

Chloride Channels in Mast Cells: Block by DIDS and Role in Exocytosis

JÜRGEN DIETRICH and MANFRED LINDAU

From the Abteilung Biophysik, Freie Universität Berlin, D-14195 Berlin, Germany

ABSTRACT In rat peritoneal mast cells, we have investigated the influence of the chloride transport blocker 4,4'-diisothiocyanato-stilbene-2,2'-disulfonic acid (DIDS) and the extracellular chloride concentration on the chloride current induced by intracellular application of cyclic AMP (cAMP) and on hexosaminidase secretion from intact cells stimulated with compound 48/80. The inhibition of the Cl^- current by extracellular DIDS is voltage and time dependent. Upon depolarization from -10 to $+70$ mV, the outward current diminishes with millisecond kinetics. The size of the steady state current and the time constant of the decrease both decrease with increasing DIDS concentrations. The steady state current at $+70$ mV is blocked by DIDS with an IC_{50} of $2.3 \mu\text{M}$. The number of open channels at -10 mV is reduced with an IC_{50} of $22 \mu\text{M}$. The electrophysiological and pharmacological properties of this current are most similar to those of the Cl^- current in T lymphocytes activated by osmotic stress (Lewis, R. S., P. E. Ross, and M. D. Cahalan. 1993. *Journal of General Physiology*. 101:801–826). Extracellular DIDS also inhibits exocytosis. At optimal stimulation with $10 \mu\text{g/ml}$ compound 48/80 secretion is inhibited with an $\text{IC}_{50} = 50 \mu\text{M}$ and a Hill coefficient $n = 10$. At half optimal stimulation with $1 \mu\text{g/ml}$ inhibition occurs with an $\text{IC}_{50} = 10 \mu\text{M}$ and $n = 1$. Substitution of extracellular chloride by glutamate has only very small effects on secretion stimulated with $10 \mu\text{g/ml}$ compound 48/80. We conclude that activation of the chloride current in mast cells is not essential for stimulation of exocytosis but may enhance secretion at suboptimal stimulation. Alternatively, the channel may play a role in volume regulation following degranulation.

INTRODUCTION

In mast cells, exocytosis is stimulated via activation of GTP-binding proteins (for review see Lindau and Gomperts, 1991). Although large calcium transients occur in

J. Dietrich's present address is Zentrale Biologische Forschung, Schering AG, Sellerstrasse 31, D-13342 Berlin, Germany.

Dr. Lindau's present address is Abteilung Molekulare Zellforschung, Max-Planck-Institut für Medizinische Forschung, Jahnstrasse 29, D-69120 Heidelberg, Germany.

Address correspondence to Dr. Manfred Lindau, Abteilung Molekulare Zellforschung, Max-Planck-Institut für medizinische Forschung, Jahnstrasse 29, D-69120 Heidelberg, Germany.

response to stimulation by extracellular application of compound 48/80 (Almers and Neher, 1985) or intracellular application of GTP γ S (Neher, 1988), the significance of the calcium change for activation of secretion has been questioned because granule fusion occurs at resting and even lower intracellular calcium concentrations (Neher and Almers, 1986; Lindau and Nüße, 1987; Neher, 1988). Negative membrane potentials drive a calcium influx across the plasma membrane (Penner, Matthews, and Neher, 1988; Matthews, Neher, and Penner, 1989a). The resting potential rat peritoneal mast cells, however, is unstable as measured in the permeabilized patch configuration using ATP in the pipette and fluctuates between 0 and -30 mV (Lindau and Fernandez, 1986). An inward Ca $^{2+}$ current would thus easily depolarize the cell. Activation of the Cl $^{-}$ channels would clamp the membrane potential at E_{Cl} . Although the cytoplasmic [Cl $^{-}$] in mast cells is not exactly known, E_{Cl} may be expected to be ~ -40 mV, thus, supporting Ca $^{2+}$ influx driven by hyperpolarized membrane potentials (Penner et al., 1988; Matthews, Neher, and Penner, 1989b). However, when mast cells are stimulated with compound 48/80 or substance P, the development of the Cl $^{-}$ current (Penner et al., 1988; Matthews et al., 1989b) is much slower than the exocytotic response (Fernandez, Lindau, and Eckstein, 1987) suggesting that the Cl $^{-}$ permeability may not be essential for exocytosis.

Stilbene disulfonic acid anion channel blockers have been reported to inhibit exocytosis of platelets (Pollard, Tack-Goldman, Pazoles, Creutz, and Shulman, 1977) and neutrophils (Korchak, Eisenstat, Hoffstein, Dunham, and Weissmann, 1980). However, it was suggested that these compounds may interfere with the agonist rather than the exocytotic response in the case of thrombin stimulation of platelets (Vostal, Reid, Jones, and Shulman, 1989). In neutrophils, the effects of DIDS and 4-acetamido-4'-isothiocyano-2,2'-disulfonic acid (SITS) are not limited to the anion channel (Korchak, Eisenstat, Smolen, Rutherford, Dunham, and Weissmann, 1982) and they may inhibit the activity of the secreted product β -glucuronidase, rather than inhibiting exocytosis by an effect on Cl $^{-}$ channels (Vostal et al., 1989). We have compared the influence of the Cl $^{-}$ channel blocker DIDS and the extracellular Cl $^{-}$ concentration on the Cl $^{-}$ current and exocytosis in intact mast cells. The results indicate that, as in platelets, the inhibitory effect of DIDS on secretion is mostly not due to Cl $^{-}$ channel block.

MATERIALS AND METHODS

Solutions

The compositions of the solutions used for cell preparation and for the experiments are listed in Table I. The variable concentration of glucose in the external saline (ES) for patch clamp experiments was used to adjust the osmolality to 290 mosm. The pipette solution was supplemented with variable amounts of NaCl, MgCl $_2$, EGTA, CaCl $_2$, GTP, ATP, cAMP, or GTP γ S as described in the text. In most experiments we used 20 mM NaCl, 300 μ M GTP, 200 μ M EGTA and 50 μ M cAMP.

Cell Preparation

Peritoneal mast cells were obtained from Sprague Dawley rats by peritoneal lavage with isotonic NaCl containing 1 mg/ml BSA. For purification, cells were centrifuged at 220 g, resuspended in HEPES buffer, layered on top of an isotonic percoll layer and centrifuged for 15 min at 400 g. The pellet was washed twice with HEPES buffer and the cells were stored at room temperature. The resulting cell suspension had a purity > 85% and a yield of $0.4\text{--}1.1 \times 10^6$ cells.

TABLE I
Composition of Solutions. K-glu Stands for Potassium-L-Glutamate

	HEPES buffer	ES buffer	Pipette solution
NaCl	137 mM	140 mM	see text
KCl	2.7 mM	5 mM	—
CaCl ₂	1.8 mM	2 mM	see text
MgCl ₂	1 mM	1 mM	see text
K-glut	—	—	125 mM
HEPES	20 mM	10 mM	10 mM
glucose	5.6 mM	15–20 mM	—
BSA	1 mg/ml	—	—
pH	7.2–7.4	7.2–7.4	7.3–7.4

Patch Clamp Experiments

100–200 μ l of a suspension of nonpurified peritoneal cells or of purified mast cells were placed in a recording chamber. After a few minutes, allowing the cells to settle on the glass bottom, the dish was perfused with ES buffer to remove floating cells. Recordings were made at room temperature in the patch clamp whole-cell configuration using an EPC-7 patch clamp amplifier (List Electronics, Darmstadt, Germany) in the voltage clamp mode without capacitance compensation. The gain was usually set to 2 mV/pA. The command input was controlled by a PDP 11/73 via a D/A converter. The current monitor output was filtered at 29 kHz using an 8-pole Bessel filter (LPF 902B, Frequency Devices, Haverhill, MA). The internal filters of the EPC-7 were switched off. In a typical experiment, the holding potential was -10 mV and a sequence of 10 pulses was repeatedly given. All potentials are corrected for the liquid junction potential which develops at the pipette tip when the pipette is immersed into the bath (Fenwick, Marty, and Neher, 1982) as previously described for the solutions used here (Lindau and Fernandez, 1986). First, a 1.4-ms pulse with -20 mV amplitude was given. The current was sampled (12-bit A/D converter, DT2782A, Data Translation, Marlboro, MA) with a dwell time of 7 μ s before, during, and after the pulse. 256 data points were stored on disk for off-line determination of the cell capacitance (Lindau and Neher, 1988). Subsequently, 200-ms pulses were given to test potentials between -90 and $+70$ mV in 20-mV intervals. For each pulse, the current was sampled with a dwell time of 25 μ s. A total of 10,240 data points was sampled with 1,280 points before the pulse, 8,000 points during the pulse and the remaining 960 points after repolarization. 40 subsequent current values were added and divided by 4 reducing the amount of data to 256 points per pulse (1-ms averages) with reduced noise and improved resolution. These data were also stored on disk for later analysis. A complete sequence required 9.1 s and was continuously repeated. The interval between individual pulses was ~ 1 s. To illustrate the time course of the currents during individual 200 ms pulses the data point containing the capacitive transient was set equal to the preceding point. External solutions were exchanged by bath perfusion.

Secretion Measurements

All experiments were done in HEPES or ES buffer. After incubating the cells at 37°C for 5 min, 25 μ l of the cell suspension was added to 25 μ l of buffer containing DIDS at twice the final concentration. After 2 min, 50 μ l of buffer containing the final DIDS concentration and the appropriate amount of the stimulator compound 48/80, was added. After 10 min at 37°C, the samples were cooled on ice and cells were pelleted at 3,000 rpm at 4°C. 50 μ l of the supernatant were transferred to 5-ml tubes and 50 μ l of buffer containing a 4-methyl-umbelliferyl β -glucosaminidase substrate were added and the mixture was kept at 37°C for 30–120 min. The hexosaminidase reaction was quenched by adding 1 ml of ice-cold carbonate buffer (pH 10). To determine a possible direct effect of DIDS on the β -glucosaminidase activity DIDS was added 2 min after, rather than before stimulation. At this time, the secretory response to compound 48/80 is complete. A similar protocol was used to determine the effects of anion substitution in the buffer.

Fluorescence of the reaction product 4-methyl-umbelliferone (4-MU) was measured in a fluorescence spectrometer (Shimadzu RF 540) (excitation, 360 nm; emission, 448 nm). DIDS exhibits strong fluorescence in the same spectral range. After 30 min of the enzyme reaction the DIDS fluorescence was comparable to the 4-MU fluorescence. Recording of the fluorescence time course after opening of the excitation light beam revealed time-dependent bleaching of DIDS in the fluorimeter which had to be accounted for. When the time for the enzyme reaction was prolonged to 2 h, an irreversible decay of the DIDS fluorescence was observed which, together with the increased amount of the reaction product, reduced the DIDS fluorescence to less than 7% of the 4-MU fluorescence.

Curve Fitting

The concentration dependence of the DIDS inhibition was analyzed using the Hill equation $Y([\text{DIDS}]) = Y(0) \cdot \{1 - I_{\text{max}}[\text{DIDS}]^n / ([\text{DIDS}]^n + \text{IC}_{50}^n)\}$. For the secretion experiments the IC_{50} , the Hill coefficient n and the maximal inhibition I_{max} were determined by a nonlinear least squares fit. For the inhibition of the chloride current only IC_{50} and n were used as free parameters. Small leak currents were neglected assuming that the maximal block at high DIDS concentrations was complete ($I_{\text{max}} = 1$).

RESULTS

cAMP-activated Cl^- Current

Intracellular dialysis of a mast cell with a pipette solution containing 50 μ M cAMP induced the slow development of an outward rectifying current as previously described (Penner et al., 1988; Matthews et al., 1989a,b) (Fig. 1). Fig. 1A shows the currents during 200 ms pulses given from a holding potential of -10 mV to test potentials between -90 and $+70$ mV in 20-mV intervals. To follow the time course of membrane conductance, the pulse sequence was continuously repeated. A complete cycle was acquired every 9.1 s. In Fig. 1B, the mean currents during each pulse are plotted as a function of time and the points measured at a particular potential were connected by straight lines. When external $[\text{Cl}^-]$ was reduced from 151 to 11 mM by exchange for L-glutamate, the outward currents were markedly reduced. The anion substitution effect is reversible. The outward rectification is illustrated in the I/V relationship for normal extracellular $[\text{Cl}^-]$ (Fig. 1C, open circles). At low extracellular $[\text{Cl}^-]$, the I/V relationship became almost linear and the reversal potential was shifted to ~ 0 mV (Fig. 1C, filled circles).

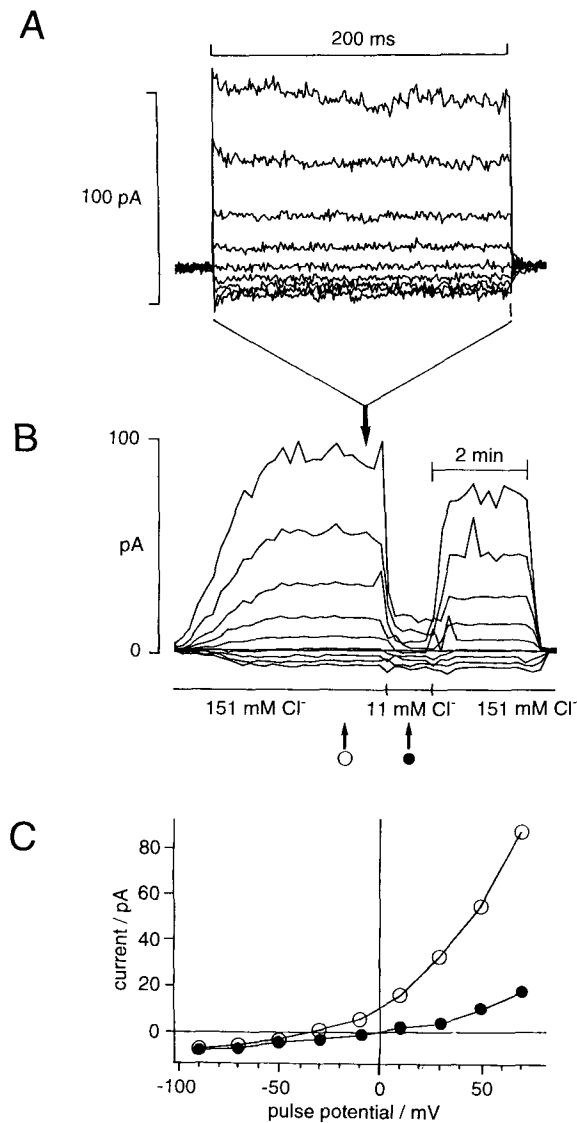


FIGURE 1. Development of the Cl^- current in response to intracellular application of $50 \mu\text{M}$ cAMP. (A) The currents during 200-ms pulses to nine different potentials between -90 and $+70$ mV in 20-mV intervals are time independent. (B) The time course of the mean current at the different potentials. The external solution was transiently exchanged for a solution containing 11 mM Cl^- as indicated. At the end of the experiment, an outside-out patch was pulled to check the pipette-membrane seal (*horizontal bar*). (C) The voltage dependence of the mean currents during individual pulses at normal (\circ) and low (\bullet) external Cl^- measured at the times indicated by the arrows in B. In this experiment the internal Cl^- concentration was 20 mM.

The observed reversal potential shift indicates Cl^- selectivity and for a Cl^- channel a reduction of the outward currents would be expected as a result of the reduced $[\text{Cl}^-]$. Alternatively, the channel could be completely blocked by extracellular glutamate and the remaining current could be due to a nonspecific leak at the pipette-membrane seal which would shift the reversal potential to 0 mV independent of channel selectivity. A leak at the seal persists when an outside-out patch is pulled. However, when this was done at the end of the experiment (Fig. 1 B, *horizontal bar*), the remaining conductance markedly decreased (to 20 pS). The reversal potentials measured here are, thus, not distorted by leak conductances, confirming

Cl⁻ selectivity. The shift is, however, less than the 65 mV expected for perfect a Cl⁻ channel, indicating poor Cl⁻ selectivity and a significant glutamate permeability.

The amplitude of the Cl⁻ current induced by 50 μM cAMP was not affected by including GTP (0.3 mM) or MgATP (1 mM) in the pipette. However, in the presence of 23–25 mM internal Na⁺ the current amplitude was 230 ± 50 pA (SEM, *n* = 22), which is ~5 times larger than with 5–15 mM Na⁺ (48 ± 16 pA, *n* = 18). Variation of intracellular Cl⁻ between 11 and 34 mM had no detectable effect on current activation. With 0.2 or 1 mM intracellular ATP the development of the current was much slower (0.44 ± 0.26 pA/s) than without ATP (3.5 ± 1.5 pA/s). These results indicate that current activation is not mediated by activation of protein kinase A.

Voltage-dependent Block by DIDS

It was previously reported that the Cl⁻ current in mast cells is blocked by DIDS (Matthews et al., 1989b). After step depolarization from -10 mV holding potential in the presence of DIDS in the extracellular saline, the outward currents declined with time at positive potentials (Fig. 2 A). At +70 mV, the half time for the decrease was 8 ms with 30 μM DIDS and 40 ms with 3 μM DIDS. In the presence of DIDS, small tail currents are observed upon repolarization. These tail currents depend on the pulse potential and apparently reflect the kinetics of binding and dissociation of DIDS at the holding potential.

The DIDS block of the outward currents is further illustrated in Fig. 2 (B and C). The cell was first perfused with 11 mM Cl⁻ to identify the current as the Cl⁻ current. The cell was then exposed to increasing DIDS concentrations. The final and initial currents at the end (*I_F*) and onset (*I_I*) of the 200 ms pulses are plotted as a function of time where the pulse was given. The final currents are reduced by about half in the presence of 3 μM DIDS and are virtually abolished at 30 μM DIDS. After a 2-min exposure to high concentrations of DIDS the inhibition became partly irreversible (Fig. 2 B, 30 μM).

Fig. 2 indicates that the DIDS block is voltage dependent. For comparison with the effect of DIDS on secretion from intact cells, the block at physiological membrane potentials must be determined. The membrane potential of mast cells shows slow fluctuations between 0 and -30 mV (Lindau and Fernandez, 1986) and activation of the Cl⁻ current sets the membrane potential to the reversal potential of this current (Matthews et al., 1989b). However, the Cl⁻ currents are extremely small between 0 and -30 mV and the DIDS sensitivity cannot be accurately determined from the steady state currents at these potentials. As shown in Fig. 2 A the currents in the presence of DIDS show time-dependent inactivation. The instantaneous current *I_I* after depolarization from -10 to +70 mV is thus determined by the number of unblocked channels at the holding potential of -10 mV. As shown in Fig. 2 C, a 50% block of *I_I* is observed at ~30 μM DIDS which is 10 times higher than the IC₅₀ of *I_F*.

Fig. 2 D plots the relative size of *I_F* (filled circles) and *I_I* (open circles) at +70 mV against the DIDS concentration. The data were fitted with the Hill equation (continuous lines). For *I_F* we obtained 50% inhibition at IC₅₀ = 2.3 ± 0.3 μM and a Hill coefficient *n* = 0.7 ± 0.1. For *I_I* the IC₅₀ is shifted by a factor of 10–22 ± 3 μM with *n* = 0.7 ± 0.1. DIDS also blocks the Cl⁻ current at -10 mV, but ~10-fold higher concentrations are required as compared to the block at +70 mV. The Hill

coefficients may be underestimated because small leak currents would artificially increase the currents at high DIDS concentrations and would flatten the dose-response curve. For comparison, the irreversible block after 50–150 s exposure to different DIDS concentrations is also shown (x).

Because the Hill coefficient for the DIDS block is close to 1 for the steady state current at +70 mV as well as for the number of open channels at -10 mV, the simplest model for the DIDS block would be

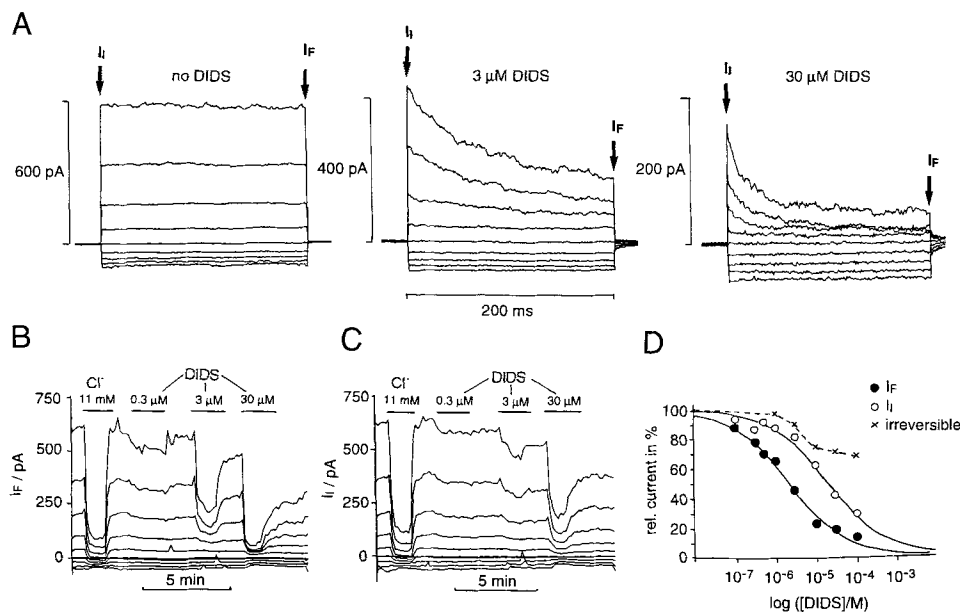


FIGURE 2. (A) The currents during 200-ms pulses in the absence and presence of extracellular DIDS at two different concentrations. The DIDS block is time and voltage dependent. (B) The currents at the end of 200-ms pulses (I_F). (C) The currents at the onset of 200-ms pulses (I_I). (D) Dependence of the Cl⁻ currents on the DIDS concentration (I_I ○, I_F ●, irreversible inhibition x). The continuous lines are fits of the Hill equation.

where O and B denote an open and a blocked channel. The forward rate constant k^+ depends on the DIDS concentration and on the membrane potential V : $k^+ = k^0(V) \cdot [\text{DIDS}]$. The backward rate constant should be independent of $[\text{DIDS}]$ ($k^- = k^-(V)$). When the potential is changed from -10 to +70 mV, the new steady state is obtained with a rate constant $k([\text{DIDS}]) = k^+([\text{DIDS}]) + k^-$. The individual rate constants k^+ and k^- at a single DIDS concentration can be calculated from the measured rate constant of the block ($k = \ln 2 / t_{1/2}$) and the steady state block according to $k^+ = kn_B / (n_B + n_O)$ and $k^- = kn_O / (n_B + n_O)$, where n_O and n_B denote the numbers of open and blocked channels. From the fitted I_F curve of Fig. 2 D, we estimate that 55% of the channels are blocked at 3 μM DIDS and ~85% at 30 μM.

With $t_{1/2} = 40$ ms, we obtain $k^+ = 10 \text{ s}^{-1}$ and $k^- = 8 \text{ s}^{-1}$ at $3 \text{ } \mu\text{M}$ DIDS. At $30 \text{ } \mu\text{M}$ DIDS with $t_{1/2} = 8$ ms the corresponding values are $k^+ = 75 \text{ s}^{-1}$ and $k^- = 13 \text{ s}^{-1}$. The off rate constants are thus similar at the two concentrations, whereas the on rate constant is nearly 10-fold higher at $30 \text{ } \mu\text{M}$. The simple reaction scheme used here thus agrees quite well with the measured properties of the block. For the single channels, the above rate constants correspond to a mean blocked time of ~ 100 ms and a mean open time of 100 ms at $3 \text{ } \mu\text{M}$ DIDS and ~ 13 ms at $30 \text{ } \mu\text{M}$.

When DIDS is added, the decrease of the current is accompanied by an increase of the variance (Fig. 2A). This indicates that without DIDS, the channels are indeed open most of the time and the channels may close only in the presence of the blocker. For high [DIDS], the open probability is small and from the variance measured by ensemble averaging, we estimate the single-channel current (Anderson and Stevens, 1973) to be $i_0 = 0.2 \pm 0.05$ pA. The voltage dependence of the block correlates with the negative charge of DIDS and suggests that the DIDS binding site is inside the channel. However, we cannot exclude the possibility that the blocking site becomes more accessible at positive membrane potentials.

DIDS Blocks Secretion Stimulated with Compound 48/80

If the cAMP activated Cl^- current plays a role in mediating stimulus secretion coupling in mast cells, then the anion channel blocker DIDS should inhibit exocytosis. GTP γ S stimulates both, exocytosis (Fernandez, Neher, and Gomperts, 1984) and activation of the Cl^- current (Penner et al., 1988; Matthews et al., 1989b). By capacitance measurements, we found that $200 \text{ } \mu\text{M}$ DIDS has no detectable inhibitory effect on GTP γ S-stimulated degranulation as expected for a voltage clamp experiment with an intracellular Cl^- concentration determined by the pipette solution. Furthermore, GTP γ S may activate exocytosis at a very late stage bypassing other second messenger-mediated events (Lindau and Gomperts, 1991). We have thus measured release of glucosaminidase from intact cells stimulated with compound 48/80. Fig. 3 shows the dose response curves obtained with two different concentrations of compound 48/80 inducing maximal ($10 \text{ } \mu\text{g/ml}$) or half maximal ($1 \text{ } \mu\text{g/ml}$) release. At $1 \text{ } \mu\text{g/ml}$, the fit of the Hill equation yields an IC_{50} of $9.7 \text{ } \mu\text{M}$ and a Hill coefficient of 1.1 ± 0.3 . At $10 \text{ } \mu\text{g/ml}$ inhibition requires much higher concentrations ($\text{IC}_{50} = 51 \text{ } \mu\text{M}$, Hill coefficient = 10). It is possible that DIDS reacts with compound 48/80 or the target of the stimulus since in the presence of $50 \text{ } \mu\text{M}$ DIDS, the 48/80 dose response curve was shifted to ~ 10 -fold higher concentrations (data not shown). A direct inhibitory effect of DIDS on the glucosaminidase activity was observed only above $200 \text{ } \mu\text{M}$ DIDS (data not shown).

Cl^- Dependence of 48/80-induced Secretion

To investigate the effect of the external Cl^- concentration we have replaced Cl^- by L-glutamate, or acetate (Fig. 4). The effect on glucosaminidase activity in the supernatant after stimulation with $10 \text{ } \mu\text{g/ml}$ 48/80 is shown in Fig. 4A. Since the anion substitution may affect the exocytotic response as well as the enzyme activity, we also measured the effect of anion substitution on enzyme activity alone by changing the anion concentration after release was complete. The strong inhibition in the presence of acetate is due to a direct effect on the glucosaminidase activity as

shown in Fig. 4 *B*. The inhibitory effect of L-glutamate substitution on secretion, evaluated as the difference between the corresponding results of Fig. 4 *A* and *B*, is shown in Fig. 4 *C*. There is a gradual decrease of secretion reaching ~60% of the control level at 16 mM external Cl⁻. Similar results were obtained using D-glutamate indicating that the observed effects are due to a reduction in Cl⁻ rather than a specific effect of L-glutamate.

DISCUSSION

Activation of the Cl⁻ Current

Intracellular application of cAMP induces an outward rectifying Cl⁻ current in the plasma membrane (Penner et al., 1988; Matthews et al., 1989*b*). The slow activation indicates that cAMP does not directly activate the channel. This current has several similarities with the cAMP-dependent Cl⁻ conductance in cardiac myocytes (Ackerman and Clapham, 1993). The cardiac Cl⁻ current has a similar voltage dependence when similar solutions are used and is also inhibited by low concentrations of stilbene

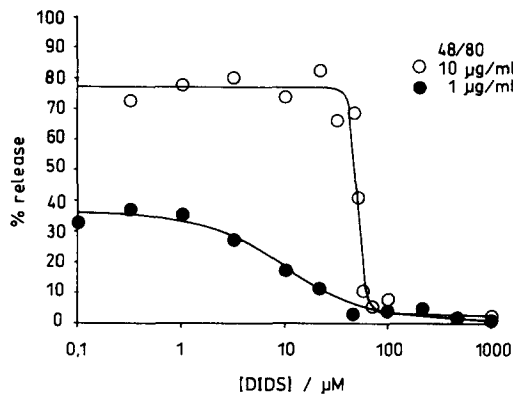


FIGURE 3. Dose-response curves for DIDS inhibition of secretion stimulated by compound 48/80 (○, 10 $\mu\text{g/ml}$; ●, 1 $\mu\text{g/ml}$) together with the fits of the Hill equation (continuous lines).

blockers (Bahinsky, Nairn, Greengard, and Gadsby, 1989). Furthermore, the cardiac current is sensitive to intracellular Na⁺ (Matsuoka, Ehara, and Nomaet, 1990; Harvey, Jurevicius, and Hume, 1991) which fits with our observation that the mast cell Cl⁻ currents are much larger in the presence of ~25 mM Na⁺ than with 5–15 mM Na⁺. It remains to be determined if this is an effect on the activation process or on the channel itself. There is, however, one major difference: the cAMP activation of the heart channel is apparently mediated by activation of protein kinase A (PKA) (Bahinsky et al., 1989) whereas in mast cells, we found that the current amplitude is independent of ATP and the activation is even slower in the presence of ATP, indicating that current activation is not mediated by a phosphorylation through PKA.

By fluctuation analysis we estimate an apparent single-channel current of ~0.2 pA at +70 mV, which agrees with the previous estimate of 0.13 ± 0.06 pA at +40 mV (Matthews et al., 1989*b*). These values are certainly smaller, but still comparable to those of the cardiac cAMP-activated Cl⁻ channel. A low conductance cAMP-activated Cl⁻ channel has been described in epithelial cells, which is defective in cystic fibrosis,

but this channel is insensitive to DIDS (Tabcharani, Low, Elie, and Hanrahan, 1990; Egan, Flotte, Afione, Solow, Zeitlin, Carter, and Guggino, 1992; Cunningham, Worrell, Benos, and Frizzell, 1992) and only weakly affected by the extracellular Cl^- concentration (Cunningham et al., 1992). It also requires the presence of PKA and ATP to be active. The outwardly rectifying Cl^- channel which is also defective in cystic

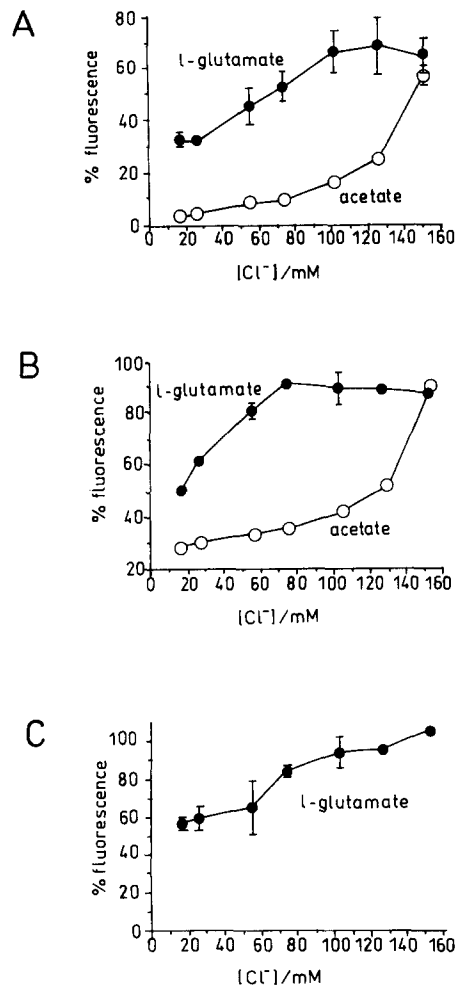


FIGURE 4. (A) Glucosaminidase activity in the supernatant obtained by stimulation with $10 \mu\text{g}/\text{ml}$ compound 48/80 when chloride is replaced by acetate (\circ) or L-glutamate (\bullet). (B) Effect of anion substitution on the enzyme activity itself. (C) Effect of Cl^- substitution by L-glutamate on secretion.

fibrosis is DIDS sensitive, but it has a much larger conductance. Furthermore, the block by 4,4'-dinitrostilbene-2-2'-disulphonic acid (DNDS) is not voltage dependent and the DIDS block is irreversible (Bridges, Worrell, Frizzell, and Benos, 1989). A low conductance, Cl^- channel similar to that in mast cells has recently been described in chromaffin cells (Doroshenko, Penner, and Neher, 1991). This channel is not activated by cAMP, but via GTP-binding protein-mediated activation of phospholipase A_2 and generation of arachidonic acid metabolites (Doroshenko, 1991). It is also blocked by micromolar concentrations of DIDS. A channel which has a similar unitary

conductance and which is blocked by DIDS in a similar voltage and time dependent manner was recently observed in T lymphocytes (Lewis, Ross, and Cahalan, 1993). The electrophysiological properties of this channels appear to be virtually identical to those of the mast cell channel described here. It also has a substantial permeability for organic anions like aspartate or gluconate and activates slowly on a time scale of minutes (Lewis et al., 1993). However, the Cl⁻ conductance in lymphocytes is activated by osmotic stress and requires intracellular ATP. It is possible that in this case ATP is required for the generation of cAMP in response to the osmotic change. It appears that the low conductance Cl⁻ channel is expressed in a wide variety of cell types, but the mechanisms for its activation may be different depending on the cell type.

DIDS Block of Secretion

An inhibitory effect of DIDS on exocytosis has been described for lysosomal enzyme secretion from neutrophils (Korchak et al., 1980; Korchak et al., 1982), but it has also been suggested that the apparent inhibition may not be restricted to an effect on anion channels (Korchak et al., 1982) and may simply be due to inhibition of β -glucuronidase activity by DIDS (Vostal et al., 1989). We found that in mast cells an inhibitory effect on the activity of the measured product β -glucosaminidase is observed above 200 μ M DIDS. At lower concentrations, DIDS also has an inhibitory effect on secretion. With 1 μ g/ml 48/80 which induced half maximal release, \sim 10 μ M DIDS were required for 50% inhibition with a Hill coefficient of $n = 1.1$. These values are close to those obtained for the Cl⁻ channel block at -10 mV ($IC_{50} = 22$ μ M, $n = 0.7$). In contrast, with 10 μ g/ml 48/80 inducing maximal release, \sim 50 μ M DIDS is required for 50% inhibition and the concentration dependence is very steep. The large Hill coefficient ($n = 10$) indicates that the inhibitory effect of DIDS under these conditions may be due to a completely different mechanism. The fact that the inhibition is relieved by elevating the agonist concentrations would be in agreement with an interference of the anionic DIDS with the cationic stimulator. The similarity of the concentration dependence for inhibition of exocytosis and inhibition of the Cl⁻ current at -10 mV, however, suggests that at low agonist concentration the activation of the Cl⁻ current may be required for a full response, whereas at high agonist concentrations this current is not required to stimulate complete degranulation.

Cl⁻ Dependence of Secretion

To overcome the difficulty of nonspecific effects of DIDS we did anion substitution experiments. Replacing most of the extracellular Cl⁻ by glutamate reduces the outward current and shifts the reversal potential close to zero. Under these conditions, activation of the Cl⁻ conductance will induce neither a significant Cl⁻ influx nor a hyperpolarization of the membrane potential. Cl⁻ substitution by L-glutamate has a relatively small inhibitory effect on 48/80 induced secretion demonstrating that exocytosis occurs in the absence of Cl⁻ influx and membrane hyperpolarization.

When exocytosis is stimulated in permeabilized mast cells with GTP γ S and Ca²⁺, replacement of most of the Cl⁻ by glutamate results in \sim 30% inhibition of secretion (Churcher and Gomperts, 1990), comparable to the effect which we observe on intact

cells. The Cl^- replacement in the external solution will probably induce also a reduction of cytoplasmic $[\text{Cl}^-]$. The inhibitory effect of Cl^- substitution described here could thus also be explained by a reduction of intracellular $[\text{Cl}^-]$ and does not necessarily indicate an enhancement of secretion by Cl^- channel activation.

We conclude that the cAMP-activated Cl^- current in mast cells is not a necessary event in stimulus-secretion coupling but its activation could possibly enhance secretion at suboptimal stimulation. Since a Cl^- channel with similar electrophysiological and pharmacological properties is activated by osmotic stress in lymphocytes, it is tempting to speculate that the Cl^- channel in mast cells may alternatively play a role in volume regulation. After exocytosis and the associated increase of plasma membrane area, mechanisms for volume regulation may indeed be required. The activation of a current involved in volume regulation may thus be needed during the degranulation process without the development of significant membrane stress. Accordingly different activation mechanisms might be required for the Cl^- channel in different cell types depending on the physiological conditions where volume regulation is necessary.

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