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Valinomycin, a degranulating agent in rat mast cells which inhibits calcium-uptake

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Abstract

The effect of valinomycin on both, mast cell histamine release and on calcium (^{45}Ca)-uptake processes was examined. Pleural and peritoneal mast cells were purified in isotonic Percoll (pH=7) and mixed populations were used in the experiments.

Valinomycin (10^{-9}–$10^{-5}M$) stimulated histamine release in isolated rat mast cells when the incubation medium contained high K^+ concentrations (Tris-K^+ with 150 mM K^+), but not in ohter media such as Tris-Na^+ (120 mM Na^+) or Tris-sucrose (300 mM sucrose). In contrast, in the absence of valinomycin, elevated K^+ levels in the external environment did not activate mast cell secretion. Optimum response in valinomycin-treated mast cells was obtained when the cells were incubated for 60 min. Also valinomycin (10^{-5} M) induced substantial inhibition of ^{45}Ca-uptake while lower doses (10^{-9}–10^{-7} M) did not affect or only slightly incrased uptake.

In this paper valinomycin is shown to be a degranulating agent eliciting mediator release in mast cells incubated in the presence of high K^+ levels, which does not require extracellular calcium and inhibits ^{45}Ca uptake. The possibility that valinomycin acts as a K^+ ionophore, as in other secretory systems, is discussed.

Introduction

Mast cells release their chemical mediators by a specific exocytotic process [1]. The mechanisms regulating exocytosis are not completely understood, although it is clear that monovalent and divalent ions play a key role [2, 3]. The function of Ca^{+2} as the second messenger in stimulus-response coupling has been amply dealt with the literature, while the function of K^+ and Na^+ ions is not clearly understood. Uvnas' classic hypothesis considers extracellular sodium to be an ions which is exchanged for histamine molecules of the granular matrix during the final phase of exocytosis [4]. The same author has recently suggested the existence of an intracellular cation exchange Hi^+/K^+ instrumental in the release process [5].

Ionophores selectively transport ions across the plasma membrane and are thus useful for studying the role of ions in mediator release [6]. In mast cells, the ionophore X-537-A has been discovered for monovalent ions; it provokes histamine release in the presence of high levels of Na^+ or K^+ by means of a Ca^{++}- and energy-independent process [7, 8]. Valinomycin is another lipophilic compound which specifically transports K^+ ions and stimulates catecholamine release in bovine adrenal chromaffin cells [9]. In this work we have studied the effect of valinomycin on rat

mast cell secretory response; since valinomycin has a depolarizing action which opens specific calcium channels, ^{45}Calcium uptake in valinomycin permeabilized cells was also measured.

Material and methods

Isolation of mast cells. Mast cells were removed from old male and female Sprague-Dawley rats (400–800 g). Rats were sacrified by decapitation subsequent to ether anaestesia. Physiological saline solution was injected into pleural and peritoneal cavities. Following a gentle massage the fluid was extracted and decanted into plastic tubes. The cellular suspension, which contained 4–6% mast cells, was washed twice for 5 min at 50 g_{max} before purification (Kontron centrifugue, mod. TGA-6).

Umbreit physiological saline solution was slighly modified as follows (10) (m*M*): Na^+, 142.3; K^+, 5.94; Ca^{++}, 1; Mg^{++}, 1.2; Cl^-, 126.1; CO_3^-, 22.85; $PO_4H_2^-$, 1.2; SO_4^{-2}, 1.2; finally, 0.5 mg/ml of BSA was added (300 ± 5 mOsm/Kg H_2O, pH = 7).

Pure mast cell preparations were obtained by using the method described by Enerbäck and Svensson for crude cell suspensions with minimal red blood cell contamination [11]. An aliquot of 0.75–1 ml of cellular suspension containing 10^6 cells was mixed with 3.5–4 ml of isotonic Percoll (pH = 7) and centrifuged at 1000 g_{max} for 15 min. Erytrocytes, macrophages and leucocytes were removed and discarded together with the supernatant. The cells at the bottom contained 95–98% pure mast cells, of which 98–100% were impermeable to trypan blue dye. $1–1.5 \times 10^6$ and $3–5 \times 10^5$ pure mast cells were obtained from the peritoneal and pleural cavities of each rat.

Incubation. Samples for cell incubation were prepared as follows: 25 µl of valinomycin concentrated solution was added to sufficient incubation medium to attain a final volume of 0.9 ml, and then preincubated. When the medium reached 37 °C, 100 µl of cell suspension containing $1–1.5 \times 10^5$ mast cells was added to each tube. Incubation was carried out in a shaking bath (160 cycles/min) at 37 °C. Incubation time is indicated for each experiment. The modified media, which contained elevated ionic concentrations, were buffered with Tris-base to achieve isosmotic pressure. The presence or absence of external calcium has been indicated for each experiment. When incubation was carried out in the absence of calcium, purified mast cell suspensions were washed twice before incubation in Umbreit saline solution without 1 m*M* Ca^{+2}.

Reaction was prevented by immersing the tubes in a cold bath. After centrifugation at 1000 g_{max} for 5 min the supernatants were collected and decanted into other tubes for histamine determination.

Spontaneous histamine release in the absence of valinomycin was measured in the control tubes of each experiment.

Since the difference in response between pleural and peritoneal mast cells treated with valinomycin has been to shown to be insignificant [12, 13], mixed purified populations was used.

Histamine release measurement. Histamine was assayed spectrofluorometrically both in the pellet – residual histamine – and supernatants – released histamine – using a modified Shore's method (0.1% OPT) [14]. Trichloroacetic acid 14% was added to precipitate protein in the medium, which otherwise would interfere with histamine assay. In order to ensure the measurement of total histamine, pellets were first boiled for 10 min in 0.8 ml of 0.1 N ClH.

Results are expressed as the percentage of histamine released with respect to total histamine content.

^{45}Calcium-uptake. Cell incubation for ^{45}Ca-uptake determination was performed in 500 µl Akes tubes. First 100 µl of immersion oil (d = 1.02) was placed at the bottom of each tube. Then 200 µl of pure mast cells ($300 \pm 25 \times 10^3$) were suspended in the buffer and preincubated for 20 min at 37 °C. The reaction was started by the addition of valinomycin and the aliquot of ^{45}Ca which contained 5×10^5 dpm.

The cells were separated from the radioactive incubation medium by centrifugation for 30 s at 10000 rpm in a Beckman model 11 microfuge. The tubes were frozen rapidly at –25 °C and the bottoms were then clipped and transferred to glass scintillation vials. In order to ensure total ^{45}Ca measurement, cells were disrupted with 0.1 N NaOH and the solution was homogenized by shaking. Four ml of scintillation liquid was added to the samples and radioactivity was mea-

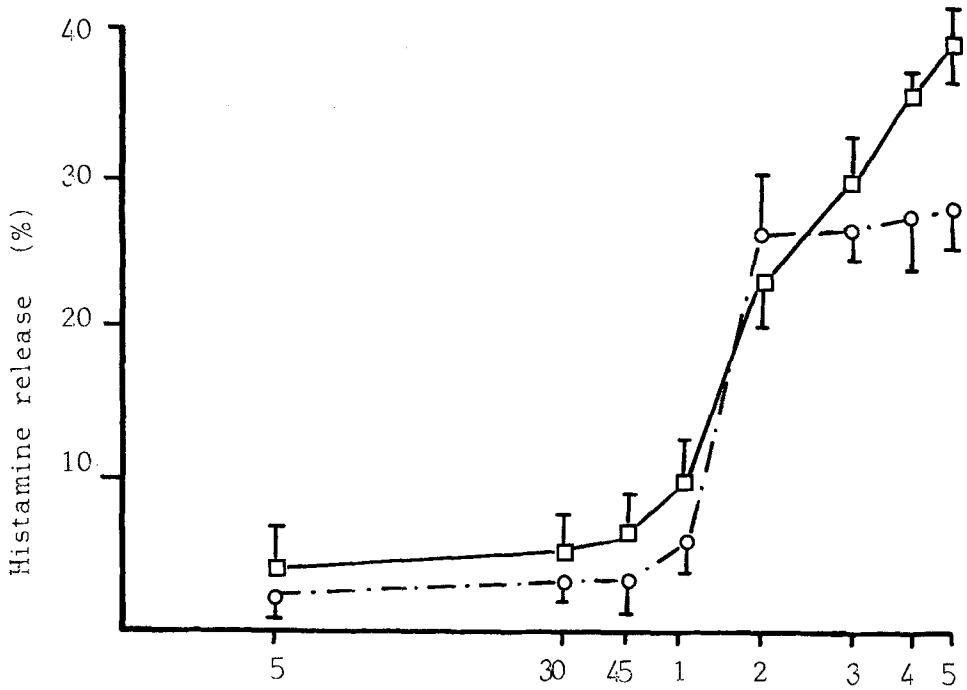

Figure 1
Histamine-release in rat mast cells incubated in the presence of 100 m*M* K^+ (—□—) and spontaneous release in mast cells incubated in Umbreit solution (—○—) for times ranging between 5 min–5 h. Media ± sem for $n = 5$.

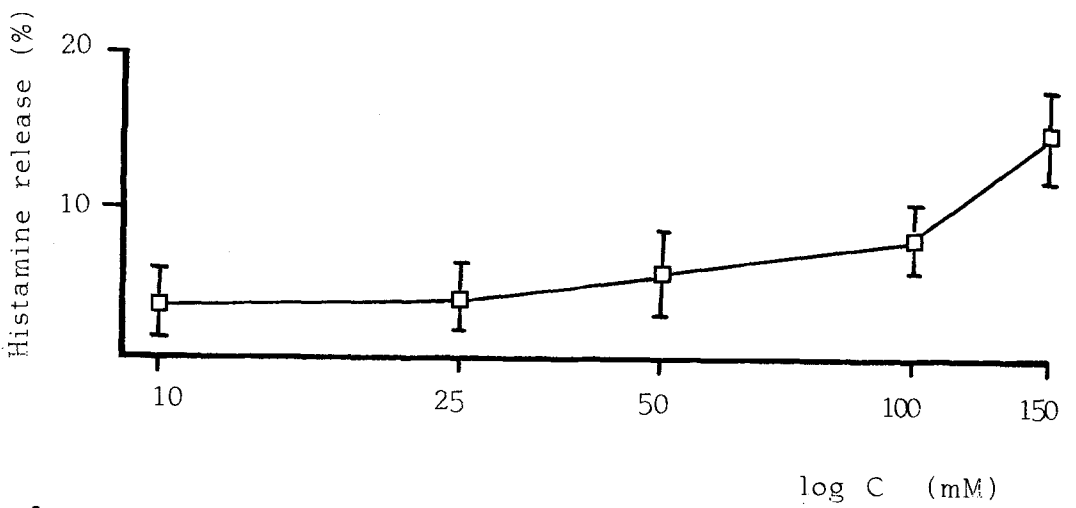

Figure 2
Secretory response induced by elevated K^+ concentrations (10–150 m*M*) in rat mast cells incubated for 1 h. The values were rectified for spontaneous histamine secretion. Media ± sem for $n = 7$.

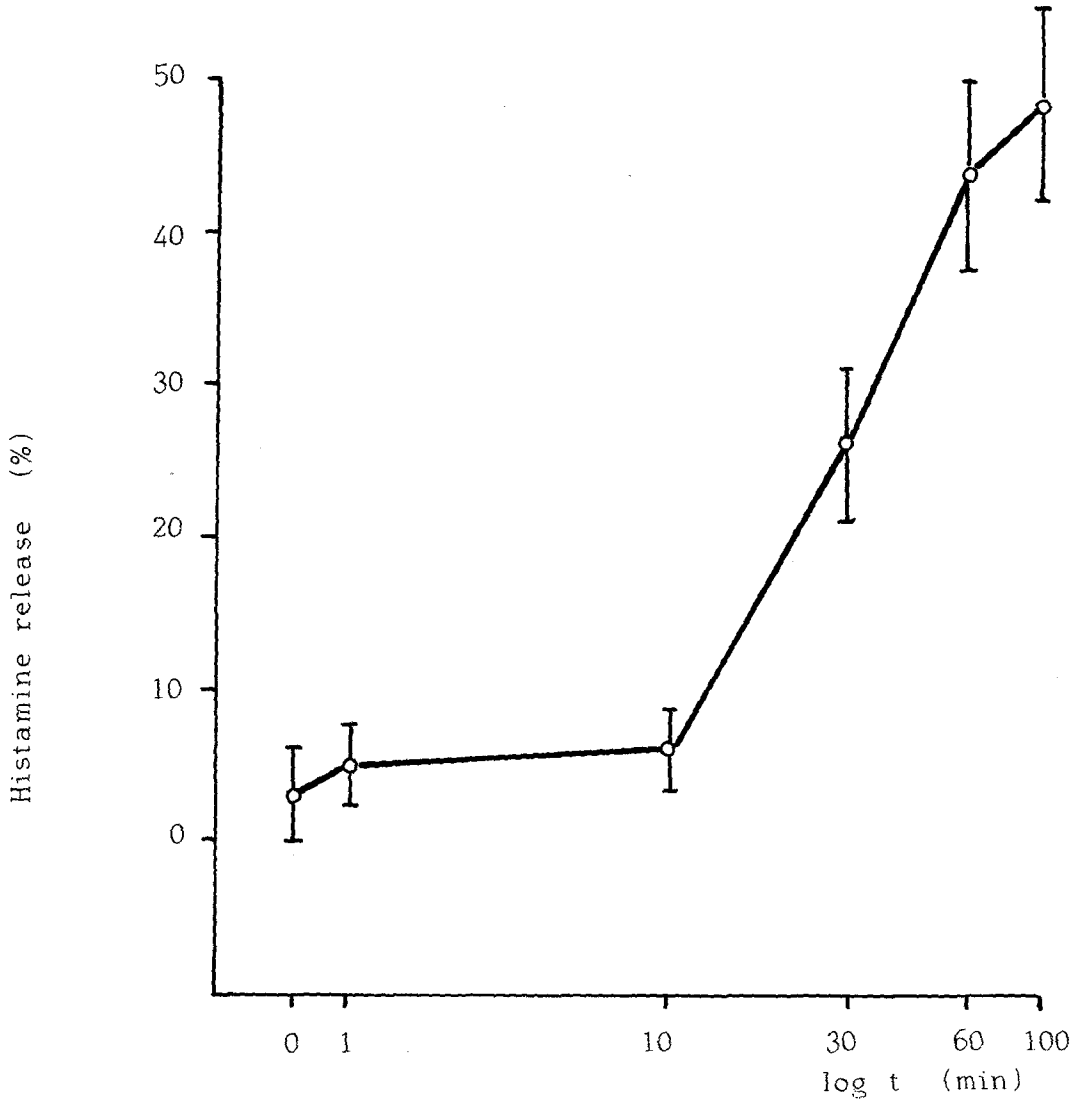

Figure 3
Valinomycin-induced histamine release for increased incubation time. Mast cells were preincubated for 10 min in Tris-K^+ (150 m*M*), and valinomycin 1 μM was then added; the incubation was prolonged for time indicated in x-axis. Media ± sem for $n = 3$.

sured in a Beckman beta counter (model LS-3801). The counting efficiency was 90–100%. Incubation was continued until saturation of calcium-uptake in non-stimulated cells was reached (NSU = non-stimulated uptake).

Appropriate controls for measuring non-specific binding and calcium bound to the external membrane were included in each experiment.

Results are expressed as the percentage of increase of ^{45}Ca-uptake – cpm – in cells stimulated

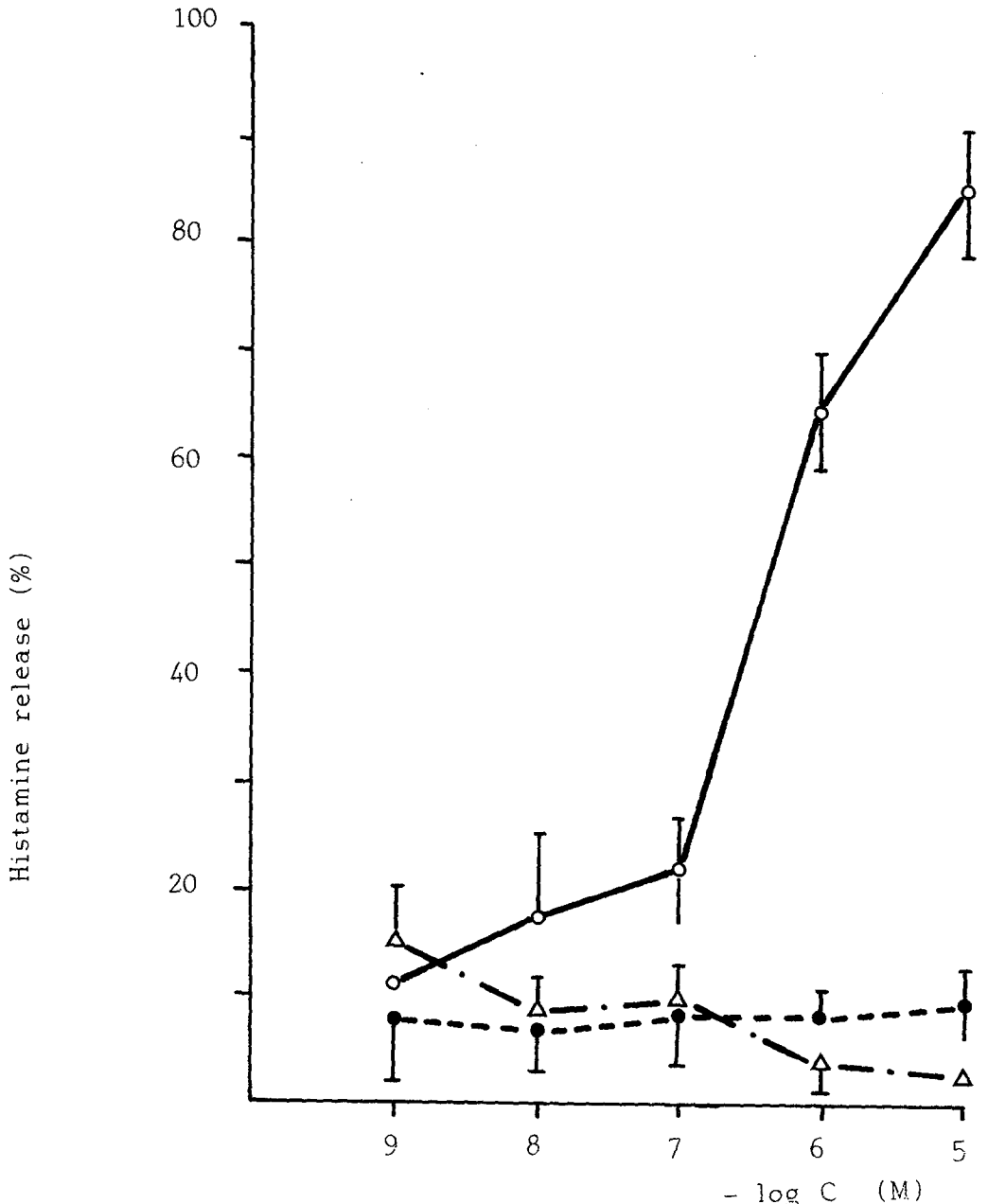

Figure 4
Dose-response pattern of histamine release induced by valinomycin in Tris-K (150 m*M*) (—○—), Tris-Na (120 m*M*) (—●—) and Tris-sucrose (300 m*M*) (—△—). Media ± sem for $n = 3$.

with valinomycin (SU = stimulated uptake) with respect to control ^{45}Ca-uptake (% = (SU-NSU)/NSU × 100).

Statistical analysis of the results. Results were analysed using Student's *t*-test for unpaired data. Values of p 0.05 were considered significant. Results are expressed as the Mean ± SEM.

Reagents. Valinomycin, Bovine serum albumin (BSA) and Tris-base were obtained from Sigma Chemical Co. (St. Louis, USA), and O-phtaldehyde (OPT) and immersion oil from Merck (Darmstadt, FRG). ^{45}Ca was purchased from Amersham Int. (Buckinghamshire, England) and Percoll, Pharmacia Fine Chem. (Uppsala, Sweden). Scintillation liquid Ready-Solv was obtained from Beckman Instruments (Spain), and Trypan blue dye from Flow Lab. (Virginia, USA). The other reagents were of analytical grade.

Results

The secretory response of rat mast cells induced by the presence of elevated K^+ concentrations was determined. The percentage histamine release when pure mast cell suspensions were incubated for 5 min–5 h with 100 mM K^+ are shown in Figure 1. The response increased from 23% at 2 h to 40% at 5 h. However, the levels of spontaneous histamine-release in mast cells incubated in physiological saline solution – 5.94 mM K^+ – were insignificant up to 60 min but increased quickly with longer periods of incubation, suggesting the existence of a non-specific exocytotic process. The incubation media of the experiments presented in Figures 1 and 2 were buffered with Tris base to achieve isosmotic pressure and did not contain external calcium.

When increasing K^+ concentrations (10–150 mM) were added to the incubation media and incubated for 1 h, histamine secretion increased slightly to 15 ± 2.5% at 150 mM K^+ (Figure 2). However, addition of valinomycin elicited a concentration-dependent histamine release profile in mast cells (10^{-9}–10^{-5} M) (Figure 4): valinomycin 10^{-6} M induced 64 ± 5% and maximum percentage 84 ± 8% was achieved with valinomycin 10^{-5} M.

It is interesting to note that valinomycin activates secretion in mast cells in the presence of high K^+ concentrations (Tris buffer with 150 mM K^+) but not in a Tris-Na^+ (with 120 mM Na^+) or Tris-sucrose (with 300 mM sucrose) medium. Optimal response values (44 ± 10%) were seen after 60 min of incubation (Figure 3). The percentage of spontaneous histamine-release was 5 ± 3%.

High doses of valinomycin which induce mediator release were also to found to inhibit calcium-uptake in rat mast cells: 35% inhibition with 10^{-5} valinomycin, whereas 10^{-6}–10^{-7} M only slightly stimulated ^{45}Ca entry. Lower doses did not affect either secretory or ^{45}Ca-uptake processes in the same way as did higher doses (Figure 5).

Discussion

Purified mast cells incubated in K^+ concentrations of up to 150 mM, exhibited a basal histamine-secretion response (Figure 2). When valinomycin (10^{-9}–10^{-5} M) was added to the incubation medium rat mast cells were activated and released their granular content by a process which maintained cell viability (Figure 4).

Valinomycin is known to be a powerful ionophore which transports monovalent cations across plasma membranes [6]. The only known ionophore for monovalent ions in mast cells is the compound X-537-A, which promotes exocytotic mediator release by an energy-independent process not requiring extracellular or intracellular calcium [8]. X-537-A activates mast cells in the presence of Na^+ or K^+ ions, but not in an isotonic Tris-glucose solution [7]. We have here demonstrated that valinomycin-treated mast cells incubated at 37 °C and in the absence of external calcium do elicit histamine release in Tris-K^+ (150 mM) although not in isotonic Tris-Na or Tris- sucrose media (Figure 4). Therefore, valinomycin induces histamine release in mast cells and shows a higher potassium selectivity than X-537-A. Previous studies have shown that valinomycin-based electrodes exhibit at 4000:1 K:Na discrimination ratio [6] and selectively transport K^+ ions across the plasma membrane of adrenal chromaffin cells [9].

It is not clear if the process of mast cell activation and the onset of secretory response in valinomycin-permeabilized cells is associated with a change in membrane potential. Sugiyama's paper shows that valinomycin (0.2 μg/ml) added to mast cell suspensions containing diS-C_3-(5) induced changes in fluorescence which were directly cor-

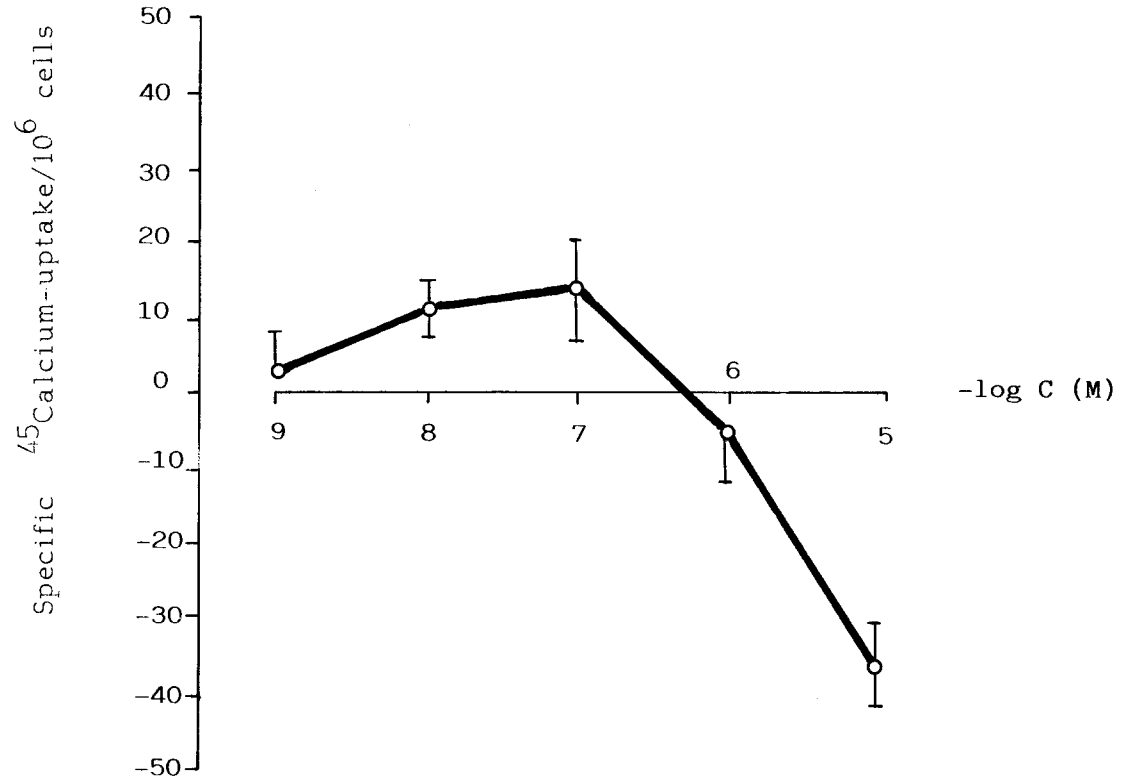

Figure 5
Calcium-uptake in valinomycin-permeabilized mast cells incubated for 1 h in Tris-K (150 m*M*). Results are expressed as percentage of increase of specific ^{45}Ca-uptake in stimulated cells (SU) in respect to ^{45}Ca-uptake in non-stimulated cells (NSU) ((SU-NSU)/NSU × 100). Each tube contains 300 ± 25.10^3 mast cells. Media ± sem for $n = 4$.

related to the log K^+-concentration in the range of 2.5 to 100 m*M* [15], but kinetics analyses of histamine release and fluorescence indicate that depolarization of the cells is not associated with degranulation. Also, Tasaka et al., obtained similar results using the microelectrode technique [16]. Moreover, membrane depolarization activates voltage-dependent calcium channels leading to a rise in cytosolic free-Ca^{++} which in turn evokes exocytotic secretion [17]. We have here shown that high doses of valinomycin which stimulate mediator release promote a notable inhibition of ^{45}Ca-uptake in isolated rat mast cells (Figure 5). These results suggest that with valinomycin there is no cell depolarization leading to Ca^{++} penetration via specific calcium channels. Also valinomycin has been shown to evoke significant levels of catecholamine-secretion without depolarization in adrenal chromaffin cells [9].

The presence of high potassium-concentrations in the external environment depolarizes plasma membranes and promotes activation of excitable cells [19]. However the presence of elevated K^+-levels (up to 150 m*M*) in the incubation medium does not stimulate histamine release in isolated rat mast cells (Figure 2). This suggests that the secretory process of mast cells is not modulated by permeability of K^+ ions. Thus, discrepancies were found in membrane permeability to K^+ ions between excitable and non-excitable systems; in the former the resting potential is due to K^+-diffusion while in non-excitable systems K^+ concentrations of up to 80 m*M* do not modify the membrane potential [18].

Also, prolonged treatment is required for optimal response in valinomycin-stimulated rat mast cells (Figure 3), while the response induced by depolarizing stimuli in excitable secretory cells appears immediately.

In conclusion, valinomycin is a degranulating agent which promotes histamine-release in rat pleural and peritoneal mast cells by a mechanism requiring elevated K^+ concentrations alone in the medium. If valinomycin acts as a monovalent cation carrier, one would expect it to release histamine from rat mast cells via an exchange process with K^+ ions [5].

It is significant that valinomycin either inhibits or has no effect on calcium-uptake in rat mast cells at concentrations that stimulate secretion. This result throws into question the function of calcium ions in stimulus-secretion coupling process in rat mast cells [20–22].

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