IgE-receptor activated chloride uptake in relation to histamine secretion from rat mast cells

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1 Antigen-stimulated histamine secretion from rat peritoneal mast cells was inhibited when extracellular chloride was replaced by either isethionate or gluconate anions, but the histamine release still remained quite substantial.

2 Rat peritoneal mast cells take up ^{36}Cl and the uptake reaches a steady state after 60 min incubation with the isotope. At steady state, the intracellular chloride level in the cells was calculated to be $29 \pm 11.5 \text{ mM}$.

3 The chloride uptake in mast cells was exponential with a rate constant of 0.036 min^{-1} in resting cells. When the cells were stimulated with antigen, and rate constant for chloride uptake increased to 0.90 min^{-1} : an increase of 25 fold. Under identical experimental conditions histamine release increased 3 fold.

4 The rate of chloride uptake in either resting cells or in antigen-stimulated cells was not changed when the extracellular medium was nominally calcium-free but histamine release was almost completely inhibited in the absence of extracellular calcium.

5 The putative chloride channel blocker DIDS (4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid) 0.3 to $30 \mu\text{M}$, produced a concentration-related inhibition of antigen-stimulated histamine secretion but DIDS ($30 \mu\text{M}$) did not inhibit the antigen-stimulated increase of chloride uptake.

6 The cyclic AMP analogue, dibutyryl cyclic AMP (1 mM) produced a delayed increase in chloride uptake in resting mast cells but neither dibutyryl cyclic AMP nor 8-bromo cyclic AMP *per se* induced any histamine secretion.

7 Ouabain (1 mM) which inhibits the Na^+/K^+ ATPase in rat peritoneal mast cells, failed to affect the uptake of chloride in resting mast cells.

8 The $\text{Na}/\text{K}/2\text{Cl}$ -cotransport inhibitor, furosemide (0.7 mM), slowed the unstimulated chloride uptake in resting mast cells and abolished the increased antigen-induced chloride uptake when added together with antigen. In contrast, spontaneous and antigen-induced histamine release were unaffected by the presence of furosemide. However, when furosemide was added to the cell suspension 5 min before stimulation, furosemide was without effect on the antigen-induced chloride uptake.

9 In addition to the chloride uptake mediated by chloride channels which may be related to the mechanism of histamine secretion, crosslinking of the high affinity membrane receptors for IgE is followed by a fast chloride uptake that is likely to occur through a furosemide-sensitive $\text{Na}/\text{K}/2\text{Cl}$ -cotransporter.

Keywords: Mast cells; chloride transport; histamine secretion; IgE receptors

Introduction

Secretion from mast cells stimulated by crosslinking of the high affinity membrane receptors for IgE is dependent on extracellular calcium, although a small part of the secretion still occurs when extracellular calcium is removed (Foreman & Mongar, 1972). In addition, elevation of intracellular calcium is capable of activating the secretory process of these cells (Foreman *et al.*, 1973). Further, the rise in intracellular calcium in rat mast cells and rat basophilic leukaemia cells which follows crosslinking of the high affinity receptor for IgE is largely dependent upon extracellular calcium (Beaven *et al.*, 1984; White *et al.*, 1984). The evidence would suggest an important role for extracellular calcium in secretion from mast cells or rat basophilic leukaemia cells in response to crosslinking of the high affinity receptor for IgE. Early attempts to identify the putative calcium channel opened by crosslinking IgE receptors failed (Lindau & Fernandez, 1986).

This apparent paradox concerning the role of calcium has recently been resolved by the discovery of a membrane cal-

cium current in the mast cell which is activated by release of calcium from intracellular stores (Hoth & Penner, 1992).

Previous studies in mast cells by Matthews and coworkers (1989a,b) have revealed a stimulus-induced, delayed chloride current which may also be activated by adenosine 3':5'-cyclic-monophosphate (cyclic AMP) or GTP- γ -S. This chloride current was large and would tend to drive the mast cell membrane potential towards E_{Cl} (Matthews *et al.*, 1989b). The authors assume that E_{Cl} in the mast cell is about -35 mV and that the unstimulated membrane potential is near zero mV, whereby the membrane potential would be held in the range where there is a hyperpolarization-driven calcium influx (Matthews *et al.*, 1989a,b). However, the studies on the chloride conductance did not measure secretion and the stimuli applied to the cells (compound 48/80 and substance P) are known to cause secretion which is independent of extracellular calcium (Foreman & Mongar, 1973; Sagi-Eisenberg *et al.*, 1985).

The aim of the study was to examine the role of extracellular chloride in histamine secretion stimulated by the crosslinking of high affinity IgE receptors and to examine the effect of such stimulation on the membrane permeability to chloride.

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Methods

Isolation of mast cells

Male Sprague-Dawley (specific pathogen-free) rats weighing 250 to 420 g, were used for experiments on unstimulated cells, whereas male Wistar (specific pathogen-free) rats weighing 300 to 420 g, were used for experiments with antigen- or anti-IgE-stimulated cells. Rats were killed by bleeding from the carotid arteries after asphyxiation in CO₂.

Mixed peritoneal cells were collected by injecting through a small incision in the abdominal wall a physiological salt solution buffered with 20 mM HEPES (4-(2-hydroxyethyl)-1-piperazine ethane sulphonic acid). The cell suspension was removed from the peritoneal cavity and kept at 4°C. The remaining part of the cell isolation was carried out at 4°C. After centrifugation at 220 g for 10 min, the supernatant was discarded and the cells were resuspended in a small volume (200–300 µl) of physiological salt solution and transferred to an isotonic solution of Percoll (SG = 1.017) buffered with 20 mM HEPES. A continuous gradient of Percoll was created by centrifugation at 10,000 g for 20 min. The mast cells were concentrated in the lower third of the gradient, whereas the peritoneal leucocytes and macrophages were located in the upper third. The mast cells were harvested and washed three times to remove the remaining Percoll. The cells were then suspended in physiological salt solution containing bovine serum albumin, 1 mg ml⁻¹, and glucose, 1 mg ml⁻¹, pH 7.4 at room temperature.

A Coulter counter was used to count the number of cells and inspection of a stained (toluidine blue) smear was used to determine the fraction of mast cells in the cell suspension (97.9% ± 0.5%, *n* = 35).

Sensitization of mast cells

Rats were sensitized by injection of 1 ml pertussis vaccine and 0.25 ml of a solution of ovalbumin (20 mg ml⁻¹) the first day, reinjected the following day with 0.5 ml incomplete Freund's adjuvant and 0.25 ml ovalbumin solution (20 mg ml⁻¹), and the third day, injected with 0.25 ml ovalbumin solution (20 mg ml⁻¹) alone. The sensitized rats were used 3 to 4 weeks after the sensitization was completed. Sensitized mast cells were stimulated with ovalbumin, 1 mg ml⁻¹. This rather high concentration was chosen based on dose-response experiments in the absence of phosphatidylserine.

Incubation procedure

Mast cells pooled from 5 rats were resuspended to a cell density of 0.8 to 3.4 × 10⁶ cells ml⁻¹, depending on the experimental protocol. The cells were equilibrated at 37°C for 60 min in either calcium-containing or calcium-free physiological salt solutions. In some experiments the cells were preincubated with ouabain or 4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid (DIDS) for 10 min or furosemide for 5 min before ³⁶Cl was added.

Measurement of ³⁶Cl uptake

Triplicate samples of cells were incubated in a medium containing the normal concentration of chloride, 143 mM (see below), in addition to the radioactive tracer. The specific activity of ³⁶Cl was 17.8 kBq µmol⁻¹, and the concentration was between 7.2 to 8.3 mM, leading to a total chloride concentration of 150.2 to 151.3 mM in the medium. The incubation of the cells with ³⁶Cl lasted for 1 to 120 min and was performed at 37°C. The incubation was terminated by transferring 100 µl of cell suspension to an Eppendorf tube containing 100 µl of silicone oil (37°C), followed by centrifugation at 16,000 g for 25 s at room temperature. The tubes were frozen at -50°C, and the tip containing the cell pellet was cut off and placed into a tube with 100 µl of NaOH 48 mM,

mixed vigorously for 1 min, and then 1.8 ml Ecoscint scintillation counting medium were added before mixing and counting. The samples were counted in a liquid scintillation spectrometer using the preset window for ³²P. The counting efficiency was greater than 90%. For the calculation of the cellular uptake of ³⁶Cl, the counts for each sample were corrected for space, which amounted to 128 ± 33 nl/10⁶ cells. This space value was determined with the extracellular markers ¹⁴C-inulin and ³⁵SO₄ and provide a reliable estimate of ³⁶Cl associated with the cell pellet which is not uptake into the cells but reflects ³⁶Cl trapped in the extracellular space. Based upon efficiency determinations, c.p.m. were converted into d.p.m. and the specific activity of chloride in the extracellular medium was used to calculate the cellular uptake of ³⁶Cl in nmol/10⁶ cells.

Measurement of histamine release

Mast cells, in a final volume of 0.5 ml, were incubated in duplicate at 37°C for 10 min with secretagogues (and without: spontaneous release) under the same experimental conditions as described for the associated ³⁶Cl uptake experiments. The incubation was terminated by the addition of 1.5 ml of ice-cold physiological salt solution to the samples. After centrifugation at 220 g for 10 min at 4°C, the supernatants were collected for determination of the amount of histamine released. The cell pellets were suspended in 250 µl of a physiological salt solution and boiled for 3 min. After addition of 1.75 ml of physiological salt solution and centrifugation the supernatant was collected for determination of the residual histamine. This was performed by the fluorometric method (Shore *et al.*, 1959) omitting the extraction procedure. The histamine release was expressed as a percentage of the total histamine content of the cells. The anti-IgE- and antigen-induced histamine release were adjusted for the spontaneous histamine release.

Solutions

The physiological salt solution used in these experiments had the following composition in mM: NaCl 137, KCl 4, MgSO₄ 1, CaCl₂ 1, HEPES 20. The solution contained bovine serum albumin, 1 mg ml⁻¹, and glucose, 1 mg ml⁻¹. Changes in the concentrations of calcium in the physiological salt solution were balanced by changes in the concentration of NaCl in order to maintain isotonicity. The pH of the solution was adjusted to 7.4. In those experiments in which extracellular chloride concentration was varied, sodium chloride was substituted with equimolar amounts of either sodium isethionate or sodium gluconate. The activity of Ca²⁺ in solutions containing isethionate or gluconate was checked by means of a calcium electrode and in the case of solutions containing gluconate, the total calcium concentration was adjusted to give the desired calcium ion activity by adding small volumes of calcium chloride solution, 1 M.

Data presentation

The data are presented as means ± s.e.mean.

Materials

Bovine serum albumin, HEPES, 4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid disodium salt (DIDS), sodium D-gluconate, furosemide and ouabain were supplied by Sigma Chemical Company, St. Louis, U.S.A.; sodium isethionate by Fluka, Gillingham, Dorset, U.K.; Percoll by Pharmacia Fine Chemicals, Uppsala, Sweden; silicone oil (AR 200/50 Wacker, density 1.04) by Serva, Heidelberg, Germany. ³⁶Cl was obtained from Amersham, Buckinghamshire, U.K., and the scintillation solution, Ecoscint, was from BN Plastic, Helsingør, Denmark. Pertussis vaccine and Freund's adjuvant were obtained from Statens Serum Institut, Copenhagen, Denmark. All other chemicals were of analytical grade.

Results

Relationship between extracellular chloride and histamine release

The removal of extracellular chloride inhibited antigen-stimulated histamine secretion. The extracellular calcium concentration-effect relationship was shifted to the right and the maximum depressed when chloride was replaced by isethionate or gluconate (Figure 1).

Chloride uptake

Figure 2a shows that in a physiological solution containing calcium, resting mast cells took up chloride. The rate of chloride uptake was not changed when the extracellular medium was nominally calcium-free. Equilibration of intracellular and extracellular ³⁶Cl was 50% complete in about 15 min and complete after 60 min. In six different and unrelated experiments, incubation of mast cells with ³⁶Cl lasted for 120 min, during which the steady state value remained constant (data not shown). The specific activity of intracellular ³⁶Cl at steady state gives an intracellular chloride concentration of 29 ± 11.5 mM ($n = 9$). In resting mast cells, spontaneous histamine release in the presence of extracellular calcium was $5.3 \pm 0.9\%$ ($n = 4$) and in the absence of calcium it was $6.4 \pm 3.6\%$ ($n = 4$).

Stimulation of sensitized rat mast cells with antigen (ovalbumin, 1 mg ml^{-1}) in the presence of calcium greatly increased the initial rate of chloride uptake, and again, this was not changed when the extracellular medium was made nominally calcium-free (Figure 2a). In these experiments, antigen and ³⁶Cl were added simultaneously to cells suspended in physiological salt solution which contained calcium (1 mM) or was nominally calcium-free. The histamine release after stimulation with antigen amounted to $17 \pm 1\%$ in the presence of calcium ($n = 4$) and $8.3 \pm 2.5\%$ in its absence ($n = 4$) after 10 min of incubation after which histamine release is complete (Johansen & Chakravarty, 1975).

The data in Figure 2a were fitted to the exponential $y = a(1 - e^{-kt})$ using an iterative method. In calcium-containing media, the estimated rate constants for chloride uptake were $0.036 \pm 0.015 \text{ min}^{-1}$ in resting cells and $0.90 \pm 0.1 \text{ min}^{-1}$ in antigen-stimulated mast cells. Thus, antigen stimulation of

Figure 1 Concentration-response relationships for calcium and histamine secretion from rat mast cells stimulated by anti-IgE. Normal physiological salt solution (●); physiological salt solution in which sodium chloride was replaced by sodium isethionate (○) or gluconate (■). Each point is the mean $\pm$ s.e.mean from 6 experiments.

the mast cells produced a 25 fold increase in chloride uptake. Estimates of a , the maximum value for chloride uptake, were $45 \pm 15 \text{ nmol}/10^6$ cells in resting cells and $38 \pm 1 \text{ nmol}/10^6$ cells in antigen-stimulated cells.

The action of dibutyryl cyclic AMP on ³⁶Cl uptake

Since previous studies (Matthews *et al.*, 1989b) have shown that the chloride conductance of mast cells may be activated by intracellular cyclic AMP, the effect of membrane-permeable analogues of cyclic AMP, applied extracellularly, was studied. Dibutyryl cyclic AMP, $0.1 \mu\text{M}$ to 3 mM , did not induce any histamine secretion above 3% of total. The range of histamine release in the dose-response experiments was 1.0–2.2% ($n = 33$). Similar data were obtained with the cyclic AMP analogue, 8-bromo cyclic AMP (range 0.8–3.6%, $n = 33$). Addition of dibutyryl cyclic AMP, 1 mM did not increase the rate of ³⁶Cl uptake. The rate constant in the absence of cyclic AMP was $0.084 \pm 0.006 \text{ min}^{-1}$ and in the presence of dibutyryl cyclic AMP (1 mM) was $0.034 \pm 0.003 \text{ min}^{-1}$. However, the estimated maximum chloride uptake was increased from $31 \pm 1 \text{ nmol}/10^6 \text{ cell min}^{-1}$ in the absence of dibutyryl cyclic AMP to $64 \pm 4 \text{ nmol}/10^6 \text{ cells min}^{-1}$ in the presence of dibutyryl cyclic AMP.

Figure 2 (a) Time course of chloride uptake by rat mast cells. Unstimulated cells in the absence of extracellular calcium (○), unstimulated cells in the presence of extracellular calcium, 1 mM (△); cells stimulated with ovalbumin, 1 mg ml^{-1} in the absence of extracellular calcium (●); cells stimulated with ovalbumin, 1 mg ml^{-1} in the presence of extracellular calcium, 1 mM (▲). Each point is the mean $\pm$ s.e.mean from 4 experiments. Resting cells (open symbols) were obtained from non-sensitized animals, whereas antigen-stimulated cells (closed symbols) were obtained from sensitized animals. (b) Effect of dibutyryl cyclic AMP on the rate of chloride uptake by rat mast cells. Unstimulated cells in the absence of dibutyryl cyclic AMP (○); unstimulated cells in the presence of dibutyryl cyclic AMP, 1 mM (△). Each point is the mean $\pm$ s.e.mean from 3 experiments. (c) Concentration-response curve for the inhibition of anti-IgE-stimulated histamine release by 4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid (DIDS). Cells were preincubated with DIDS for 10 min at 37°C before stimulation with anti-IgE. Each point is the mean $\pm$ s.e.mean from 6 experiments and in these experiments the anti-IgE-stimulated histamine release in the absence of DIDS was $55.5 \pm 4.7\%$. (d) Effect of DIDS on the rate of chloride uptake by sensitized rat mast cells. Cells stimulated with ovalbumin, 1 mg ml^{-1} in the absence of DIDS (●); cells stimulated with ovalbumin, 1 mg ml^{-1} in the presence of DIDS, $30 \mu\text{M}$ (▲). Each point is the mean $\pm$ s.e.mean from 4 experiments. The cells were preincubated with DIDS for 10 min before addition of ³⁶Cl and antigen.

Effect of DIDS on histamine release and ^{36}Cl uptake

The putative chloride channel blocker DIDS, 0.3 to 30 μM , caused a concentration-dependent inhibition of antigen-stimulated histamine secretion from rat mast cells (Figure 2c). However, DIDS had no effect on chloride uptake in antigen-stimulated mast cells (Figure 2d). The cells were preincubated with DIDS, 30 μM , for 10 min before addition of ^{36}Cl and antigen. The initial rate of chloride uptake in the first minute after antigen-stimulation of the cells was $27 \pm 7.5 \text{ nmol}/10^6 \text{ cells min}^{-1}$ in the absence of DIDS ($n = 4$) and $24.5 \pm 7.5 \text{ nmol}/10^6 \text{ cells min}^{-1}$ in the presence of DIDS, 30 μM ($n = 4$). Further, DIDS, 30 μM , did not change the steady state value of chloride uptake.

The effect of ouabain on ^{36}Cl uptake

Rat mast cells have a Na^+/K^+ ATPase which is sensitive to relatively high ouabain concentrations (Knudsen *et al.*, 1993). Since ouabain is likely to cause sodium accumulation by mast cells, the possibility that sodium accumulation might result in a secondary change in chloride uptake was examined. The cells were preincubated with ouabain, 1 mM, in physiological salt solution containing calcium, 1 mM for 10 min before addition of ^{36}Cl but the chloride uptake was unaffected by this treatment (Figure 3a).

The effect of furosemide on ^{36}Cl uptake

Furosemide was used to investigate the role of a $\text{Na}/\text{K}/2\text{Cl}$ cotransport. When furosemide (0.7 mM) was added to uns-

Figure 3 (a) Effect of ouabain on the rate of chloride uptake by rat mast cells. Unstimulated cells in the absence of ouabain (●); unstimulated cells in the presence of ouabain, 1 mM (▲). The cells were preincubated with ouabain for 10 min before addition of ^{36}Cl . Each point is the mean $\pm$ s.e. mean from 4 experiments. (b) Effect of furosemide on the rate of chloride uptake by rat mast cells. Unstimulated cells in the absence of furosemide (●); unstimulated cells in the presence of furosemide, 0.7 mM (▲). Each point is the mean $\pm$ s.e. mean from 4 experiments. (c) Effect of furosemide on the rate of chloride uptake by sensitized rat mast cells. Furosemide, antigen and ^{36}Cl were added simultaneously to the cell suspension. Cells stimulated with ovalbumin, 1 mg ml $^{-1}$ in the absence of furosemide (●); cells stimulated with ovalbumin, 1 mg ml $^{-1}$ in the presence of furosemide, 0.7 mM (▲). Each point is the mean $\pm$ s.e. mean from 4 experiments. (d) Effect of furosemide on the rate of chloride uptake by sensitized rat mast cells. Cells stimulated with ovalbumin, 1 mg ml $^{-1}$ in the absence of furosemide (●); cells stimulated with ovalbumin, 1 mg ml $^{-1}$ in the presence of furosemide, 0.7 mM added 5 min before stimulation with antigen (▲). Each point is the mean $\pm$ s.e. mean from 5 experiments.

stimulated mast cells, a small delay in obtaining steady state was observed (Figure 3b). Under identical experimental conditions, the spontaneous histamine release was $7.9 \pm 1.1\%$ in the absence of furosemide ($n = 4$) and $6.6 \pm 1.1\%$ in its presence ($n = 4$).

This experiment was repeated on antigen-stimulated sensitized mast cells, where furosemide, antigen and ^{36}Cl were added simultaneously to the cell suspension. Under these conditions, furosemide was able to abolish the chloride uptake induced by stimulation with antigen (Figure 3c). In these experiments, the antigen-induced histamine release was $30.8 \pm 3.6\%$ in the absence of furosemide ($n = 4$) and $51.9 \pm 3.7\%$ in its presence ($n = 4$).

When furosemide was added to sensitized mast cells 5 min before addition of antigen and ^{36}Cl , however, the inhibitory effect of furosemide had almost disappeared (Figure 3d). The antigen-induced histamine release in the presence of furosemide was $34.7 \pm 2.5\%$ ($n = 5$), whereas the unstimulated histamine release in the presence of furosemide was $8.0 \pm 1\%$ ($n = 5$).

Discussion

In the electrophysiological studies of Matthews *et al.* (1989b) an outward membrane current (corresponding to influx of chloride) was observed in rat peritoneal mast cells when they were stimulated with substance P or compound 48/80. They also demonstrated the existence of a hyperpolarization resulting from chloride influx into the cell which created a driving force to facilitate the entry into the cytosol of calcium required to initiate histamine secretion (Matthews *et al.*, 1989a).

We have shown that removal of extracellular chloride shifts the calcium concentration-effect curve for antigen-stimulated histamine secretion to the right and depresses its maximum. This observation is consistent with the hypothesis that chloride-mediated hyperpolarization of the cell acts as a driving force for calcium that is required to enter the cell to cause secretion. It seems unlikely that the inhibition of secretion seen when chloride was replaced in the extracellular medium was due to the chemical nature of the substituting anion since both gluconate and isethionate produced similar effects.

We have also shown that when rat mast cells are stimulated with antigen, there is a large increase in the rate of chloride uptake into the cell. Extracellular calcium had no effect on either the resting uptake of chloride or on the antigen-stimulated uptake. Removal of extracellular calcium produced a marked reduction of antigen-stimulated histamine secretion but did not change the chloride uptake, implying that chloride uptake is not a consequence of secretion.

Matthews *et al.* (1989b) were able to mimic the substance P- or compound 48/80-induced chloride conductance in rat mast cells by the intracellular application of cyclic AMP, although the effect was slower than that produced by substance P or compound 48/80. Using the dibutyl analogue of cyclic AMP, we have shown that this compound produces a small and slow increase in chloride uptake, and it is clear that the effect is by no means as marked as that caused by antigen-stimulation. It is possible that the level of intracellular cyclic AMP achieved with the analogue is insufficient because of metabolism or diffusion limitations. However, both of these explanations seem unlikely because dibutyl cyclic AMP inhibits histamine secretion (Kaliner & Austen, 1974) and hence presumably gains access to the intracellular compartment. The antigen-stimulated increase in chloride permeability that we observe is large and may represent mechanisms in addition to the opening of the chloride channels described by Matthews *et al.* (1989b). Whilst cyclic AMP activates the chloride channels, it may not affect other mechanisms of increased chloride permeability.

The chloride conductance described in rat peritoneal mast

cells by Matthews *et al.* (1989b) was shown to be blocked by DIDS. We have shown that DIDS inhibits antigen-induced histamine secretion at concentrations similar to those used to block chloride channels, although the inhibition was incomplete even at the higher concentrations. We were, however, unable to demonstrate any effect of DIDS on the antigen-induced increase of chloride uptake, and this uptake may represent a DIDS-insensitive mechanism of increased permeability to chloride, other than the DIDS-sensitive chloride channels described by Matthews and coworkers (1989b).

Besides these chloride channels, the Na⁺/K⁺ ATPase is the only other electrolyte transporter that has been characterized in mast cells. Ouabain, 1 mM, has been shown to inhibit the Na⁺/K⁺ ATPase in rat peritoneal mast cells (Knudsen *et al.*, 1993). In the present study, inhibition of the Na⁺/K⁺ ATPase with ouabain did not change the rate of chloride uptake.

The chloride uptake that we measure is probably different from the chloride current described by Matthews *et al.* (1989b) because of the apparently contradictory results with cyclic AMP and DIDS as discussed above, and because the delayed chloride current was only fully developed 6 min after stimulation (Matthews *et al.*, 1989b), whereas the antigen-induced increase in chloride uptake in the present study was near maximal 1 min after stimulation. Since this rapid chloride uptake was not detected in the electrophysiological studies, it may represent chloride uptake via electroneutral transporters such as the Na/K/2Cl cotransporter or the Cl⁻/HCO₃⁻-exchanger, described in other cell types. Experiments with the Na/K/2Cl-cotransport inhibitor, furosemide, confirmed that the antigen-induced uptake of chloride may be due to the activity of the cotransporter, since the increase in chloride uptake was almost abolished in the presence of furosemide. In addition to this cotransporter, our data have revealed that other chloride transporters may also be operating in the early events after stimulation, since furosemide added 5 min before stimulation was without notable effect on the rapid antigen-induced increased chloride uptake. A possible candidate for this chloride transport is the

Cl⁻/HCO₃⁻-exchanger, which is at present under investigation in our laboratory.

The antigen-induced increased chloride uptake is not directly related to histamine release, since the absence of extracellular calcium or the presence of DIDS inhibited antigen-induced histamine release without affecting the chloride uptake. Furthermore, furosemide added simultaneously with antigen inhibited the increase in chloride uptake without inhibiting histamine release. Chloride uptake by the Na/K/2Cl cotransporter would not be involved in membrane hyperpolarization, as it is electroneutral. Our data so far do not point to any possible functions of this chloride uptake in the signal-transduction mechanism. It coincides with the large increase in the activity of the Na⁺/K⁺ pump that follows a secretory response (Knudsen *et al.*, 1993). It may be that these observations reflect an increased activity of an electroneutral chloride transport that contributes to compensatory ion transport necessary for the cell to recover during histamine secretion.

In conclusion, crosslinking of the high affinity membrane receptors for IgE initiates a large and fast transport of chloride into the mast cell which occurs independently of the secretory response and is thus not directly related to the signal-transducing mechanism. The inhibition of the fast chloride uptake by furosemide points to an increased activity of the Na/K/2Cl cotransporter. However, inward movement of chloride through chloride channels may be part of the mechanism controlling histamine secretion since removal of extracellular chloride or addition of DIDS inhibits histamine secretion.

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