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Effect of ryanodine on histamine release from rat peritoneal mast cells induced by anti-IgE

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Abstract—Ryanodine strongly inhibited histamine release from rat peritoneal mast cells induced by anti-IgE. Ryanodine also inhibited Ca^{2+}-mobilization from the intracellular Ca^{2+}-store as well as histamine release in mast cells activated by anti-IgE. These results suggest that the effect of ryanodine on histamine release from rat mast cells might be due to the inhibition of Ca^{2+} release from the intracellular Ca^{2+} store.

Ca^{2+} acts as a second messenger during cell activation (Rasmman & Goodman 1977). An increase in the intracellular Ca^{2+} level has been proposed as an essential trigger for mast cell activation. Many workers have demonstrated an absolute requirement for calcium in histamine release induced by a variety of secretagogues. Histamine release from mast cells has been shown to be preceded by an increased mobilization of intracellular Ca^{2+} (White et al 1985). We have previously shown that the mobilization of calcium from the intracellular Ca^{2+} store is obligatory for histamine release induced by various secretagogues (Takei et al 1989, 1991).

Ryanodine, an alkaloid from *Ryania speciosa* Vahl, acts specifically on the Ca^{2+} release channel of the sarcoplasmic reticulum (SR) (Alexandre 1985). Ryanodine also diminishes the rate of Ca^{2+} accumulation into the SR.

We have investigated the effects of ryanodine on the change in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and on the histamine release, induced by the secretagogues, anti-IgE, concanavalin A or compound 48/80, from rat mast cells.

Materials and methods

Rats were killed by asphyxiation in an atmosphere of CO_2. Rat peritoneal mast cells were obtained from male Wistar rats, 200–300 g, and mast cells were purified using the method of Németh & Röhlich (1980). Viability of the cells was >97% as assessed by trypan blue occlusion. Passively sensitized rat mast cells were prepared as described previously (Takei et al 1988).

Assay of histamine. Purified mast cells from normal rats were incubated for passive sensitization with sensitized rat sera for 1 h at 4°C. The mast cell suspensions in Tyrode-HEPES solution (pH 7·4) were incubated in duplicate at 37°C with or without ryanodine for 5 min before the addition of secretagogues. Tyrode-HEPES solution contained (mM): NaCl 124, KCl 4·0, $CaCl_2$ 1·0, $NaHCO_3$ 10, glucose 5·6, NaH_2PO_4 0·64, $MgSO_4$ 0·5, 4-(2-hydroxyethyl)-1-piperazine-ethane sulphonic acid (HEPES) 5, and 50 mg mL^{-1} of phosphatidylserine and 50 mg L^{-1} bovine serum albumin. Bovine serum albumin was omitted from the solution in experiments on histamine release. At intervals, samples of the solution were withdrawn and the reaction was stopped by the addition of 1 mL of Tris-EDTA buffer. The Tris-EDTA buffer contained (mM): Tris 25, NaCl 120, KCl 5, EDTA 1 and 0·2 g L^{-1} human serum albumin. The cells were separated from the released histamine by centrifugation at 1300 *g* for 10 min at 4°C. Residual histamine in the cells was released by disrupting the cells with trichloroacetic acid (final concentration 10% trichloroacetic acid), and centrifugation at 1500 *g* for 15 min at 4°C. Histamine content was determined fluorometrically (Shore et al 1959). The amount of histamine released was calculated as percentage of the total histamine present in the control suspension.

Mast cells were incubated with 3 mM EGTA for 3 min at 37°C and then an optimal concentration of anti-IgE (200 μg mL^{-1}) was added to the mixture.

Measurement of intracellular calcium concentration. Fluorescence was recorded using a fluorimeter (model 650-40 Fluorescence 100; Hitachi, Japan) with a temperature-controlled cuvette and a magnetically driven stirrer. Purified mast cells (1×10^6 cells mL^{-1}) were incubated at 37°C for 10 min with 100 μM Quin-2/AM (2-[(2-amino-5-methyl-phenoxy)methyl]-6-methoxy-8-aminoquinoline-*N*,*N*,*N*′,*N*′-tetraacetic acid, tetraacetoxymethyl ester) in Tyrode-HEPES solution. The cell suspension was diluted (1 : 10) with Tyrode-HEPES solution, left to stand for 60 min, and then washed twice with the complete solution. Samples of the cell suspension (1×10^5 cells mL^{-1}) were placed in the cuvette described above, and all reagents were added with a microsyringe directly into the cuvette, without interrupting the recording. The cell suspension was incubated at 37°C for 5 min with ryanodine and challenged with the secretagogue. Fluorescence excitation and emission wavelengths

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were 339 and 492 nm, respectively. The concentration of intracellular Ca^{2+} was calculated by the method of Tsien et al (1982).

Ryanodine and Quin-2/AM were obtained from Wako Pure Chemical Industries, Osaka, Japan. *Bordetella pertussis* vaccine was purchased from the Ciba Serum Institute, Ciba, Japan. Compound 48/80 and L-γ-phosphatidylserine were purchased from Sigma Chemical Company, St Louis, MO, USA. Anti-IgE was purchased from Miles Laboratories, USA.

Results

Anti-IgE at its optimal concentration of 200 μg mL^{-1}, induced a time-dependent release of histamine from rat peritoneal mast cells. The histamine release was essentially complete within 4–5 min, and 51 ± 2·1% of the total histamine was released after 5 min (n = 5). When added to the reaction medium, ryanodine strongly inhibited histamine release from rat mast cells induced by anti-IgE. At 0·1 and 100 μM, the degrees of inhibition by ryanodine on histamine release from rat mast cells 5 min after challenge with anti-IgE were 47 ± 2·2 and 72 ± 3·2%, respectively (n = 5) (Fig. 1).

Purified passively sensitized rat mast cells were challenged with 200 μg mL^{-1} of anti-IgE, which induced an initial increase in $[Ca^{2+}]_i$ within several seconds, followed by a slower increase of $[Ca^{2+}]_i$ (second rise) reaching the maximum 4 min after the challenge. Histamine release followed the initial rise in $[Ca^{2+}]_i$, and in parallel reached a maximum within 4 min. The initial and maximal increase were 88 and 119 nM of $[Ca^{2+}]i$, respectively. The effects of the various concentrations of ryanodine on the initial rise in $[Ca^{2+}]_i$ in mast cells activated by anti-IgE were examined. Ryanodine inhibited the initial rise in $[Ca^{2+}]_i$. In the presence of 0·1 and 100 μM of ryanodine, the initial rise in $[Ca^{2+}]_i$ reached a maximum of only 45 and 18 nM, respectively. The inhibitory effect was 48 and 80% for the initial rise, respectively (n = 5) (Fig. 2). Ryanodine did not cause a dose-dependent inhibition of the initial rise in $[Ca^{2+}]_i$ and histamine release in mast cells activated by anti-IgE, but these two effects were closely correlated (data not shown).

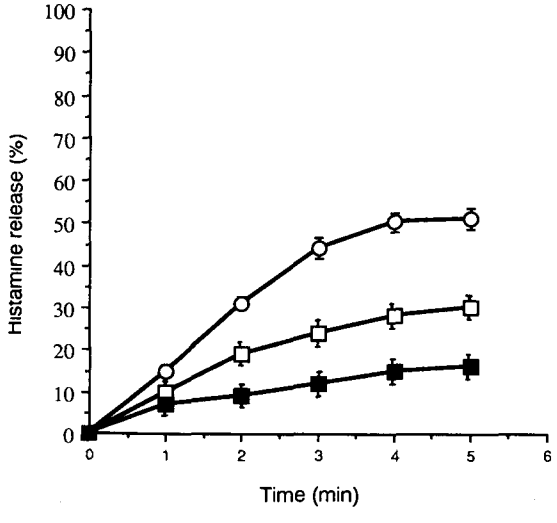

FIG. 1. Kinetics of histamine release induced by anti-IgE 200 μg mL^{-1} (○), and in the presence of ryanodine at 0·1 μM (□), and 100 μM (■). Mast cells were preincubated for 5 min in the presence of ryanodine before the addition of anti-IgE. Spontaneous histamine release from mast cells was 5·2 ± 0·3%, and this value was subtracted from the experimental value. Vertical bars represent the s.e.m. for 5 experiments.

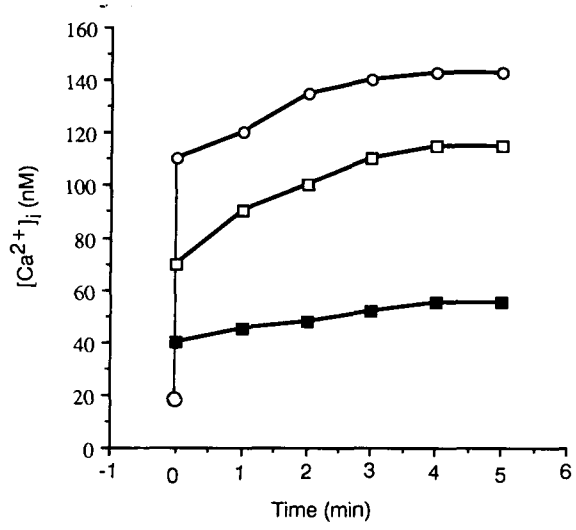

FIG. 2. Effect of ryanodine on the increase in $[Ca^{2+}]_i$ in passively sensitized mast cells induced by anti-IgE. Passively sensitized mast cells (1×10^5 cells mL^{-1}) were challenged with 200 μg mL^{-1} of anti-IgE and incubated at 37°C with or without ryanodine. Mast cells were preincubated for 5 min in the presence of ryanodine. ○, $[Ca^{2+}]_i$ without ryanodine; □, $[Ca^{2}]_i$ with 0·1 μM of ryanodine; ■, $[Ca^{2+}]_i$ with 100 μM of ryanodine. Each value represents the average of 5 experiments.

Table 1. Effect of ryanodine on the histamine release and the increase in $[Ca^{2+}]_i$ in passively sensitized mast cells induced by anti-IgE (200 μg mL^{-1}) in the absence of extracellular Ca^{2+}. After pre-incubation with ryanodine for 5 min, mast cell suspension was incubated with anti-IgE for 10 min at 37°C. EGTA (3 mM) was added to all cell suspensions 3 min before the addition of anti-IgE. Each value represents the average of 5 experiments.

	Histamine release (%)	Increase in $[Ca^{2+}]_i$ (nM)
Without ryanodine	35·3 ± 2·4	62
With ryanodine		
0·1 μM	18·8 ± 1·2	36
100 μM	9·4 ± 0·71	16

The effect of ryanodine on the intracellular calcium ion mobilization and histamine release induced by anti-IgE in the presence of 3 mM EGTA is shown in Table 1. Ryanodine (0·1 and 100 μM) inhibited the anti-IgE-induced increase in $[Ca^{2+}]_i$ and histamine release in the absence of extracellular Ca^{2+}.

Similar results were obtained for concanavalin A. However, compound 48/80 (1·0 μg mL^{-1}) rapidly caused histamine release, the process being complete within 60 s. Ryanodine (100 μM) did not inhibit histamine release and the initial rise in $[Ca^{2+}]_i$ induced by compound 48/80 (Fig. 3).

Discussion

These experiments were undertaken to clarify the effect of ryanodine on histamine release from rat isolated peritoneal mast cells induced by various secretagogues. Ryanodine strongly inhibited histamine release from rat peritoneal mast cells induced by anti-IgE and concanavalin A.

Ryanodine appears to antagonize sarcoplasmic reticulum (SR) Ca^{2+} release in cardiac muscle (Sutko et al 1986). Ryanodine inhibited three types of Ca^{2+} release from the SR, which have different mechanisms; Ca^{2+}-induced release of Ca^{2+} triggered by a rapid and transient increase of free Ca^{2+} at the outer surface of the SR; caffeine-induced release of Ca^{2+};

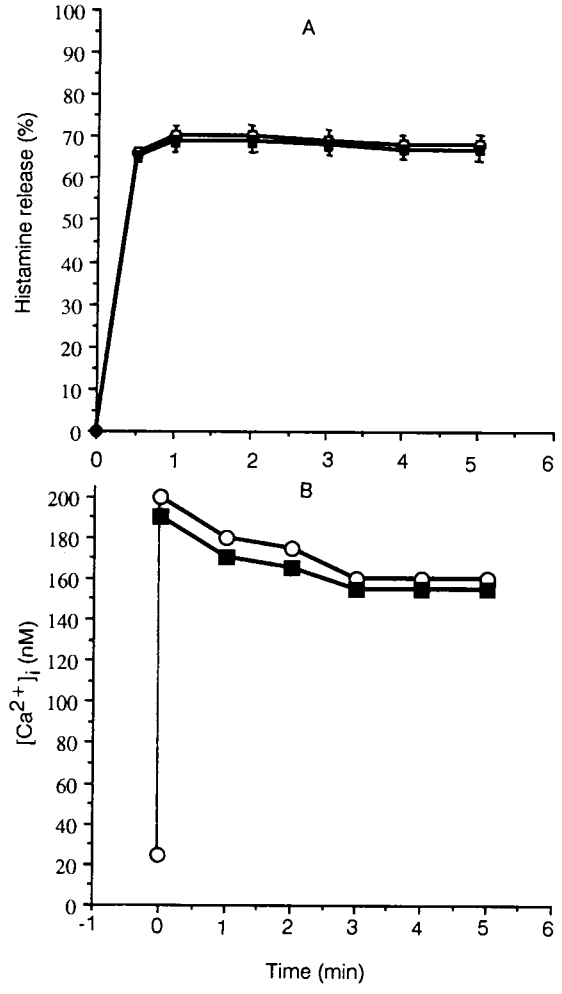

FIG. 3. A. Kinetics of histamine release induced by compound 48/80 1 $\mu g\ mL^{-1}$ (○), and in the presence of ryanodine at 100 μM (■). Mast cells were preincubated for 5 min in the presence of ryanodine before the addition of compound 48/80. Spontaneous histamine release from mast cells was 5·5 ± 0·6%, and this value was subtracted from the experimental value. Vertical bars represent the s.e.m. for 5 experiments. B. Effect of ryanodine on the increase in $[Ca^{2+}]_i$ in mast cells induced by compound 48/80. Mast cells (1×10^5 cells mL^{-1}) were challenged with 1 $\mu g\ mL^{-1}$ of compound 48/80 and incubated at 37°C with or without ryanodine. Mast cells were preincubated for 5 min in the presence of ryanodine. ○, $[Ca^{2+}]_i$ without ryanodine; ■, $[Ca^{2+}]_i$ with 100 μM of ryanodine. Each value represents the average of 5 experiments.

spontaneous cyclic release of Ca^{2+} occurring in the continuous presence of concentrations of free Ca^{2+} sufficient to overload the SR (Alexandre 1985). These suggest that the three types of Ca^{2+} release are through the same channel across the SR membrane, although the gating mechanisms are different for each type.

Ryanodine showed inhibition of the initial rise in $[Ca^{2+}]_i$, Ca^{2+} mobilization from the intracellular Ca^{2+} store, i.e. the plasma membrane-endoplasmic reticulum pool and the mitochondria matrix. The effect of ryanodine on the intracellular-mobilization induced by anti-IgE and concanavalin A is closely correlated with the effect of ryanodine on the histamine release, whereas the influx of extracellular Ca^{2+} is not. Moreover, ryanodine inhibited anti-IgE- and concanvalin A-induced increase in $[Ca^{2+}]_i$ and histamine release in the absence of extracellular Ca^{2+}.

We have previously shown that the initial rise in $[Ca^{2+}]_i$, due to Ca^{2+}-mobilization, correlates with the histamine release promoted by various secretagogues (Takei et al 1989). Other workers (Foreman et al 1977; Ishizaka et al 1980) have confirmed that Ca^{2+} uptake is accompanied by histamine secretion from rat mast cells following stimulation by various secretagogues. These findings suggested that the activation of mast cells is mediated by changes of the intracellular calcium concentration.

It has been reported that ryanodine is effective on the skeletal muscles (Désilets et al 1989). However, in the rat peritoneal mast cells, ryanodine inhibited Ca^{2+} mobilization from the intracellular Ca^{2+} store, indicating that there might be a common factor for the calcium ion storage of the skeletal muscle and rat peritoneal mast cells.

On the other hand, the histamine release induced by compound 48/80 was not affected by ryanodine. The present results suggested that there are some differences between the process of histamine release induced by anti-IgE and compound 48/80. The histamine release induced by compound 48/80 may not involve Ca^{2+} mobilization from the intracellular Ca^{2+} store.

From these results, it is suggested that the effect of ryanodine on histamine release from rat mast cells might be due to the inhibition of Ca^{2+} release from the intracellular Ca^{2+} store and that Ca^{2+} mobilization is necessary to elicit the release of histamine from mast cells.

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