# Histamine release by pharmacological agents in the absence of external free $Ca^{2+}$

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The involvement of extracellular free $Ca^{2+}$ in histamine release was investigated in rat peritoneal mast cells. Incubation of non-antigenized cells in a media with high extracellular potassium did not increase histamine release. Secretion induced by A23187 and compound 48/80 in the presence of $Ca^{2+}$ requires metabolic energy. In the absence of external free $Ca^{2+}$ (2.5 $\mu$M) histamine release induced by A23187 is reduced but not abolished. Secretion induced by compound 48/80 is independent of extracellular $Ca^{2+}$. These results lead us to suggest that mast cell plasma membranes probably lack voltage-gated $Ca^{2+}$ channels and that external $Ca^{2+}$ may not be an absolute requisite for histamine secretion.

*$Ca^{2+}$ ionophore* *Compound 48/80* *Exocytosis* *Histamine secretion* *Mast cell* *Potassium depolarization*

## 1. INTRODUCTION

It is generally accepted that an increase of ionic calcium at some critical point inside the cytosol is the control signal that triggers exocytosis [1]. In a variety of cells this rise of intracellular $Ca^{2+}$ seems to be mainly due to an increase of $Ca^{2+}$ influx through the plasma membrane and therefore secretion heavily depends on extracellular $Ca^{2+}$ concentration [2,3]. However, this general scheme may not apply to all secretory cells. In parathyroid cells, for example, it has been shown that fully secretory activity can be attained with an external $Ca^{2+}$ concentration as low as 2.5 $\mu$M [4].

Although mast cells have been extensively used to study the basic mechanisms of secretion, the role played by extra- and/or intracellular $Ca^{2+}$ in stimulus-secretion coupling in these cells is not fully understood. It has been generally agreed that histamine secretion induced by the antigen–antibody reaction is almost totally dependent on extracellular calcium [5,6], however, more recent studies have shown otherwise [7]. The same lack of agreement exists with respect to the dependency on external $Ca^{2+}$ of histamine released by agents such as the calcium ionophore A23187 and the polycationic compound 48/80 [7,8].

Here, we have studied the effect of cell depolarization by increased external potassium on histamine secretion to ascertain if a voltage-gated $Ca^{2+}$ channel participates in histamine release. We have also compared the effects of A23187 and 48/80 on histamine release in the presence and absence of external $Ca^{2+}$. Our results suggest that mast cells probably lack the voltage-dependent $Ca^{2+}$ channel seen in other secretory cells [9] and that secretion evoked by pharmacological agents does not necessarily depend on external calcium.

## 2. MATERIALS AND METHODS

Mast cells were obtained from Wistar rats (either sex) weighing 250–350 g. The animals were decapitated after anesthetizing with ether. Cells were isolated as in [10]. Briefly, 10 ml buffer (137 mM NaCl, 2.7 mM KCl, 0.4 mM $NaH_2PO_4$, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM Hepes, 5.6 mM glucose, 1% BSA, 25 U/ml heparin; pH 7.4) was injected into the peritoneal cavity of each rat. The abdomen was massaged for 1 min and then the fluid contained in the peritoneal cavity was withdrawn with a plastic pipette through a mid-line incision. The fluid from several rats was pooled for an experiment and contained about

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Table 1

Histamine release at different concentrations of external potassium

| External potassium concentration (mM) | Histamine released (% of total content indicated by mean ± SD) |
|---|---|
| 2.7 (basal) | 0.04 ± 0.9 (14) |
| 5 | 0.09 ± 0.9 (4) |
| 10 | 0.20 ± 0.4 (4) |
| 25 | 0.02 ± 0.05 (4) |
| 50 | 0.02 ± 0.04 (4) |
| 100 | 0.04 ± 0.08 (4) |
| 2.7 (1 μg/ml A23187) | 90.00 ± 10 (6) |

Basal histamine secretion was measured in a solution with the same composition as indicated in fig.1 (1.8 mM $Ca^{2+}$ and 2.7 mM $K^+$). When the external concentration of potassium was increased the concentration of NaCl was decreased to maintain constant the osmolarity of all solutions. In all experiments incubation time was 20 min. Between parentheses is the number of observations

3–5% mast cells. Because of the consistent and uniform response of such a cell mixture to histamine-releasing agents, no effort was made to obtain homogeneous mast cell preparation by density gradient centrifugation procedure.

The cell suspension was centrifuged for 2 min at 90 × g, and the pellet washed with 4–6 ml buffer. Histamine release was initiated by resuspending centrifuged mast cells in fresh media at 37°C. The composition of solutions used in each experiment is indicated in the figure legends. At different times aliquots (200 μl) of incubating cells were withdrawn and diluted into 1.1 ml ice-cold buffer (without calcium and with 100 μM EGTA added), and centrifuged at 200 × g for 5 min: 1.2 ml of each supernatant was diluted with 2.8 ml HCl (0.1 N) for ulterior histamine determination. The pellets obtained from each sample were resuspended with 200 μl $HClO_4$ (6%), stirred for 10 s and sonicated for 20 min. The suspension was centrifuged for 5 min in a microfuge and the supernatant diluted with HCl (0.1 N) to determine the histamine content. Histamine was measured fluorimetrically as in [11] as modified in [12]. Internal controls were made with A23187 and 48/80 and no fluorescence was observed at the concentrations used. In all figures histamine release is expressed as the percentage of the total histamine content of the cells and is calculated as the ratio:

$$\frac{\text{histamine released}}{\text{residual cell histamine + histamine released}} \times 100$$

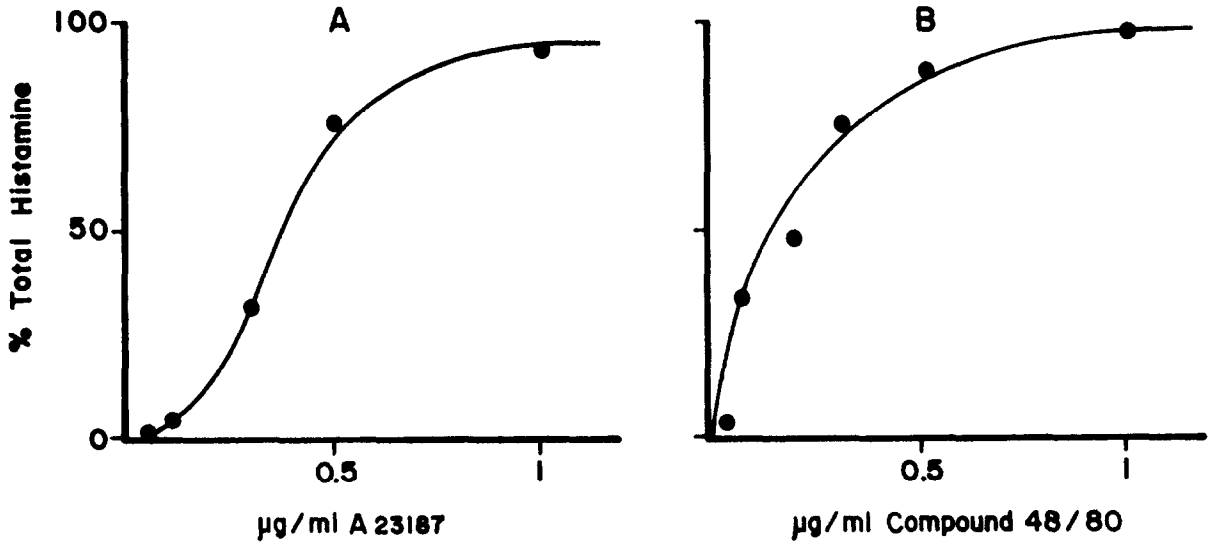


Fig.1. Histamine secretion at different concentrations of calcium ionophore A23187 and compound 48/80. (A,B) Dose-response curve for A23187 and 48/80, respectively, when added to a solution containing 137 mM NaCl, 2.7 mM KCl, 0.4 mM $NaH_2PO_4$, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5.6 mM glucose, 10 mM Hepes and 1% BSA (pH 7.4); temperature 37°C. The incubation time in all cases is 10 min.

In all experiments basal histamine secretion was less than 1% and this value was subtracted from those measured in any other situation.

## 3. RESULTS AND DISCUSSION

The effect of raising external $K^+$ concentration on mast cell histamine secretion has been studied by measuring histamine release in solutions containing a fixed $Ca^{2+}$ concentration (1.8 mM) and either 2.8 mM $K^+$ (control) or 5, 10, 25, 50 and 100 mM $K^+$. The results summarized in table 1 show that high extracellular potassium does not significantly increase histamine secretion in non-antigenized cells. The intactness of the cell membrane in our preparations is indicated by the low basal secretion and by its clear increase after the addition of A23187. The lack of effects of raising external $K^+$ concentration on mast cell secretion contrasts with findings in many secretory cells [13] and is viewed as a first indication that voltage-gated $Ca^{2+}$ channels may not participate in the calcium-induced histamine release. We suggest that if external $Ca^{2+}$ is required for histamine release (see below) it must permeate the membrane by a mechanism other than the regular voltage-dependent $Ca^{2+}$ channel observed in other secretory cells [9]. It has been reported that basophilic leukemia cells depolarize during the histamine secretion that follows the antigen-antibody reaction [14]; this depolarization may be a consequence rather than a previous step in the events leading to secretion.

The calcium ionophore A23187 and compound 48/80 can induce mast cells to secrete histamine. Fig.1 shows the concentration range at which these agents are active. The concentration-effect curve for these compounds show some differences in kinetics but both curves are very steep. Maximal histamine secretion (about 100% of the total histamine content) was obtained with either A23187 or 48/80 at 1 $\mu$g/ml. Histamine release induced by any of these compounds is functionally similar and probably occurs through the same energy-dependent exocytotic mechanism. Fig.2 shows that the addition of 100 $\mu$M CNK to buffers containing either A23187 or 48/80 completely abolishes the ability of these agents to evoke histamine release. These results show that exocytotic degranulation of mast cells evoked by A23187 or 48/80 is dependent on intracellular ATP and is supported by oxidative phosphorylation as in other secretory cells [3,15].

The level of external calcium has a different effect on histamine release by A23187 and 48/80. Fig.3a shows the time dependence of histamine release evoked by 1 $\mu$g/ml A23187 in the presence (1.8 mM) and absence (~2.5 $\mu$M [4]) of external free $Ca^{2+}$. Secretion induced by A23187 greatly depends on external $Ca^{2+}$ although a significant amount of histamine (about 15% of the value with 1.8 mM $Ca^{2+}$) is released in the free $Ca^{2+}$ solution. By contrast, fig.3b shows that histamine release evoked by 1 $\mu$g/ml 48/80 is practically independent of external $Ca^{2+}$. The rise in intracellular $Ca^{2+}$ that is probably needed for exocytosis must then be the result of calcium release from $Ca^{2+}$-sequestering organelles. A23187 may be less potent than 48/80 in releasing calcium from in-

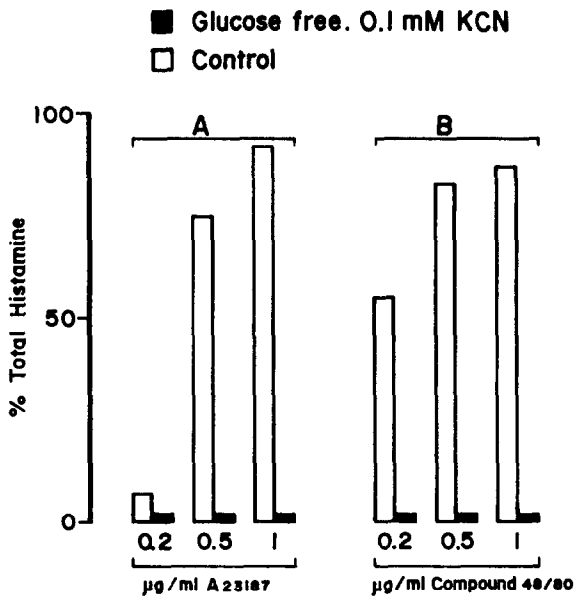


Fig.2. Effect of glucose removal and the addition of KCN on histamine release induced by A23187 and 48/80. Cell suspensions were preincubated for 20 min in a solution with the same composition as indicated in fig.1 with the exception that glucose was removed and 0.1 mM KCN was added. Histamine release (filled bars) was measured after incubating the cells for 5 min in this solution and with variable amounts of A23187 (A) and 48/80 (B). Control measurements of histamine secretion (empty bars) induced by A23187 (A) and 48/80 (B) were obtained with the same protocol as in fig.1.

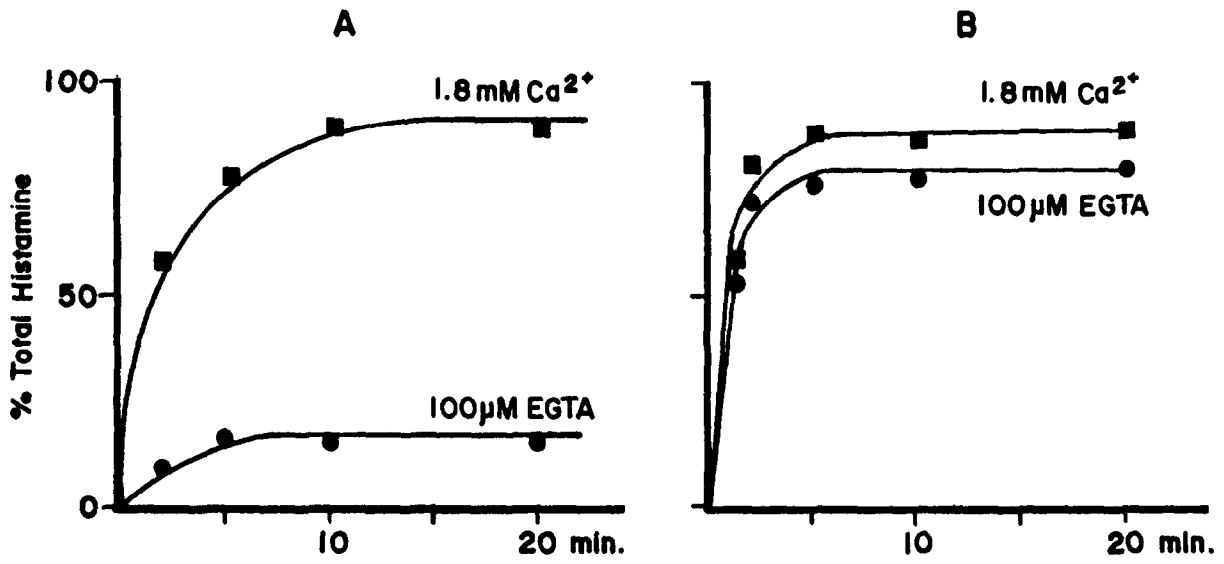


Fig.3. Time dependence of histamine secretion evoked by A23187 and 48/80 in the presence and the absence of external free calcium. Histamine release induced by A23187 (A) and 48/80 (B) at a final concentration of 1 μg/ml was measured after incubating the cells in a buffer of the same composition as indicated in fig.1 (■, 1.8 mM $Ca^{2+}$). Aliquots were obtained at different incubation times to measure histamine secretion. With the same protocol histamine release was also measured in the absence of external free calcium and with 100 μM $Na_2EGTA$ added (● in A,B). For these experiments deionized double-distilled water was used. True concentration of ionized calcium as measured with a calcium electrode was 25 μM in the presence of 1% BSA and no added calcium. In the presence of 100 μM $Na_2EGTA$, 2.5 μM free calcium was present [4]. Basal histamine secretion in the absence of releasing agents was in all cases (regardless of external calcium concentration) less than 1%.

tracellular stores and therefore its ability to induce histamine release depends more strongly on an increase in $Ca^{2+}$ influx through the plasma membrane.

Our findings indicate that mast cells plasma membrane probably lack voltage-dependent $Ca^{2+}$ channels and that external free $Ca^{2+}$ is not an absolute requisite for histamine secretion. This agrees with recent observations concerning the mechanism of antigen-induced histamine release in mast cells, which attribute to intracellular $Ca^{2+}$ release a role much more important than previously thought [7]. On the other hand, it has been shown in some secretory systems that secretagogues induce both, an increase in intracellular $Ca^{2+}$ and the enhancement of phosphatidylinositol breakdown. It has been proposed that these two events can independently trigger exocytosis. In the absence of an increase of intracellular $Ca^{2+}$ a metabolite of phosphatidylinositol (1,2-diacylglycerol) can activate a protein kinase, which in turn activates secretion [16].

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