the secretory response, and this was complete after 75 min (65.1%) when compared with the control value (68.3%) from cells preincubated in parallel with calcium and magnesium ($P>0.1$). Addition of EGTA to the cells concomitantly with ouabain dose-dependently inhibited the restoration (Fig. 6). While 3 μM had no effect, a partial effect was observed with 10 μM, and complete blockade required 30 μM EGTA.

4. DISCUSSION

The decreased secretory responsiveness observed after 60 min preincubation of the cells in presence of

magnesium in a calcium-free medium confirms previous observations [12]. The partial recovery of the responsiveness by removal of extracellular magnesium, its reversal by the subsequent addition of EGTA and the complete blockade of the recovery by 10 μM EGTA support the idea that magnesium may influence a pool of cellular calcium that is utilized in the stimulus-secretion coupling. This cellular calcium pool is available to EGTA from the external side of the plasma membrane. The enhancement of the secretion of histamine by ouabain is not likely to be caused by a counteraction of the mechanism of inhibition by magnesium, since it occurred after removal of magnesium from the extracellular compartment as well as after preincubation of the cells in the absence of the divalent cations.

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REFERENCES

[1] Horsfield, G.I. (1965) J. Path. Bact. 90, 599-605.
[2] Mousli, M., Bueb, J.-L., Bronner, C., Rouot, B. and Landry, Y. (1990) Trends Pharmacol. Sci. 11, 358-362.
[3] Mousli, M., Bronner, C., Landry, Y., Bockaert, J. and Rouot, B. (1990) FEBS Lett. 259, 260-262.
[4] Mousli, M., Bronner, C., Bockaert, J., Rouot, B. and Landry, Y. (1990) Immunol. Lett. 25, 355-358.
[5] Uvnäs, B. and Thon, L.-L. (1961) Exp. Cell Res. 23, 45-57.
[6] Douglas, W.W. and Ueda, Y. (1973) J. Physiol. 234, 97P-98P.
[7] Kagayama, M. and Douglas, W.W. (1974) J. Cell Biol. 62, 519-526.
[8] Cochrane, D.E. and Douglas, W.W. (1974) Proc. Natl. Acad. Sci. USA 71, 408-412.
[9] Atkinson, G., Ennis, M. and Pearce, F.L. (1979) Br. J. Pharmacol. 65, 395-402.
[10] Rubin, R.P. (1970) Pharmacol. Rev. 22, 389-428.
[11] Foreman, J.C. and Mongar, J.L. (1972) J. Physiol. 224, 753-769.
[12] Johansen, T. (1980) Life Sci. 27, 369-375.
[13] Johansen, T., Knudsen, T. and Bertelsen, H. (1990) FEBS Lett. 262, 228-230.
[14] Knudsen, T. and Johansen, T. (1989) Br. J. Pharmacol. 96, 773-778.
[15] Shore, P.A., Burkhalter, A. and Cohn, V.H. (1959) J. Pharmacol. Exp. Ther. 127, 182-186.
[16] Bergendorff, A. and Uvnäs, B. (1972) Acta Physiol. Scand. 84, 320-331.
[17] Siegel, S. (1956) Nonparametric Statistics for the Behavioral Sciences, McGraw-Hill, New York.