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SHORT COMMUNICATIONS

Veratridine elicits histamine release and promotes calcium uptake in mast cells

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The alkaloid veratridine evokes secretion in adrenal chromaffin cells and in beta cells of Langerhans' islets [1, 2]. In these two cellular systems, veratridine binds to Na^+-channels of the plasma membrane [3] stabilizing the open state of the channel and thus increasing sodium influx [4]. Increased intracellular sodium levels depolarize the plasma membrane activating the secretory process.

Calcium has been considered the universal intracellular messenger in a wide variety of cell types. Thus, there is evidence that the increase of intracellular Ca^{2+} concentration is a necessary event in the activation of exocytotic histamine release in mast cells [5–7]. When secretory cells are activated, there is fast calcium entry via specific sodium channels [8], prior to the main calcium influx via voltage-dependent or receptor-operated specific calcium channels [9, 10]. However, the ways by which calcium ions cross plasma membrane are not clearly established and are a matter of controversy [11–15].

In the present study, we report that veratridine releases histamine from mast cells and promotes calcium uptake by an unknown mechanism. Mast cell secretion requires the simultaneous presence of sodium and potassium ions in the external environment. The secretory response was calcium-independent, though millimolar concentrations of external Ca^+ were necessary for optimal response. The possible existence of veratridine-sensitive channels in mast cells is discussed in the light of our results and the recent suggestion that there are specific sodium channels blocked by tetrodotoxin in these cells [16].

Materials and methods

Mast cell isolation. Mast cells were obtained by lavage of pleural and peritoneal cavities of Sprague–Dawley rats (400–800 g) as previously described [17]. Physiological saline composition was (mM): Na^+, 142.3; K^+, 5.94; Ca^{2+}, 1; Mg^{2+}, 1.2; Cl^-, 126.1; CO_3^-, 22.85; $PO_4H_2^-$, 1.2; SO_4^{-2}, 1.2 giving a final osmotic pressure of 300 ± 5 mOsm/kg H_2O. Bovine serum albumin (0.5 mg/ml) was added and the pH was adjusted to 7.0.

The unpurified cellular suspension contained 4–6% mast cells.

Pure mast cell preparations were obtained by using the method described by Enerbäck and Svensson [18]. An aliquot of 0.75–1 ml of cellular suspension was mixed with 3.5–4 ml of isotonic Percoll (pH = 7) and centrifuged at 1000 g_{max} for 15 min. Leucocytes, macrophages and erythrocytes were removed and discarded together with the supernatant. Cellular suspensions at the bottom contained 95–98% pure mast cells. Following the purification procedure, purified mast cells were impermeable to trypan blue dye (98%–100%).

Pure mast cells were obtained from the peritoneal (1–1.5 × 10^6) and pleural (3–5 × 10^5) cavity of each rat respectively.

Cell incubation. Twenty-five microlitres of veratridine concentrated solution was added to sufficient incubation medium to attain a final volume of 0.9 ml, and preincubated. When the medium reached 37°, 100 µl of cell suspension, containing 1–1.5 × 10^5 mast cells, was added to each tube. Incubation was carried out in a shaking bath (160 cycles/min) at 37°. Incubation time is indicated in each experiment. In the sodium and/or potassium-free media, the isosmotic pressure was maintained with Tris-buffer.

Incubations were stopped 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.

Appropriate controls determining spontaneous histamine release in the absence of stimuli were executed in every experiment.

Histamine release assay. Histamine was assayed spectrofluorometrically both in the pellet—residual histamine—and supernatants—released histamine—by Shore's method [19]. However, 0.1% OPT was employed. Trichloroacetic acid was added (7%, final concentration) to prevent reaction because protein interferes with histamine assay.

In order to ensure the measurement of total histamine, pellets were previously boiled for 10 min in 0.8 ml of 0.1N ClH.

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

^{45}Calcium-uptake measurement. Cell incubation for ^{45}Ca-uptake determination was performed in polypropylene Akes-tubes of 500 µl. First, 100 µl of immersion oil (d = 1.02) was deposited at the bottom of each tube. Then 200 µl of pure mast cells (300 ± 25 × 10^3) suspended in Umbreit (without protein) were preincubated for 20 min at 37°. Reaction was started by the addition of veratridine 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 sec at 10000 rpm in a Beckman (model 11) microfuge. The tubes were rapidly frozen at −25° and the tips containing the cell pellets were cut and transferred into glass scintillation vials. In order to ensure total ^{45}Ca measurement, cells were disintegrated by addition of 0.5 ml of NaOH (0.1 N) and vigorously shaken. Then 4 ml of scintillation liquid cocktail were added to the vials, which were left in the dark for 2 hr at room temperature before taking radioactive measurements. Radioactivity was assayed by liquid scintillation spectrometry (Beckman model LS-3801) at a ^{45}Ca counting efficiency of 95%.

Incubation time was prolonged until saturation of calcium-uptake in non-stimulated cells was reached.

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

Results are expressed as the percentage of increase of ^{45}Ca-uptake (CPM) in cells stimulated with veratridine with respect to ^{45}Ca-uptake in controls.

Statistical analysis. Results were analysed using the Student's t-test for unpaired data. Values of $P < 0.01$ were considered significant.

Results were expressed as the mean ± SEM.

Chemicals. The chemicals used were obtained from the following sources: veratridine, compound 48/80, bovine serum albumin (BSA) and Tris, Sigma Chemical Co. (St. Louis, MO). *O*-phtaldehyde (OPT) and immersion oil from Merck (Darmstadt, F.R.G.). ^{45}Ca was purchased from Amersham Int. (Buckinghamshire, U.K.) Percoll, Pharmacia Fine Chem. (Upsala, Sweden). A high performance scintillation cocktail Ready-Solv HP was obtained from

Beckman Instruments (Spain). Trypan blue dye from Flow Labs. (Virginia, U.S.A.). The other reagents were of analytical grade.

Results

Veratridine (10–200 μM) induced histamine release in purified mast cells incubated in physiological saline solution. The dose–response profile showed that in the absence of extracellular calcium, histamine release declined (Fig. 1). Thus, veratridine (50 μM) induced 45.5 ± 6% histamine release in a calcium-free medium, while 25 μM stimulated 48 ± 8% histamine release when mast cells were incubated with 1 mM Ca^{2+}; the maximum response was attained at 100 μM (89.5 ± 3.5%) and 50 μM (85.7 ± 4.5%), respectively. Therefore, veratridine-stimulated histamine release in mast cells appears to be calcium-independent; however, the addition of 1 mM Ca^{2+} to the buffer was required for optimal release.

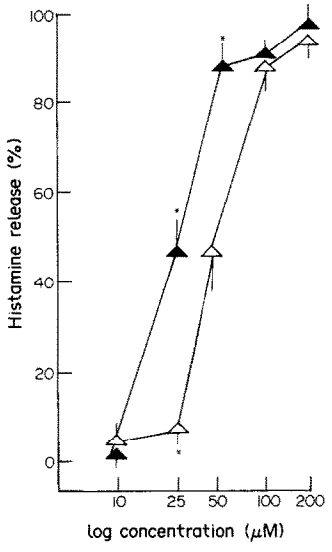

Fig. 1. Dose–response profile of histamine release in rat mast cells stimulated with veratridine (10–200 μM) in physiological saline solution with 1 mM Ca^{2+} (▲) and without Ca^{2+} (△) for 1 hr. Results are expressed as Mean ± SEM (N = 5). * Significant differences for $P < 0.01$.

The trypan blue dye test showed 98% cell viability in mast cells after incubation with veratridine 10–200 μM.

The presence of physiological concentrations of both sodium and potassium ions appears to be essential in the veratridine-activated mast cell secretory process (Table 1). When incubation was carried out in a Na^+- and K^+-free medium (isotonic Tris–sucrose, 300 mM), veratridine failed to induce secretion, with histamine release never greater than 10%. Na^+-free mediums which contained 5.94 mM K^+ (physiological concentrations) or 120 mM K^+ showed only basal histamine values. Similarly, in a K^+-free medium (Tris–Na^+, 120 mM) veratridine induced no significant response.

A second effect of veratridine is to promote specific calcium uptake in isolated rat mast cells in a concentration range 1–200 μM (Fig. 2). A control experiment of calcium-uptake in tubes containing unstimulated mast cells was carried out prior to the veratridine's assay. The control uptake is saturable in a time-dependent fashion, reaching saturation at 4 min, with saturation values of 3.6% relative to the total ^{45}Ca-activity added.

Discussion

As far as we know, this is the first description of veratridine as a histamine releaser. This alkaloid is a sodium-permeabilizing drug which induces membrane depolarization in excitable cells and stimulates catecholamine secretion from adrenal chromaffin cells [2] and insulin release from beta-cells [1]. We observed that this drug requires calcium for its releasing effect in a similar manner to the compound 48/80, showing increased sensitivity in the presence of millimolar Ca^{2+}. While the exact mechanism of action of compound 48/80 is unknown [20], it is assumed to act by releasing calcium from the intracellular pools [21]. Veratridine may act in a similar fashion. In any case, our results show clearly that veratridine requires both Na^+ and K^+ ions in the external environment.

Veratridine increases the intracellular Na^+ level in excitable cells by opening specific sodium channels [3, 4]. Thus, this alkaloid could induce Ca^{2+}-influx by two mechanisms a fast Ca^{2+} entry via specific sodium channels, and a slow Ca^{2+} entry via voltage-dependent calcium channels [8, 22]. The existence of sodium channels in mast cells has been proposed to explain the tetrodotoxin blockade of beta-blocker activated mast cells [16]. Thus, it is conceivable that veratridine might open such channels, permitting fast calcium entry and thus accounting for the linear increase of ^{45}Ca uptake seen with veratridine as reported here. Several authors working with the patch-clamp technique, have shown that while mast cells lack true calcium channels (opened by membrane depolarization or by agonist–receptor coupling), they do have non-specific voltage-inde-

Table 1. Values of histamine release induced by veratridine in isolated rat mast cells incubated in Tris–K^+ (5.94 mM), Tris–K^+ (120 mM), Tris–Na^+ (120 mM) and Tris–sucrose (300 mM). 1 mM Ca^{2+} was always present

	Ionic concentrations (mM)		Veratridine concentration (μM)			
Medium	Na^+	K^+	10	25	50	100
Umbreit	120	5.94	4.5 ± 2.6	45 ± 2.5	83 ± 5.6	89 ± 4.8
Tris–Na^+	120	–	4 ± 3.5	6.5 ± 2.8	7 ± 2.1	9 ± 3.5
Tris–K^+	—	5.94	6.3 ± 2.5	4.5 ± 2.1	8 ± 3.3	12 ± 2.6
Tris–K^+	—	120	5.7 ± 2.7	6.5 ± 3.2	7.3 ± 2.5	8 ± 1.5
Tris–sucrose	—	–	6 ± 2.5	3.5 ± 2	4 ± 2.3	9 ± 4.1

The incubation media were buffered with Tris buffer to attain the isosmotic pressure of 300 ± 5 mOsm/kg H_2O. The control was assayed in Umbreit medium. Results are expressed as Mean ± SEM (N = 5).

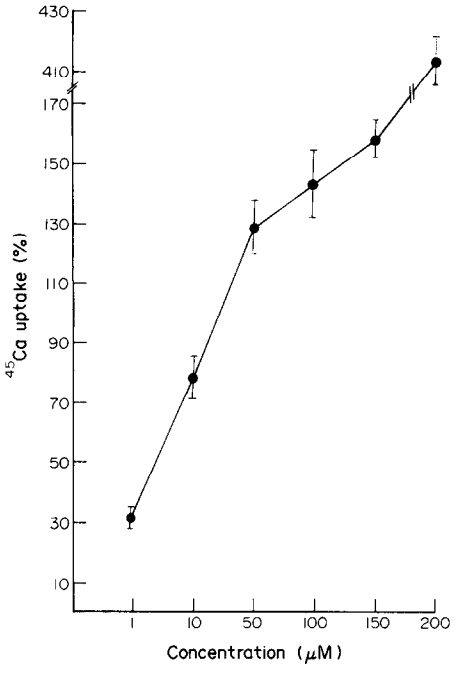

Fig. 2. Calcium-uptake in mast cells treated with veratridine. Results are expressed as percent of increase of specific ^{45}Ca-uptake in the presence of veratridine with respect to ^{45}Ca-uptake in unstimulated cells. Media ± SEM (N = 5). Each sample contains $300 \pm 25 \times 10^3$ mast cells. Unspecific binding of ^{45}Ca entrained by cells was 0.01% with respect to the total activity (measured at 0°).

pendent calcium channels, used also by Na^+ and K^+ ions [11, 12]. Therefore, given that mast cells appear to be non-excitable [11, 13], we believe a more likely explanation for veratridine's mechanism of action is that it opens these non-specific ionic channels. Further studies on ^{22}Na fluxes and tetrodotoxin could confirm or rule out the existence of specific sodium channels in mast cells.

In summary, veratridine was shown to induce histamine release and calcium uptake in isolated rat mast cells. Dose-dependent secretion was induced in the range 10–200 μM, with the presence of 1 mM of extracellular calcium necessary for optimal response. In mast cells incubated in an isosmotic buffer without sodium and/or potassium, only basal levels of histamine release was seen, indicating that the presence of both ions in the incubation medium may be required. Veratridine also promoted ^{45}Ca uptake linearly in the range 1–200 μM. On the basis of these results, we present veratridine as a new histamine-releasing drug, and offer a critical evaluation of the possible existence of non-specific ionic pathways for sodium in mast cells.

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