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# EFFECTS OF MEMBRANE DEPOLARIZATION AND DIVALENT CATIONS ON ANAPHYLACTIC HISTAMINE SECRETION

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## SUMMARY

The effects of membrane depolarization and divalent cations on histamine release have been studied in sensitized mast cells. Membrane potential of these cells has been measured with intracellular microelectrodes. Our results show that mast cells have a large resting potential (-61±12 mV) however they do not generate active membrane electrical responses when are depolarized by passing current through the recording microelectrode. High external $K^+$ does not increase histamine release. Histamine secretion is supported by alkali-earth divalent cations ($Ca^{2+} > Sr^{2+} > Ba^{2+}$) but strongly inhibited by transition metals. $Ca^{2+}$ concentrations above 1 mM inhibit histamine release, however, this effect is not mimicked by $Sr^{2+}$ and $Ba^{2+}$.

## INTRODUCTION

Antigen-induced degranulation of sensitized mast cells is believed to be triggered by a transient elevation of cytosolic $Ca^{2+}$ (1,2). This rise of intracellular $Ca^{2+}$ has been generally thought is due to an increase of $Ca^{2+}$ influx through the plasma membrane since anaphylactic histamine secretion heavily depends on the presence of external $Ca^{2+}$ (2,3). However the mechanism whereby mast cell membrane permeability to $Ca^{2+}$ increases is not known and it has been recently shown that histamine can be released in the absence of external $Ca^{2+}$ (4,5).

Membrane electrical events play an important role in stimulus-secretion coupling in many secretory cells (6). Although in mast cells no much electrophysiological information is available it is generally accepted that histamine secretion does not involve the activation of voltage-sensitive membrane channels. In non-sensitized mast cells high external $K^+$ does not increase histamine secretion (5,7) and it has been re-

cently shown that mast cell degranulation can occur without any change of membrane conductance (8).

This paper includes the first direct measurement of membrane potential in mast cells and shows that they have a large resting potential although do not generate active membrane electrical responses by direct stimulation. High external $K^+$ does not increase histamine release in sensitized mast cells; nevertheless, a critical amount of external $Ca^{2+}$ seems to be required for anaphylactic histamine secretion measured at 10 min incubation time. Other divalent cations can replace external $Ca^{2+}$, however in presence of 2 mM $Ca^{2+}$ transition metals inhibit histamine release.

## METHODS

### Mast cell preparation

Mast cells were obtained from Wistar rats (either sex) weighing 200-300 g. Animals were sensitized to egg albumin (Sigma) by intramuscular injection into each hind leg of 0.25 ml of a suspension containing egg albumin (10 mg/ml) in Freund's incomplete adjuvant (Difco). An intraperitoneal injection of Bordetella pertussis (Difco) was used to amplify the anaphylactic response (9,10). Animals were used 25-35 days after this treatment. The procedure to obtain mast cells has been previously described (5). Cells used for electrophysiological recording were purified using a density gradient of Percoll (11). In this preparation mast cell content as detected with Toluidine blue was about 90%.

### Release and assay of histamine

Peritoneal cells suspension was centrifuged for 2 min at 90 x g and the pellet washed with 4-6 ml buffer (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, pH 7.4). Histamine release was initiated by resuspending centrifuged mast cells in fresh media at 37°C and processed as previously described (5). The composition of solutions used in each experiment is indicated in the figure legends. Histamine was measured fluorimetrically as in (12) and is expressed as the percentage of the total histamine content of the cells.

### Electrophysiological recording

Purified mast cells were resuspended in 4 ml of Eagle's Minimum Essential Medium (Flow Laboratories) supplemented with autologic serum (20%), antibiotics and glucose (5.6 mM). Cells were used for recording 2-3 days after plating in small culture dishes. Inmediatelly before recording the culture medium was replaced by a solution of the following composition, in mM: 141 NaCl, 5 KCl, 2 $CaCl_2$, 1.1 $MgSO_4$, 5 glucose and Hepes 10, pH 7.4. Cellular viability (as indexed by Tripan blue exclusion) was never less than 95%. Mast cell membrane potential was recorded with microelectrodes fabri-

cated from borosilicate glass and filled with 3 M potassium acetate. Electrode resistance ranged between 60-90 MΩ. Microelectrodes were electrically connected to the headstage of an intracellular recording amplifier built in our laboratory (13). The indifferent electrode was a chlorided silver wire connected to ground. Before cell impalement stray capacitance and voltage drop across the microelectrode were electronically compensated. Good impalements were characterized by a sudden change in potential that remained stable for at least 3-5 min. Square pulses of variable amplitude and duration obtained from a constant-current pump were injected through the microelectrode to measure input resistance. After an impalement a check for voltage drift and electrode resistance compensation was made. The membrane potential was monitored with an oscilloscope and stored on an analog magnetic tape. The preparation was electrically isolated by a metallic shield and placed on a heavy granite table sitting on inner tubes for shock absorption.

## 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 (control) or 5, 10, 25, 50 and 100 mM $K^+$. Table I shows that high extracellular $K^+$ does not significantly increase histamine secretion in sensitized mast cells. However there is a clear increase of histamine release in the presence of the antigen (ovoalbumin, 50 µg/ml). These results, as those previously obtained in non-sensitized cells (5,7), contrast with findings in other secretory cells (14,15) and support the view that voltage-gated $Ca^{2+}$ channels do not participate in the control of membrane permeability in mast cells.

| External potassium concentration (mM) | Histamine released (% of total content, mean±SD) | |
|---|---|---|
| 2.7 (basal) | 7.5±0.6 | (8) |
| 5 | 6.4 | (1) |
| 10 | 7.9 | (1) |
| 25 | 6.5±0.5 | (2) |
| 50 | 6.2±0.5 | (4) |
| 100 | 9.3±1.2 | (2) |
| 2.7 (ovoalbumin 50 µg/ml) | 47.5±22 | (12) |

Table I. Histamine release at different concentrations of external $K^+$. Solution composition was as indicated in Methods. When KCl was increased NaCl was decreased to maintain constant the osmolarity. Incubation time 10 min. Temp. 37°C.

Electrophysiological measurements were done in cultured cells with intracellular microelectrodes. A few seconds after impalement mast cells reached a stable resting potential with an average value of -61±12 mM (mean±SD, n=14). The injection of square current pulses evoked passive depolarizing and hyperpolarizing voltage responses which amplitude increased linearly with the strength of the current; however, we have never seen active membrane electrical responses even with depolarizations above 0 mM. The slope of current-voltage curves calculated from several cells gives an average value for the input resistance of 65±28 MΩ(n=8). Dispersion of input resistance values around the mean probably reflects the variability in cell size.

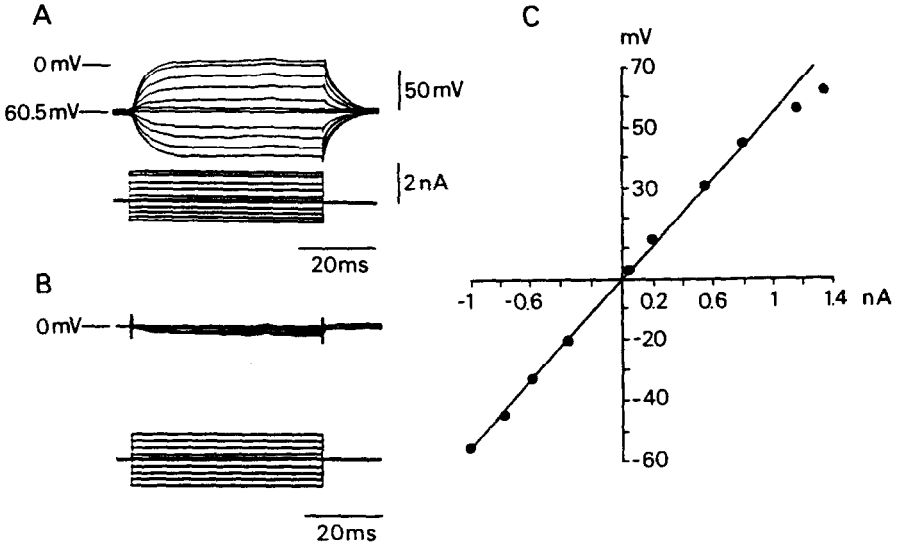


Figure 1. Intracellular recording in cultured purified mast cells. A. Passive electrical responses elicited by the injection of square current pulses through the recording microelectrode. B. Check for microelectrode balance after withdrawal from the cell. C. Current-voltage plot made from the recordings shown in A. Calibration as indicated in the figure. Experiments performed at room temperature (approx. 20-22 °C).

An example of electrical recordings obtained from sensitized mast cells is shown in Fig. 1. Part A illustrates the passive electrical potentials elicited by positive and negative current pulses of variable amplitude and the absence of autorregenerative voltage responses. After the microelectrode came out of the cell a check for microelectrode

balance was made (B). Part C is a current-voltage plot made with the values shown in A. The data points are fitted by a straight line with a slope of 57 MΩ which is the input resistance of the cell.

The effect of divalent cations, both alkali-earth and transition metals, on histamine secretion provides information about the interaction of these cations with the plasma membrane which may be useful to elucidate the possible molecular mechanism implicated in signal transduction in mast cells. Figure 2 illustrates the relationship between external $Ca^{2+}$ concentration and histamine release in the presence of a fixed concentration of ovoalbumin (50 µg/ml). In the

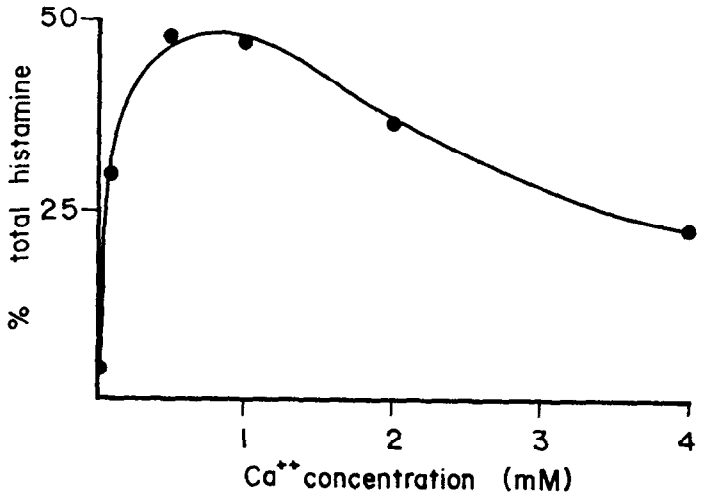


Figure 2. Anaphylactic histamine release at different external $Ca^{2+}$ concentrations. Solution composition was, in mM: 137 NaCl, 2.7 KCl, 0.4 $NaH_2PO_4$, 1.8 $CaCl_2$, 5.6 glucose, 10 Hepes, pH 7.4. When $CaCl_2$ concentration was changed the amount of NaCl was also modified to maintain constant the osmolarity. Incubation time 10 min. Temp. 37°C.

absence of external $Ca^{2+}$ (and with 100 µM EGTA added) anaphylactic histamine release is strongly depressed although an appreciable amount of histamine is still released probably due to activation of a stimulus-release coupling mechanism independent of external $Ca^{2+}$ (4). Interestingly, histamine release progressively decreases with $Ca^{2+}$ concentrations above 1 mM. This effect may be due to inactivation of membrane $Ca^{2+}$ influx or to inhibition of the secretory machinery by high $Ca^{2+}$ (16,17).

The effect of several divalent cations on antigen-induced histamine release is summarized in Fig. 3. Both, $Sr^{2+}$ and $Ba^{2+}$, can replace $Ca^{2+}$ and support histamine secre-

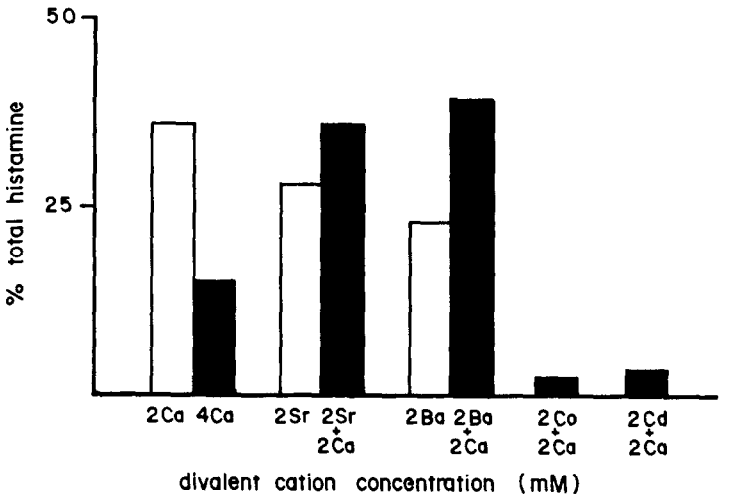


Figure 3. Effect of divalent cations on anaphylactic histamine release. Solution composition is the same as in Fig. 2 and divalent cation concentrations as indicated in the figure. Measurements done 10 min after addition of ovoalbumin (50 µg/ml). Temp. 37°C.

tion (open bars); however a nearly complete absence of histamine secretion is seen when the ions replacing $Ca^{2+}$ are the transition metals $Co^{2+}$ or $Cd^{2+}$ (not shown). $Ba^{2+}$ and $Sr^{2+}$ ions are less effective than $Ca^{2+}$ in activating secretion, the order of effectiveness being $Ca^{2+} > Sr^{2+} > Ba^{2+}$. This order is the same as the affinity of these divalent cations for $Ca^{2+}$-binding proteins that probably participate in the process of exocytosis (13,18,19) and their permeability through voltage-dependent $Ca^{2+}$ channels (20,21). With 4 mM $Ca^{2+}$ there is, as also shown above in Fig. 2, a decrease of histamine release. The inhibitory effect of high $Ca^{2+}$ is not observed when 2 mM $Ca^{2+}$ are replaced by $Sr^{2+}$ or $Ba^{2+}$ (filled bars) probably because the last two cations are not as effective as $Ca^{2+}$ to inactivate the secretory process. In presence of 2 mM $Ca^{2+}$ the addition of 2 mM $Co^{2+}$ or $Cd^{2+}$ strongly depresses histamine secretion (filled bars).

Divalent cations affect histamine release in a way that closely resembles their action on secretion in cells where $Ca^{2+}$ influx through voltage-dependent $Ca^{2+}$ channels is a critical step in the process of exocytosis (14,15). However our data further support the view that the putative $Ca^{2+}$ influx that triggers anaphylactic histamine release is not mediated by voltage-dependent channels. Mast cells have a large resting potential but no detectable signs of autorregenerative electrical activity after membrane depolarization. Furthermore, depolarization by high external $K^+$ does

not increase histamine secretion. $Ca^{2+}$ permeability in these cells may be regulated by voltage-independent and antigen-activated ionic channels with similar properties as those identified in basophilic leukemia cells (22). However in a recent patch-clamp study on passively sensitized mast cells it has been shown that degranulation occurs without any change of membrane conductance. Based on this observation it has been suggested that $Ca^{2+}$ influx is not required for histamine release and that the inhibition of secretion seen in the absence of external $Ca^{2+}$ at long incubation time could be explained by a possible alteration of some membrane constituent (8). Although our data partially support these ideas it is difficult to explain the effects of divalent cations on histamine secretion and the inhibitory action of transition metals in presence of 2 mM $Ca^{2+}$. A detailed characterization of the molecular mechanisms underlying signal transduction in mast cells must await for future experimental work.

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