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# Reversal by EGTA of the enhanced secretory responsiveness of mast cells due to treatment with ouabain

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The effect of EGTA on the enhancement by ouabain of compound 48/80-induced secretion from mast cells was compared with the effect on the $Na^+$-$K^+$ pump activity. The time-dependent secretory enhancement by ouabain was blocked by addition of EGTA to the cell suspension concomitantly with the addition of ouabain, and EGTA caused a large increase in the pump activity. Addition of 10 μM EGTA to ouabain-treated cells stopped but did not reverse the enhancement. The experiments show that the effect of ouabain was due to changes in a calcium pool utilized in compound 48/80-induced secretion following changes in the $Na^+$,$K^+$ pump activity.

Ouabain; Mast cell; EGTA; Chelating agent; ATPase, $Na^+$,$K^+$-; $Ca^{2+}$

## 1. INTRODUCTION

Digitalis glycosides are known to enhance the secretory responses of various tissues, and this is apparent in rat mast cells only in calcium-free medium [1-4]. The mechanism of histamine secretion induced by compound 48/80 from mast cells is considered to utilize a cellular pool of calcium that may be removed by EGTA [5-8]. We have studied if removal of cell calcium available to externally added EGTA influences the enhanced secretory responsiveness of the cells due to the treatment with ouabain.

## 2. MATERIALS AND METHODS

Male Sprague-Dawley rats, 315-425 g, were used for the experiments. The rats were killed by decapitation under light ether anaesthesia. The mast cells were isolated by differential centrifugation of mixed peritoneal cells in a self-generating gradient of Percoll as described previously [9]. Following the isolation procedure, the mast cells were washed and suspended in a calcium-free Krebs-Ringer solution. The cells were kept at 4°C during the isolation procedure. A coulter counter (Model 134, Analys Instrument AB, Sweden) was used to count the number of cells, and inspection of stained smears (Toluidine blue) were used to determine the fraction of mast cells, which was 97.7% ± 1.3% (mean and standard deviation, $n = 16$).

Mast cell suspensions pooled from 1-4 rats were divided into samples with the same cell density in a final volume of 0.5 ml. Each sample contained between $1.89 \times 10^5$ and $2.50 \times 10^5$ cells for the determination of $Na^+$, $K^+$ pump activity and between $2.56 \times 10^4$ and $9.87 \times 10^4$ cells for the histamine secretion experiments. The samples were equilibrated at 37°C in presence of potassium 4.75 mM and in the absence of calcium for 60 min. Then the cells were preincubated under various experimental conditions before the incubation with $K^+$ ($^{86}Rb^+$) was carried out in order to determine the $Na^+$, $K^+$ pump activity, and before the incubation with compound 48/80 (1 μg/ml) was performed in order to induce the secretion of histamine. Measurement of $Na^+$, $K^+$ pump activity was performed as described previously [9]. The $Na^+$, $K^+$ pump activity is equivalent with the cellular uptake of $K^+$ ($^{86}Rb^+$) corrected for the ouabain-resistant uptake of $K^+$ ($^{86}Rb^+$) taking place in presence of 1 mM ouabain (for further details see legends to figures). Measurement of cellular histamine release was performed essentially as described before [10]. Mast cells suspensions in a final volume of 0.5 ml were equilibrated and then preincubated as mentioned above and in the legends to figures. Incubation with compound 48/80 for activation of the secretion mechanism lasted for 10 min. After centrifugation, the supernatant was collected and the pellet was boiled for the release of residual histamine. Measurement of histamine was performed by the fluorimetric method [11] omitting the extraction procedure. The histamine release was expressed as a percentage of the total histamine content of the cells. The Krebs-Ringer solution was calcium-free and had the following composition (mM): NaCl 136.8, KCl 4.75, $MgCl_2$ 1.2, Tris-HCl 12.5. All solutions contained bovine serum albumin, 1 mg/ml, and glucose, 1 mg/ml. The pH was adjusted to 6.9-7.0 (37°C).

Bovine serum albumin was supplied by Sigma Chemical Company (St. Louis, MO, USA), glucose by E. Merck (Darmstadt, FRG), Percoll by Pharmacia Fine Chemicals (Sweden), Ecoscint by BN Plastics (Helsinge, Denmark) and $^{86}Rb^+$ by Amersham (Buckinghamshire, UK). Ouabain (Mecobenzon, Denmark) and all other chemicals were of analytical grade.

## 3. RESULTS

In the absence of EGTA the cellular uptake of $K^+$ ($^{86}Rb^+$) was stabilized after the preincubation period (60 min) at a value of 500-550 pmol/$10^6$ cells/ml (fig.1). Addition of EGTA increased the uptake and it remained very stable at a value of 800-850 pmol/$10^6$ cells/min. The ouabain-resistant uptake was 150 pmol/$10^6$ cells/min, and EGTA had no effect on the uptake. After 60 min preincubation of the cells in a calcium-free medium histamine secretion was less than 10%, and the addition of EGTA had no effect on the

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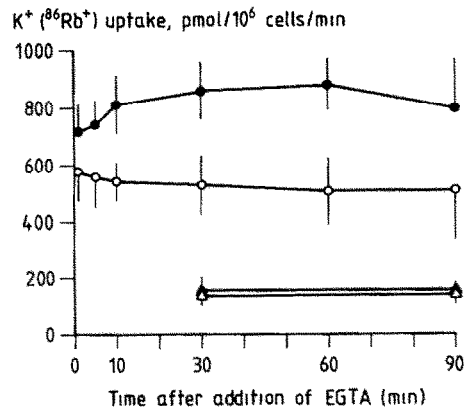



Fig.1. Time course of the effect of EGTA on the $Na^+$, $K^+$ pump activity. The cells were preincubated for 60 min in calcium-free Ringer solution containing potassium 4.75 mM, pH 7.0 (37°C). Then a second preincubation of the cells for 1–90 min (abscissa) with (●) or without (○) EGTA 0.1 mM was performed before the cells were incubated with $K^+$ ($^{86}Rb^+$) for 10 min in order to measure the activity of the pump mechanism. The specific activity of $^{86}Rb^+$ in the suspending medium was 0.4–1.0 $\mu$Ci/ml (range). This was used to calculate the cellular $K^+$ ($^{86}Rb^+$) uptake. The ouabain-resistant uptake (ouabain 1 mM) was measured in presence (▲) and absence (△) of EGTA. Ordinate scale: The cellular uptake of $K^+$ ($^{86}Rb^+$). Mean values from 3–5 experiments are shown; vertical lines show SD.

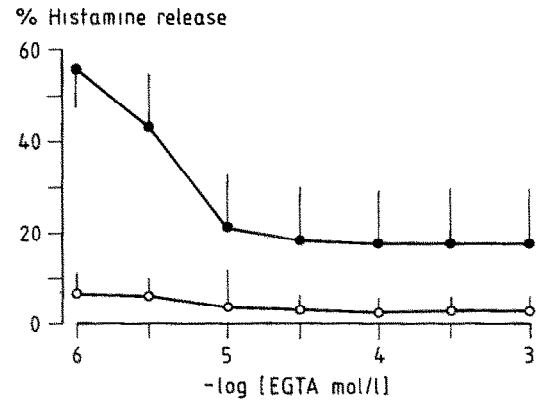



Fig.3. Dose-dependent inhibition of the effect of ouabain on histamine secretion. The cells were preincubated for 60 min in calcium-free Ringer solution containing potassium 4.75 mM, pH 6.9 (37°C). Before activation of the secretion by the addition of compound 48/80, the cells were exposed for 105 min to EGTA, 1 $\mu$M to 1 mM (abscissa) with (●) or without (○) the concomitant addition 1 mM ouabain. Control values of histamine secretion in presence of ouabain was 57.8% ± 7.7%, and the secretion from cells incubated in parallel in calcium-containing Ringer solution with potassium but without EGTA and without ouabain was 74.5% ± 8.6% (mean values and SD). Mean values from four experiments are shown; vertical lines show SD.

secretory response. Ouabain induced a time-dependent enhancement of the secretory mechanism activated by compound 48/80 (fig.2). This effect was completely blocked if EGTA was added to the cell suspension concomitantly with the addition of ouabain. The inhibitory effect of EGTA was dependent on the concentration of EGTA added to the cell suspension, while 3 $\mu$M partially inhibited the effect, it was maximally inhibited by EGTA 10 $\mu$M (fig.3). Addition of EGTA after incubation of the cells with ouabain for 105 min did not reverse the enhanced secretory responsiveness of mast cells due to the treatment with ouabain (fig.4).

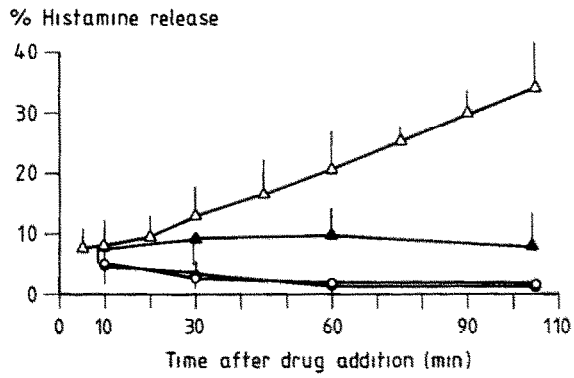



Fig.2. Enhancement by oaubain of histamine secretion and reversal of the enhancement by EGTA. The cells were preincubated for 60 min in calcium-free Ringer solution containing potassium 4.75 mM, pH 7.0 (37°C). Before activation of the secretion by the addition of compound 48/80, the cells were exposed to 1 mM ouabain for 5–105 min with (▲) or without (△) the presence of 0.1 mM EGTA. Control values of histamine secretion in the absence of ouabain and with (●) or without (○) EGTA are also shown. Control values of histamine secretion from mast cells incubated in parallel in calcium-containing Ringer solution but in the absence of ouabain and EGTA were 71.5% ± 8.6% after 60 min and 69.3% ± 13.3% after 120 min incubation (mean and SD). Mean values from three experiments are shown; vertical lines show standard deviations.

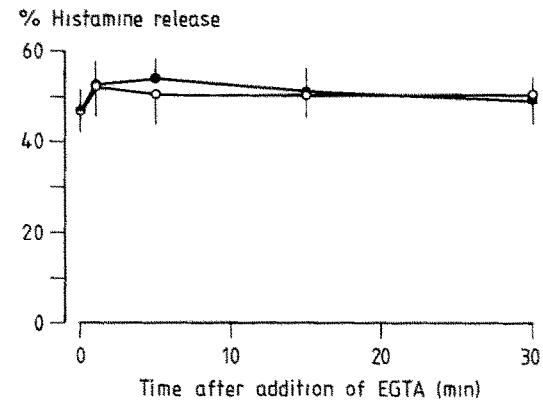



Fig.4. Effect of EGTA on the enhancement of histamine secretion by ouabain. The cells were preincubated for 60 min in calcium-free Ringer solution containing potassium 4.75 mM, pH 6.9 (37°C) followed by preincubation for 105 min with ouabain 1 mM. Then the cells were incubated for 1–30 min with EGTA 0.01 mM (●) and 0.1 mM (○) before the addition of compound 48/80. Control value of histamine secretion from cells incubated in parallel in calcium-containing Ringer solution with potassium but without EGTA and without ouabain was 68.9% ± 12.3% (mean value and standard deviation). The spontaneous histamine release from control samples was 3.2% ± 0.8%, and neither ouabain (4.2% ± 1.9%) nor ouabain and EGTA (4.6% ± 1.9%) changed the spontaneous histamine release. Mean values from four experiments are shown; vertical lines show SD.

## 4. DISCUSSION

The time dependency of the effect of ouabain on the secretory activity confirms previous observations [2], and it supports the idea that the enhancement is due to changes in the ionic composition of the cell or to the effect of this on the secretory mechanism. The identical effects of ouabain and potassium-free medium on the secretory activity [2] argue against a direct effect of ouabain on the secretory mechanism. The counteracting effect of EGTA on the enhancement by ouabain (fig.2) may be related to our previous observation [12] which is confirmed by data in this study, that EGTA increased the activity of the $Na^+$, $K^+$ pump. This increase may be due to chelation of calcium ions causing calcium deprivation of the plasma membrane, and this seems to increase the permeability of the plasma membrane to sodium [12]. The activity of the $Na^+$, $K^+$ pump is the main mechanism that creates and maintains the polarization of the plasma membrane of rat mast cells [13]. It has been proposed that depolarization of the plasma membrane by inhibition of the $Na^+$, $K^+$ pump mechanism may decrease the leakage of cellular calcium into a calcium-free medium and preserve the reactivity of the cells to stimulation with compound 48/80 [14]. In accordance, Pocock [15] provided evidence that ouabain brings about a rise in the free intracellular concentration of calcium in adrenal medullary cells. The cellular pool of calcium considered to be involved in compound 48/80-induced secretion of histamine is known to be unavailable for the secretory mechanism by pretreatment of the cells with a calcium chelator [5–8]. The present experiments argue in favour of the idea that the enhancement by ouabain may be due to an increased supply from cellular sources of calcium or preservation of calcium that is available for the stimulus-secretion coupling, and this is brought about by the inhibition of the $Na^+$, $K^+$ pump activity.

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