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Immunologically activated chloride channels involved in degranulation of rat mucosal mast cells

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Crosslinking of type I Fc_ϵ receptors ($Fc_\epsilon RI$) on the surface of basophils or mast cells initiates a cascade of processes leading to the secretion of inflammatory mediators. We report here a correlation between mediator secretion and the activation of Cl^- channels in rat mucosal-type mast cells (line RBL-2H3). Stimulation of RBL cells by either IgE and antigen or by a monoclonal antibody specific for the $Fc_\epsilon RI$, resulted in the activation of Cl^- ion channels as detected by the patch-clamp technique. Channel activation occurred slowly, within minutes after stimulation. The channel has a slope conductance of 32 pS at potentials between 0 and −100 mV, and an increasing open-state probability with increasing depolarization. Activation of apparently the same Cl^- channels could be mimicked without stimulation by isolating inside-out membrane patches in tyrode solution. Parallel inhibition of both Cl^- channel activity and mediator secretion, as monitored by serotonin release, was observed by two compounds, the Cl^- channel blocker 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB) and the anti-allergic drug cromolyn. NPPB inhibited both the antigen-induced Cl^- current and the serotonin release, where half-maximal inhibition occurred at similar doses, at 52 μM and 77 μM, respectively. The drug cromolyn, recently found to inhibit immunologically induced mediator secretion from RBL cells upon intracellular application, also blocks Cl^- channels (IC_{50} = 15 μM) when applied to the cytoplasmic side of an inside-out membrane patch. The observed Cl^- channel activation upon immunological stimulation and the parallel inhibition of channel current and of serotonin release suggests a functional role for this Cl^- channel in mediator secretion from the mast cells studied.

Key words: cromolyn/5-nitro-2-(3-phenylpropylamino)benzoic acid/patch-clamp/rat basophilic leukemia cells/secretion

Introduction

Secretion of inflammatory mediators such as histamine and leucotrienes by tissue mast cells and blood basophils is a major event in the involvement of these cells in immediate-type hypersensitivity. Mediator release is the final outcome of a cascade initiated immunologically by aggregation of the $Fc_\epsilon RI$ by the corresponding antigen (Ishizaka *et al.*, 1984). Rat peritoneal mast cells and the rat basophilic leukemia (RBL)-2H3 cell line are widely used in mechanistic studies of the stimulus−secretion coupling process. Molecular events following stimulation include (i) hydrolysis of phosphatidyl inositides (Cockroft, 1981; Beaven *et al.*, 1984a), (ii) transient increase in the intracellular free Ca^{2+} ion concentration (Ishizaka *et al.*, 1979; Beaven *et al.*, 1984b; Fewtrell and Sherman, 1987), (iii) generation of arachidonic acid (Sullivan and Perker, 1979; Crews *et al.*, 1981), (iv) protein tyrosine phosphorylation (Benhamou *et al.*, 1990) and (v) partial membrane depolarization (Kanner and Metzger, 1983; Sagi-Eisenberg and Pecht, 1983, 1984; Mohr and Fewtrell, 1987b; Labrecque *et al.*, 1989). The involvement of ion transport mechanisms in stimulus−secretion coupling remains, however, a controversial issue. In early studies no significant changes in membrane permeability in response to antigen stimulation have been observed in either rat peritoneal mast cells (Lindau and Fernandez, 1986a,b) or RBL cells (Lindau and Fernandez, 1986a). By contrast, recent patch-clamp studies (Penner *et al.*, 1988; Kuno *et al.*, 1989; Matthews *et al.*, 1989a,b) of rat peritoneal mast cells revealed the induction of ion currents upon stimulation by IgE and antigen or other secretagogues.

The present study aimed at examining the relationship between changes in the cells' membrane permeability and mediator release of serotonin, both induced by specific aggregation of $Fc_\epsilon RI$. For this purpose we used the RBL-2H3 cell cline (Barsumian *et al.*, 1981; Metzger and Ishizaka, 1982). It appears particularly suited, since the slow process of mediator release, i.e. 20−30 min (McGivney *et al.*, 1981) as compared with 1−2 min for the mast cells (Ishizaka, 1981), enables considerably better time-resolution of cellular events. We report here that $Fc_\epsilon RI$ aggregation leads to activation of Cl^- channels in RBL-2H3 cells. The involvement of these channels in mediator secretion is supported by the use of two compounds, NPPB and cromolyn. NPPB, known as a Cl^- channel blocker (Wangemann *et al.*, 1986; Jetten *et al.*, 1989; Kunzelmann *et al.*, 1989), was found to inhibit these Cl^- channels as well as serotonin secretion. Cromolyn, which is widely employed in the prophylactic treatment of allergic asthma (Cox, 1967; Cox *et al.*, 1970), is well known to inhibit mediator secretion from rat peritoneal mast cells (Cox, 1967; Foreman *et al.*, 1977). In contrast, secretion from mucosal mast cells (Pearce *et al.*, 1982) or from cells of the RBL-2H3 line (Seldin *et al.*, 1985; Hemmerich *et al.*, 1991) is not inhibited. Very recent findings indicate (Hemmerich *et al.*, 1991), however, that cytosolic application of cromolyn inhibits mediator release from RBL cells. We now report that cromolyn, when applied to the intracellular side of the membrane, also markedly inhibits Cl^- channel activity.

Results

Single channel activity induced by $Fc_\epsilon RI$ aggregation in cell-attached patches

In applying patch-clamp techniques to study RBL cells we first chose the cell-attached patch configuration, since whole-cell recordings did not reveal changes in membrane permeability upon RBL cell stimulation (Lindau and Fernandez, 1986a). We repeated these experiments in initial studies using the whole-cell recording configuration and confirmed that it is not possible to detect induction of ionic currents following immunological stimulation, probably due to the wash-out of intracellular components involved in stimulus–secretion coupling. In cell-attached patches, stimulation was attained by application of polyvalent ligands through the patch pipette. Crosslinking of the immunoglobulin E (IgE)–$Fc_\epsilon RI$ complexes by dinitrophenyl$_{11}$–bovine serum albumin (DNP_{11}-BSA) elicited single channel events (see Figure 1A) in 12 out of 53 RBL cells. Single channels with virtually identical characteristics (see below) were observed in four out of 20 RBL cells following stimulation with the $Fc_\epsilon RI$-specific monoclonal antibody (mAb) F4 as an independent means of stimulating the cells. In either case of stimulation channel activation occurred at a slow rate (histogram in Figure 1A). Due to the long time period required for channel activation in some experiments, the patch pipette position was carefully controlled to be still cell-attached at the end of the experiment. Once activated, channels remained observable for at least 30 min. Usually one to three channels were active as judged from overlapping events. Concomitant application of antigen through the bath solution enhanced neither the yield nor the rate of channel activation. Single channel events exhibited a slope conductance of 32 ± 6 pS (seven experiments) between −100 and 0 mV as exemplified in Figure 1B. The open probability increased from essentially 0 to 1 with depolarization from −100 to 0 mV as shown in the inset of Figure 1B. The reversal potential of single channel currents was estimated to be −45 ± 11 mV (n = 8, see Figure 1B) based on a resting potential of −53 ± 10 mV (n = 18) of the RBL cells used. The negative value clearly excluded Ca^{2+} as the predominant charge carrier and suggested Cl^- or K^+ as possible current carriers. The latter is less likely, however, since for a K^+-selective channel the reversal potential would be expected to be more negative (< −70 mV, no K^+ was present in the patch-pipette).

Ionic selectivity assignment to Cl^-

The inside-out patch configuration was employed to investigate the apparent Cl^- selectivity further. In two experiments patch excision was performed after having observed DNP_{11}-BSA-activated channels in the cell-attached patch. Channel events reappeared immediately after forming the inside-out patch in tyrode buffer. They exhibited slope conductances of 32 and 30 pS (between −60 and 20 mV) similar to 33 and 29 pS, respectively, observed in the cell-attached patch. The reversal potentials shifted upon patch excision from −40 and −42 mV to 4 and −4 mV (see Figure 1B), respectively, which is in reasonable agreement with the equilibrium potential for Cl^- ions of −1.4 mV ($[Cl^-]_o/[Cl^-]_i$ of 160/151 mM). In order to exclude the possibility that the current originated from a non-selective permeation of cations (which would also exhibit a reversal potential around 0 mV), all cations (except for 10 mM Na^+) in the tyrode solution were then substituted by the impermeant cation choline. Both the reversal potentials and the slope conductances of single channel events remained

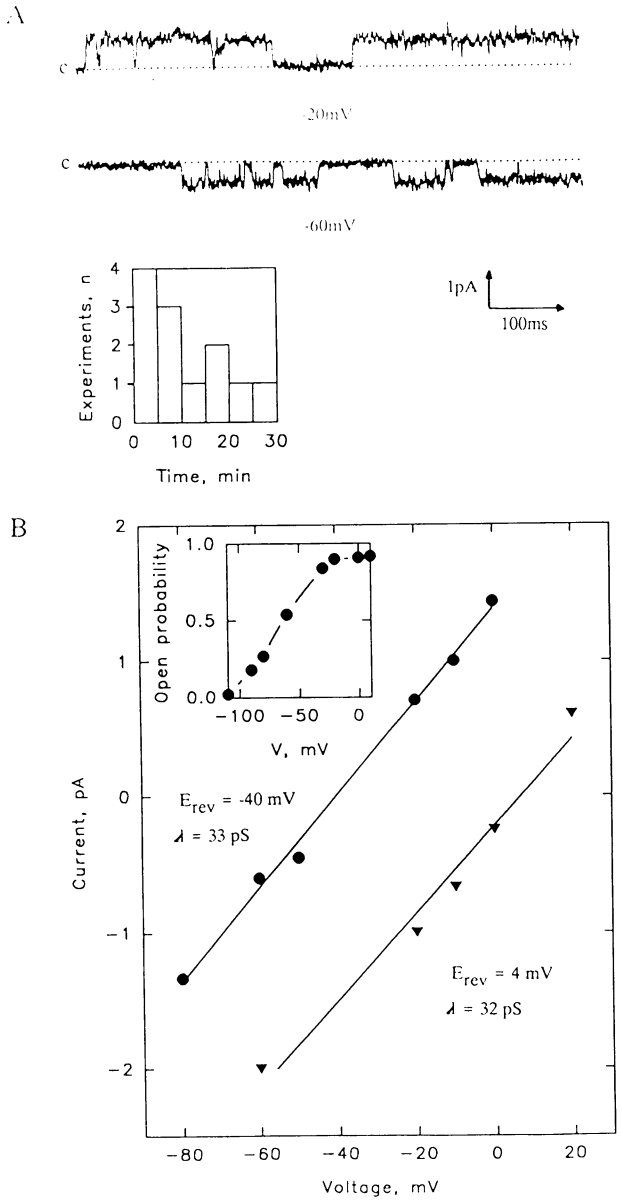

Fig. 1. Cl^- channel activity induced by $Fc_\epsilon RI$ aggregation. (**A**) Single channel events in the cell-attached patch configuration elicited by 50 nM DNP_{11}-BSA in the patch pipette. The two records show representative single channel activities at two membrane voltages. The activity appeared 6 min after cell-attached patch formation and lasted unchanged for 25 min; the traces were recorded at 6 min (−60 mV) and at 8 min (−20 mV), and were filtered at 300 Hz. The dotted lines indicate the closed state of the channel denoted as c. The histogram shows the distribution of times at which stable Cl^- channel activity appeared for the first time. Time scale starts (0 min) with the formation of a cell-attached patch. The data summarize 12 experiments, including both means of $Fc_\epsilon RI$ aggregation, i.e. IgE + DNP_{11}-BSA (eight experiments) and F4 mAb. (**B**) Current–voltage relationships of Cl^- channel activities determined in the cell-attached patch (●) and in the inside-out patch (▼). Formation of the inside-out patch into tyrode solution was performed 14 min after formation of the cell-attached patch. Upon patch excision the reversal potential exhibited a shift of +44 mV, while slope conductance remained unchanged. Inset shows the voltage dependence of the open-state probability of the Cl^- current in the cell-attached patch configuration. For cell-attached data voltage is defined as the applied potential plus mean resting potential of −53 mV (see text). Traces in A and data in B were obtained from the same experiment.

unchanged within experimental errors of ±2 mV and ±2 pS, respectively. Lowering the Cl^- concentration in tyrode solution could not be used to establish Cl^- selectivity, since it led to a change in the permeability characteristics of the channel. This phenomenon is currently under investigation. From these data we conclude that the observed immunologically activated channels are Cl^--selective ones.

Cl^- channel activity in unstimulated RBL cells

In 37 control experiments performed in the cell-attached patch configuration using either IgE-sensitized cells or unsensitized cells with DNP_{11}-BSA in the patch pipette (six experiments), no channel events were observed in 35 recordings over 30 min after patch formation. One cell exhibited a transient Cl^- channel activity (at 14 min for ~1 min) and the other cell showed current fluctuations, which were, however, distinct from the channel activity specified above. Controls were performed in paired comparison with stimulated cells exhibiting Cl^- channel activation. In the course of these experiments we observed that formation of an inside-out patch in tyrode solution led to the activation of apparently the same Cl^- channels as those observed upon immunological stimulation. Activation occurred in 23 out of 59 experiments. The time periods from inside-out patch formation to the first occurrence of channel events were distributed similarly to these in Figure 1A. Single channel currents exhibited a slope conductance of 28.5 ± 4.6 pS between −60 and 20 mV and a reversal potential of 0.1 ± 4.5 mV (n = 5). In 19 inside-out patches studied, 11 patches showed Cl^- channel activity with the same open probability characteristics as the antigen-induced Cl^- channel (see Figure 1B), while in the remaining eight experiments weaker voltage dependences were observed. Cl^- channel activity persisted in an extracellular solution containing a low level of 10^{-7} M Ca^{2+} (unpublished observation) arguing against a direct regulation by Ca^{2+}.

Blockage of Cl^- channel activity by NPPB

We examined the inhibitory capacity of NPPB on the observed Cl^- channels, since NPPB has been reported to block Cl^- channels in epithelial cells (Wangemann *et al.*, 1986; Jetten *et al.*, 1989; Kunzelmann *et al.*, 1989). NPPB effectively inhibited antigen-induced single channel events, when studied in the cell-attached patch configuration (Figure 2). Application of 500 μM NPPB (Figure 2A) led to a complete block of the channel (middle trace), which was reversed upon wash-out of NPPB (lower trace). A complete block by 500 μM NPPB was also found for the Cl^- channels observed in inside-out patches of unstimulated cells (n = 5, data not shown). The dose−response relationship for the channel block by NPPB (see Figure 2B), established for practical reasons for the inside-out patch, revealed an IC_{50} of 52 ± 21 μM (n = 4).

Inhibition of serotonin release by NPPB

In order to assess directly the functional significance of Cl^- channel activity in stimulus−secretion coupling, we studied the effect of NPPB on antigen-induced mediator release from RBL cells. Figure 3A shows that serotonin release, monitored over a period of 30 min, was markedly diminished by the Cl^- channel blocker NPPB (100 μM). Comparable inhibition was observed in a total of five experiments. In addition, similar results were obtained with RBL cells bathed in patch pipette solution (data not shown). The dose−response relationship for the inhibition of serotonin release by NPPB (see Figure 3B) yielded (determined after 30 min of DNP_{11}-BSA addition) an IC_{50} of 77 ± 60 μM NPPB (four independent experiments). In stimulation experiments in the absence of extracellular Ca^{2+} serotonin release was 12% (see Figure 3B) compared with 7% without stimulation (see Figure 3A). This confirmed the strict dependence of release on extracellular Ca^+ for the RBL cells used.

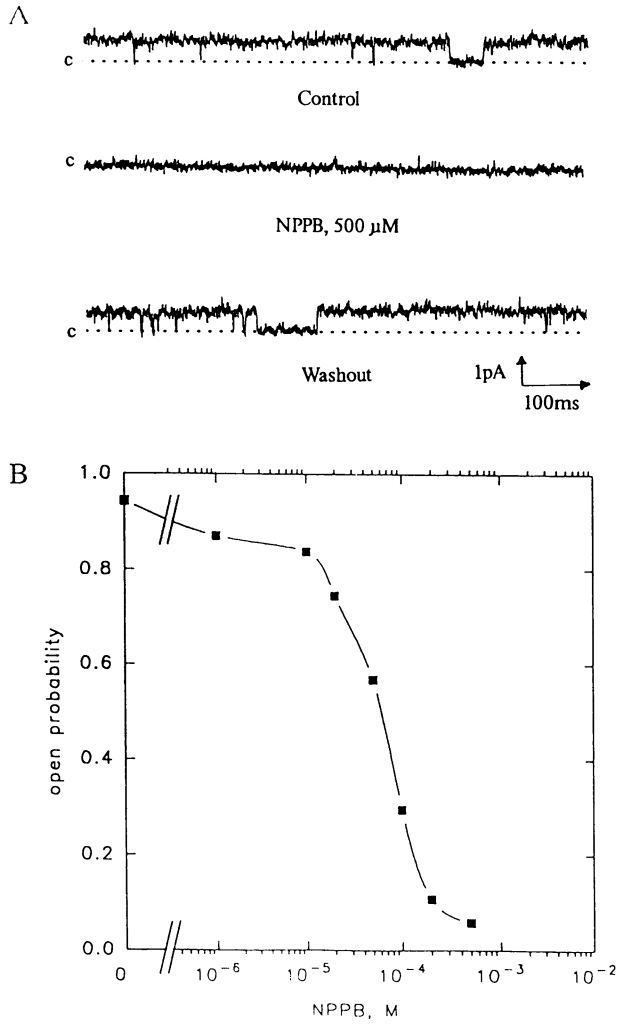

Fig. 2. Blockage of Cl^- channel activity by NPPB. (**A**) Cl^- channel activity was evoked as in Figure 1A (control trace, at 8.6 min). Activity was abolished by application of 500 μM NPPB to the bath (middle trace, at 10 min) and was completely recovered upon wash-out of NPPB (lower trace, at 12.5 min). Channel traces were recorded in the cell-attached patch configuration at a potential of −10 mV and filtered at 500 Hz. (**B**) Dependence of the open probability of Cl^- channels on NPPB concentrations. Channel open probability was decreased with an IC_{50} of ~60 μM. The dose−reponse relationship was determined in the inside-out patch configuration at a potential of 30 mV. At this potential open channel currents matched those observed in the cell-attached patch at a voltage of −10 mV as in A.

Blockage of Cl^- channel activity by cromolyn

In RBL cells, cromolyn has been found to inhibit serotonin release when applied intracellularly (Hemmerich *et al.*, 1991). Based on our finding that Cl^- channel blockade was correlated with inhibition of serotonin release, we examined the effect of cromolyn on this Cl^- channel activity. It indeed was found to be an effective Cl^- channel blocker (Figure 4). Complete inhibition of Cl^- channel activity was

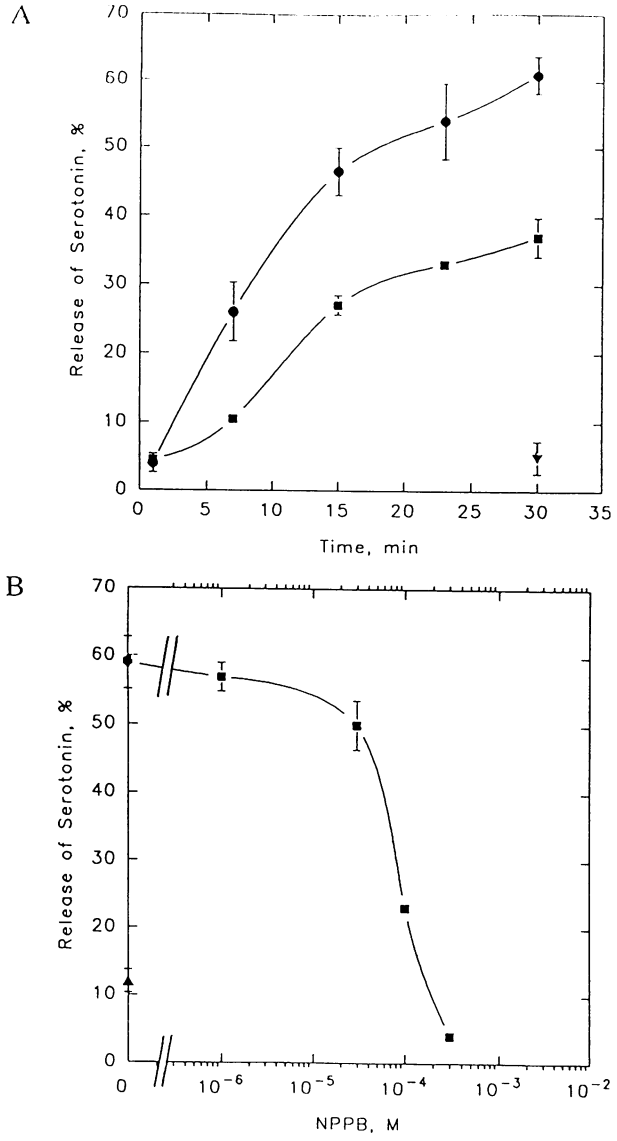

Fig. 3. Inhibition of $Fc_\epsilon RI$-mediated serotonin release by NPPB. **(A)** Time course of inhibition of serotonin release by NPPB. Serotonin release, initiated upon addition of 5 nM DNP_{11}-BSA to IgE-saturated cells, was monitored in the absence (●) and in the presence (■) of 100 μM NPPB over a time period of 30 min. NPPB was added to RBL cells 10 min before DNP_{11}-BSA. Basal serotonin release (▼), obtained in the absence of DNP_{11}-BSA, was routinely determined at 30 min and was not significantly changed by NPPB. **(B)** Dose-dependent reduction of serotonin release by NPPB after 30 min. An IC_{50} of ~70 μM is estimated. A greatly diminished serotonin release (▲, at 30 min) was observed when Ca^{2+} ions were omitted from the tyrode buffer. The respective data in (A) and (B) were from representative experiments each performed in triplicate.

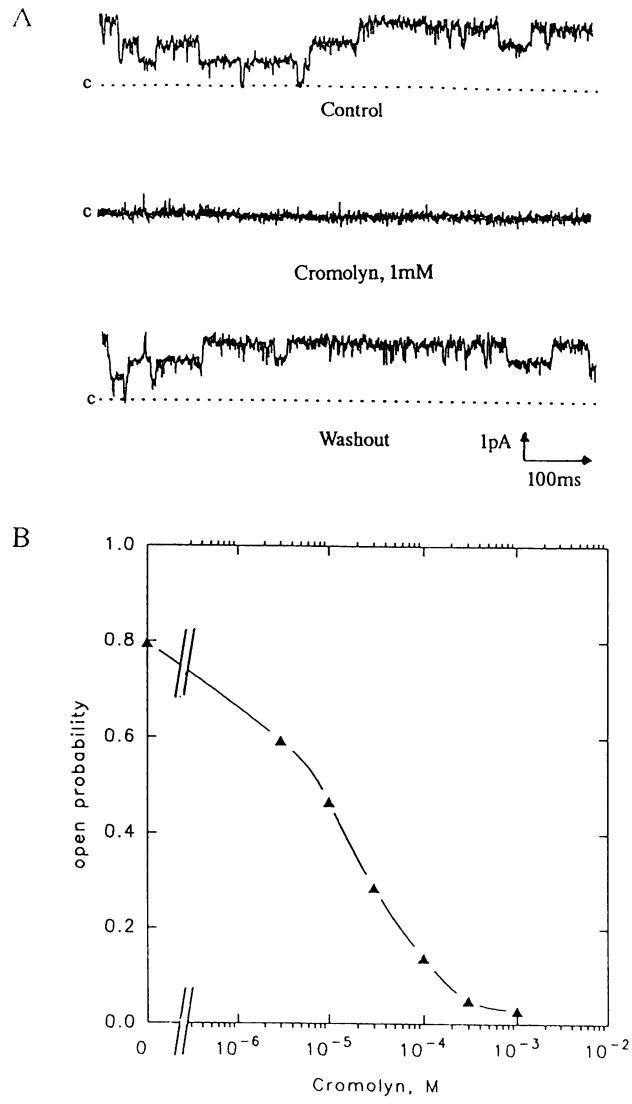

Fig. 4. Blockage of Cl^- channel activity by cromolyn. **(A)** Cl^- channel activity observed after formation of an inside-out patch (control trace, at 14 min) was completely abolished by application of 1 mM cromolyn to the bath (middle trace, at 16 min). Upon wash-out of cromolyn, activity recovered to the initial level (lower trace, at 18 min) and remained stable for at least the following 15 min. Single channel traces were recorded at a holding potential of +30 mV and filtered at 500 Hz. **(B)** Dependence of the open probability of Cl^- channels on cromolyn concentrations. The dose–response relationship exhibits a reduction in channel open probability with an IC_{50} of 14 μM. All data were obtained from one representative experiment.

observed, when 1 mM cromolyn was applied to the intracellular side of an inside-out patch (n = 5, see Figure 4A, middle trace). Block was completely reversed upon wash-out of cromolyn (lower trace in Figure 4A). The dose–response relationship for Cl^- channel block by cromolyn (see Figure 4B) revealed an IC_{50} of 15 ± 2 μM (n = 3).

Discussion

We report the observation of a Cl^- channel activated via $Fc_\epsilon RI$ and provide support for its involvement in mediator secretion from rat mucosal mast cells.

Electrophysiological evidence was obtained that immunological stimulation of mast cells of the RBL-2H3 line led to activation of Cl^- channels in the plasma membrane. Crosslinking of $Fc_\epsilon RI$ either by antigen and IgE or by a mAb specific to the $Fc_\epsilon RI$ elicited Cl^- channel activity in ~20% of RBL cells studied. This relatively low yield of response may either reflect a low density of Cl^- channels or a low degree of coupling of $Fc_\epsilon RI$ aggregation to Cl^- channel activation. Low $Fc_\epsilon RI$ density appears to be an unlikely cause, as ~500 receptors are expected in membrane patches with 2 μm^2 area (Fewtrell and Sherman, 1987). The experiments showing Cl^- channel activation after patch excision revealed a 2-fold higher efficiency of Cl^- channel activation (in 43% of RBL cells) than that observed by immunological stimulation. This may suggest that Cl^-

channels actually exist at a higher density and are only partly activated by $Fc_\epsilon RI$ aggregation.

Stimulation of rat peritoneal mast cells has been reported to evoke a delayed Cl^- current which is subject to a complex regulation (Penner *et al.*, 1988; Matthews *et al.*, 1989b). For this delayed Cl^- current a unitary conductance of 1.6 pS has been estimated from noise analysis (Matthews *et al.*, 1989). A Cl^- current with a single channel conductance of 380 pS, probably activated by Ca^{2+}, has also been reported for rat peritoneal mast cells (Lindau and Fernandez, 1986a). The Cl^- current in RBL cells reported here exhibited a unitary conductance of ~32 pS. From the reversal potential of −45 mV we estimate (assuming ideal Cl^- selectivity) an intracellular Cl^- concentration of 30 mM, which agrees with a concentration found for another non-excitable cell (Lyall *et al.*, 1988).

The functional role of the $Fc_\epsilon RI$-mediated Cl^- current in stimulus−secretion coupling was substantiated by the results obtained with the Cl^- channel blocker NPPB. Both the Cl^- current observed in patch-clamp and secretion as monitored by serotonin release were inhibited by NPPB. Moreover, both assays exhibited similar dose−response patterns for the inhibition yielding an $IC_{50} = 52 \pm 21$ μM for Cl^- current and an $IC_{50} = 77 \pm 60$ μM for serotonin release. This strongly suggests that the effect of NPPB on mediator secretion is related to Cl^- current inhibition. Comparable concentrations of NPPB have been reported to block the delayed Cl^- current in peritoneal mast cells (Matthews *et al.*, 1989b) and Cl^- channels in epithelial cells (Wangemann *et al.*, 1986; Dreinhöfer *et al.*, 1988; Kunzelmann *et al.*, 1989).

Further evidence for a linkage between Cl^- current and mediator secretion was provided by the anti-allergic drug cromolyn. Serotonin release of RBL cells is inhibited by cromolyn when present inside the cell (Hemmerich *et al.*, 1991), pointing to an intracellular site of action in these cells. This may relate to our finding that cromolyn applied to the intracellular side of an inside-out patch caused potent inhibition of the Cl^- current. The dose−response relation for cromolyn revealed a decrease in open state probability of Cl^- channels even with a lower IC_{50} (15 ± 2 μM) compared with NPPB. Inhibition of mediator secretion by cromolyn has been attributed to its inhibition of the antigen-induced Ca^{2+} uptake (e.g. Foreman and Garland, 1976), which has also been correlated with effects on protein phosphorylation (Theoharides *et al.*, 1980). Furthermore, cromolyn has been found to exert an inhibitory effect on a Ca^{2+} permeable channel protein (Mazurek *et al.*, 1984), isolated from RBL cells (Corcia *et al.*, 1988; Hemmerich and Pecht, 1988). The Cl^- channel blocking activity of cromolyn reported here may represent a further mechanism for its anti-allergic action.

The parallel inhibition of serotonin secretion and Cl^- current suggest a functional role for the Cl^- current in mediator secretion. How does this relate to other findings? Serotonin release by RBL cells has been found to be strictly dependent on extracellular Ca^{2+} (Beaven *et al.*, 1984b; Fewtrell and Sherman, 1987). Fura-II studies of RBL cells revealed the existence of a sustained Ca^{2+} influx, which is induced upon $Fc_\epsilon RI$ aggregation and apparently is necessary for serotonin release (Beaven *et al.*, 1984b; Fewtrell and Sherman, 1987). This influx should be subjected to the electromotive driving force for Ca^{2+}, since depolarization of RBL cells by 100 mM extracellular K^+ has been found to inhibit serotonin release to a similar extent to that in the absence of Ca^{2+} (Mohr and Fewtrell, 1987a,b). Accordingly, K^+-induced depolarization results in a significant reduction of the sustained Ca^{2+} influx (Mohr and Fewtrell, 1987a). Membrane potential studies of immunologically stimulated RBL cells using the fluorescent dye bis-oxonol have resolved a biphasic behaviour, i.e. an initial depolarization (attributed to the onset of Ca^{2+} influx) followed by a long-lasting repolarization (Mohr and Fewtrell, 1987b; Labrecque *et al.*, 1989). We suggest that this repolarization is caused by $Fc_\epsilon RI$-mediated activation of Cl^- channels, leading to a decay of membrane potential towards more negative values. This would maintain a sufficiently negative membrane potential required in order to sustain Ca^{2+} influx. A similar role has recently been proposed for a delayed Cl^- current in peritoneal mast cells (Penner *et al.*, 1988; Matthews *et al.*, 1989b). The observed slow activation of Cl^- channels, predominantly occuring within 10 min after stimulation, is in accord with the reported time course of repolarization starting at 2 min and reaching a steady state at 6−8 min (Mohr and Fewtrell, 1987b). Furthermore, at depolarized membrane potentials (−30 to 10 mV) the Cl^- channel is found to be almost continuously open, providing maximal efficiency in repolarizing the cell towards the Cl^- equilibrium potential. A low density of activated Cl^- channels is expected to be sufficient for this purpose, in particular as the open channel probability is still high (~60%) at the Cl^- equilibrium potential of −45 mV, which may effectively clamp the membrane potential at this value in order to maintain Ca^{2+} influx. Blockade of Cl^- channel current may lead to sustained cell depolarization and thus to inhibition of serotonin release. Serotonin release was indeed effectively inhibited by the Cl^- channel blocker NPPB. These data suggest that blockade of Cl^- channel activity is sufficient to inhibit antigen-induced mediator secretion. Hence, Cl^- channel blocking activity could represent a new mode of action for anti-allergic drugs.

Materials and methods

Materials

Murine monoclonal, IgE class (2,4-dinitrophenyl)-specific antibodies, secreted by hybridoma cells cells (HI-DNP-26.82) were isolated and purified as described (Liu *et al.*, 1980). Bovine serum albumin carrying on average 11 2,4-dinitrophenyl molecules (DNP_{11}-BSA) was prepared by derivatization of the protein (BSA, Sigma A-4503) with 1-fluoro-2,4-dinitrobenzene (FDNP, Merck) and was used as antigen. The mAb F4 ($OD_{280} = 0.7$), specific for the $Fc_\epsilon RI$, was raised and characterized as described (Ortega *et al.*, 1988). [^{3}H]serotonin ([^{3}H]hydroxytryptamin binoxalate) was purchased from New England Nuclear, Vienna, Austria. NPPB was kindly provided by Professor Dr Greger, Freiburg, FRG and dissolved in DMSO at a concentration of 77.6 mM. Cromolyn (disodium-1,3-bis(2′-carboxylate-chromone-5′-yloxy)-2-hydroxypropane or Intal) was supplied from Fisons Limited Pharmaceutical Division, Loughborough, England. Stock solutions (100 mM) were prepared in bidistilled water. Bovine serum albumin, added to the tyrode buffer and patch pipette solution, was purchased from Behring Marburg, FRG.

Solutions

Tyrode buffer consisted of (mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 glucose, 10 HEPES/Na, 0.1% BSA, pH 7.4. The patch pipette solution contained (mM): 120 choline Cl, 20 $CaCl_2$, 10 HEPES/Na, 0.1% BSA, pH 7.4.

Cell culture

All experiments were performed with the secreting subline (2H3) of RBL cells (Barsumian *et al.*, 1981) maintained in monolayer culture. The cells were grown in stationary flasks, using DMEM supplemented with 10% fetal calf serum, in a 37°C humidified incubator containing 2.3% CO_2.

When confluent (every 3 days), cells were detached by a 3 min exposure to trypsin/EDTA or EDTA alone (both yielded similar results) and used for experiments 1–3 days after subculture. Serotonin release as well as electrophysiological data were obtained from RBL cells of passages 10–50.

Serotonin release assay

Secretion from RBL cells was assayed in triplicate by measurement of released [^{3}H]serotonin (Taurog *et al.*, 1977). In brief, RBL cells were plated in 48 well microtiter plates (10^5 cells/well) and grown overnight to confluency in DMEM 10% FCS supplemented with [^{3}H]serotonin (27 800 Ci/mol, 2 μCi/ml) and monoclonal IgE class antibody specific for DNP (2 μg/ml). Then, adherent cells were washed three times with tyrode, preincubated for 10 min at 35–37°C in the presence and absence of NPPB and then challenged with 5–50 nM DNP_{11}-BSA (in this concentration range maximal serotonin secretion was obtained). Incubation was continued over a period of 30 min at 35–37°C and aliquots (200 μl) of supernatant, taken at the indicated times, were monitored by liquid scintillation counting. Total [^{3}H]serotonin content of the cells was determined following a 30 min lysis of the cells by 1% Triton X-100. All results are presented as % values of total serotonin content. The maximal DMSO concentration employed (0.4%) had no measurable effect on serotonin release and Cl^- channel activity (see below).

Immunological stimulation and drug application

For stimulation either DNP_{11}-BSA (5–50 nM) or mAb F4 (22 nM) was added to the patch pipette solution. For DNP_{11}-BSA stimulation the cells were sensitized with 2 μg/ml IgE for at least 1 h at room temperature, while for mAb F4 unsensitized cells were used. Drugs (NPPB and cromolyn) were applied to cell-attached and inside-out patches by continuous perfusion of the bath chamber (150 μl) with drug-containing tyrode solution at a flow rate of 2 ml/min. For dose–response relationships increasing concentrations of drugs were sequentially perfused.

Electrophysiology

Electrophysiological experiments were carried out at 20–24°C using the patch-clamp technique (Hamil *et al.*, 1981) either in the cell-attached patch or in the inside-out patch recording configuration. A Ag/AgCl electrode immersed into the bath solution was used as reference electrode. Hard glass pipettes (Glass Company of America, Inc., Bargaintown, NJ 08232, USA) with a resistance of 5–10 MΩ were used. The resting membrane potential of unstimulated RBL cells was measured in the current-clamp mode (Hamil *et al.*, 1981) or by the reversal potential of the inward rectifier K^+ channel with 140 mM K^+ in the patch pipette. Single channel events were detected using the L/M-EPC5 amplifier (List-Medical, Darmstadt, FRG), digitized (44 kHz) by a modified pulse-code modulator (PCM-501, Sony, Köln, FRG) and stored on video tape. For off-line analysis these analogue signals were filtered (−3 dB at 500 Hz), digitized with 10 kHz and stored on hard disk. Unitary current amplitudes and open probabilities were determined from amplitude histograms of the digitized current values. Current–voltage relationships were fitted by linear regression, leading to the calculated slope conductances. Open channel probabilities at different voltages and blocker concentrations were determined for 20–30 s time periods of recording. IC_{50} values were estimated from hand-drawn dose–response curves and Hill plot analysis. Means ± SD are presented throughout this study. Single channel currents carried by Cl^- ions flowing from the patch pipette into the cell or the bath are depicted as upward deflections. The sign of the applied potential refers to the potential at the cytosolic side with respect to that in the patch pipette interior. Liquid junction potentials were determined to be less than −3 mV and therefore not corrected.

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References

Barsumian,E.L., Isersky,C., Petrino,M.G. and Siraganian,R.P. (1981) *Eur. J. Immunol*, **11**, 317–323.

Beaven,M.A., Moore,J.P., Smith,G.A., Hesketh,T.R. and Metcalfe,J.C. (1984a) *J. Biol. Chem.*, **259**, 7317–7142.

Beaven,M.A., Rogers,J., Moore,J.P., Hesketh,T.R., Smith,G.A. and Metcalfe,J.C. (1984b) *J. Biol. Chem.*, **259**, 7129–7136.

Benhamou,M., Gutkind,J.S., Robbins,K.C. and Siraganian,R.P. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 5327–5330.

Cockroft,S. (1981) *Trends Pharmacol. Sci.*, **2**, 340–342.

Corcia,A., Pecht,I., Hemmerich,S., Ran,S. and Rivnay,B. (1988) *Biochemistry*, **27**, 7499–7506.

Cox,J.S.G. (1967) *Nature*, **216**, 1328–1329.

Cox,J.S.G., Beach,J.E., Blair,A.M., Clarke,A.J., King,J., Lee,T.B., Loveda,D.E.E., Moss,G.F., Orr,T.S.C., Ritchie,J.T. and Sheard,P. (1970) *Adv. Drug Res.*, **5**, 115–195.

Crews,F.T., Morita,Y., McGivney,A., Hirata,F., Siraganian,R.P. and Axelrod,J. (1981) *Arch. Biochem. Biophys.*, **212**, 561–571.

Dreinhöfer,J., Gögelein,H. and Greger,R. (1988) *Biochim. Biophys. Acta*, **946**, 135–142.

Fewtrell,C. and Sherman,E. (1987) *Biochemistry*, **26**, 6995–7003.

Foreman,F.C. and Garland,L.G. (1976) *Brit. Med. J.*, **1**, 820–821.

Foreman,J.C., Hallet,M.B. and Mongar,J.L. (1977) *Brit. J. Pharmacol.*, **59**, 473P–474P.

Hamil,O.P., Marty,A., Neher,E., Sakmann,B. and Sigworth,F.J. (1981) *Pflüg. Arch.*, **391**, 85–100.

Hemmerich,S. and Pecht,I. (1988) *Biochemistry*, **27**, 7488–7498.

Hemmerich,S., Sijpkens,D. and Pecht,I. (1991) *Biochemistry*, **30**, 1523–1532.

Ishizaka,T. (1981) *J. Cell. Clin. Immunol.*, **67**, 90–96.

Ishizaka,T., Foreman,J.C., Sterk,A.R. and Ishizaka,K. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 5858–5862.

Ishizaka,T. and Ishizaka,K. (1984) In Ishizaka,K. (ed.), *Progress in Allergy*. Karger, Basel, Vol. 34, pp. 188–235.

Jetten,A.M., Yankasak,J.R., Stutts,M.J., Willumsen,N.J. and Boucher,R.C. (1989) *Science*, **244**, 1472–1475.

Kanner,B.I. and Metzger,H. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 5744–5748.

Kuno,M., Okada,T. and Shibata,T. (1989) *Am. J. Physiol.*, **256**, C560–C565.

Kunzelmann,K., Pavenstädt,H. and Greger,R. (1989) *Pflüg. Arch.*, **415**, 172–182.

Labrecque,G.F., Holowka,D. and Baird,B. (1989) *J. Immunol.*, **142**, 236–243.

Lindau,M. and Fernandez,J.M. (1986a) *J. Gen. Physiol.*, **88**, 349–368.

Lindau,M. and Fernandez,J.M. (1986b) *Nature*, **319**, 150–153.

Liu,F.-T., Bohn,J.W., Ferry,E.L., Yamamoto,H., Molinaro,C.A., Sherman,L.A., Klinman,N.R. and Katz,D.H. (1980) *J. Immunol.*, **124**, 2728–2736.

Lyall,V., Croxton,T.L. and Armstrong,W.M. (1988) *Biochim. Biophys. Acta*, **903**, 56–67.

Matthews,G., Neher,E. and Penner,R. (1989a) *J. Physiol.*, **418**, 105–130.

Matthews,G., Neher,E. and Penner,R. (1989b) *J. Physiol.*, **418**, 131–144.

Mazurek,N., Schindler,H., Schürholz,Th. and Pecht,I. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 6841–6845.

McGivney,A., Morita,Y., Crews,F.T., Hirata,F., Axelrod,J. and Siriganian,R.P. (1981) *Arch. Biochem. Biophys.*, **212**, 572–580.

Metzger,H. and Ishizaka,T. (1982) *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, **41**, 7–34.

Mohr,F.C. and Fewtrell,C. (1987a) *J. Cell Biol.*, **104**, 783–792.

Mohr,F.C. and Fewtrell,C. (1987b) *J. Immunol.*, **138**, 1564–1570.

Ortega,E., Schweitzer-Stenner,R. and Pecht,I. (1988) *EMBO J.*, **7**, 4101–4109.

Pearce,F.L., Befus,A.D., Gauldie,J. and Bienenstock,J. (1982) *J. Immunol.*, **128**, 2481–2486.

Penner,R., Matthews,G. and Neher,E. (1988) *Nature*, **334**, 499–504.

Sagi-Eisenberg,R. and Pecht,I. (1983) *J. Membr. Biol.*, **75**, 97–104.

Sagi-Eisenberg,R. and Pecht,I. (1984) *EMBO J.*, **3**, 497–500.

Seldin,D.C., Adelmann,S., Austen,K.F., Stevens,R.L., Hein,A., Caulfield,J.P. and Woodbury,R.G. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 3871–3875.

Sullivan,T.J. and Perker,C.W. (1979) *J. Immunol.*, **122**, 431–436.

Taurog,D., Mendoza,G.R., Hood,W.A., Siraganian,R.P. and Metzger,H. (1977) *J. Immunol.*, **119**, 1757–1761.

Theoharides,T.C., Sieghart,W., Greengard,P. and Douglas,W.W. (1980) *Science*, **207**, 80–82.

Wangemann,P., Wittner,M., DiStefano,A., Englert,H.C., Lang,H.J., Schlotter,E. and Greger,R. (1986) *Pflüg. Arch.*, **407**, S128–S141.

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