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CHLORIDE CONDUCTANCE ACTIVATED BY EXTERNAL AGONISTS AND INTERNAL MESSENGERS IN RAT PERITONEAL MAST CELLS

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SUMMARY

1. Stimulation of mast cells by externally applied secretagogues activated a slowly developing membrane current. With high external and low internal chloride (Cl^-) concentrations, the current reversed at about −40 mV, but when external Cl^- was made equal to internal Cl^-, the reversal potential shifted to about 0 mV, demonstrating that the current carrier was Cl^-.

2. In addition to external agonists, internally applied cyclic AMP and high concentrations of intracellular calcium ($[Ca^{2+}]_i$) could also activate the Cl^- current. However, elevated $[Ca^{2+}]_i$ produced only slow and incomplete activation. This suggests that the Cl^- current is not directly Ca^{2+} activated. Also, activation of Cl^- current by external agonists and by cyclic AMP was unimpaired when $[Ca^{2+}]_i$ was clamped to low levels with internal ethylene glycol bis-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), indicating that elevated $[Ca^{2+}]_i$ is not necessary for activation of the Cl^- current. Although activation by cyclic AMP was faster than that produced by elevated $[Ca^{2+}]_i$, it still required tens of seconds; thus the effect of cyclic AMP was also likely to be indirect.

3. Internal guanosine 5′-*O*-(3-thiotriphosphate) (GTP-γ-S) could also activate the Cl^- current, suggesting the involvement of a G protein in the control of the current.

4. The variance associated with the Cl^- current was small, and noise analysis gave a lower limit of about 1–2 pS for the single-channel conductance. The Cl^- current was reduced by 4,4′-diisothiocyano-2,2′-stilbenedisulphonate (DIDS), and during DIDS blockade, the variance of the current increased. This suggests that DIDS enters and blocks the open channel.

5. Activation of the Cl^- current would make the membrane potential negative following stimulation of a mast cell, thus providing a driving force for entry of external calcium via the stimulation-induced influx pathways described in the preceding paper (Matthews, Neher & Penner, 1989).

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INTRODUCTION

In excitable cells, influx of external calcium is ordinarily coupled to membrane depolarization, which is required to open the voltage-operated channels through which calcium enters the cell. In some non-excitable cells, however, it has been recognized that depolarization is inhibitory to calcium influx (see Oettgen, Terhorst, Cantley & Rosoff, 1985; Sage & Rink, 1986; Di Virgilio, Lew, Andersson & Pozzan, 1987; Mohr & Fewtrell, 1987; Penner, Matthews & Neher, 1988). Indeed, in the preceding paper (Matthews *et al.* 1989), we showed that agonist-stimulated calcium influx in mast cells increases with membrane hyperpolarization rather than depolarization. This hyperpolarization-driven Ca^{2+} influx could be mimicked by internally applied inositol 1,4,5-trisphosphate ($Ins1,4,5P_3$), suggesting that $Ins1,4,5P_3$ released inside the cell by agonist stimulation activates a pathway through which Ca^{2+} can enter the cell at a rate governed by the electrochemical gradient for Ca^{2+} across the membrane.

For this mechanism to support Ca^{2+} influx in intact cells, a means is required to keep membrane potential sufficiently negative to drive influx. In this paper, we describe a chloride current that is also activated in mast cells by agonist stimulation and by internal cyclic AMP and calcium. This chloride conductance is much larger than other conductances in mast cells and should therefore determine the post-stimulation membrane potential. The reversal potential of the Cl^- current under our experimental conditions is about -40 mV and when this large conductance is activated, it could hyperpolarize the cell sufficiently to support increases in $[Ca^{2+}]_i$. This agonist-activated Cl^- current may thus provide the electrical driving force for $Ins1,4,5P_3$-induced calcium influx following stimulation in mast cells.

METHODS

Recordings were made from rat peritoneal mast cells prepared as described in the preceding paper. Membrane currents were measured using the whole-cell patch-clamp technique. Intracellular calcium concentration was monitored using Fura-2, loaded into cells via the patch pipette.

In experiments on the effect of external chloride concentration on membrane currents, the following low-Cl^- Ringer solution was used (mM): sodium glutamate, 140; potassium glutamate, 2·5; $CaCl_2$, 2; $MgCl_2$, 5; glucose, 5; HEPES–NaOH, 10; pH = 7·2. Details of the recording procedures and intracellular and extracellular solutions are given in Matthews *et al.* (1989). Cyclic AMP and 8-bromo cyclic AMP were purchased from Boehringer (Mannheim) and 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB) was kindly provided by Professor R. Greger (Freiburg, FRG). Solution changes were made by exchanging the bath solution. In experiments in which external chloride was manipulated, the Ag–AgCl reference electrode was connected to the bath via a 1 M-KCl agar bridge. Nevertheless, control measurements showed that during short solution changes to low chloride solution a junction potential developed between bridge and bath, which was taken into account in the reported values of membrane holding potential.

RESULTS

Agonist stimulation activates a chloride current

The effect of the secretagogue compound 48/80 on membrane current in a mast cell is shown in Fig. 1*A*. When membrane voltage was stepped to +40 mV, there was only a small outward membrane current before stimulation, but after stimulation, a large outward current slowly developed. At 0 mV, the slowly developing current was

smaller but still outward. At −40 mV, however, there was little change in current following stimulation, suggesting that this agonist-activated current has a reversal potential near −40 mV. This is confirmed in the current–voltage curves for the slowly developing agonist-activated current shown in Fig. 1*B* and *C* (circles). When external chloride was reduced from 156 to 14 mM (replacement with glutamate, which was the internal anion in the pipette solution), the outward currents (corresponding to influx of Cl^-) at 0 and +40 mV were strongly reduced, and the inward current at −40 mV was increased (Fig. 1*A*, right panel). This effect of reducing external chloride can be seen in the current voltage curves of Fig. 1*B*, where the reversal potential shifted to about 0 mV and the curve flattened in low-chloride solution. In the same cell (Fig. 1*C*), raising the external potassium concentration was without effect. Thus, the slowly developing, agonist-induced current is carried by chloride ions. In thirteen experiments like that of Fig. 1*B*, the reversal potential for the current in normal external chloride averaged -37 ± 6 mV (mean ± S.D.) and -2 ± 4 mV in low chloride. Also, when recordings were made with pipettes containing KCl rather than potassium glutamate solution, so that the chloride concentrations were nearly symmetric on the two sides of the membrane, the reversal potential for the delayed current was about 0 mV, which is again consistent with an agonist-induced increase in chloride conductance underlying the delayed current.

In addition to compound 48/80, substance P and antigen could also activate the delayed Cl^- current (see below). Although all of these are capable of stimulating secretion, development of the delayed Cl^- current was not necessarily connected to degranulation. In almost all of the experiments in this series, degranulation did not occur or was very slight, either because of the presence of internal EGTA (Neher & Penner, 1988), or cyclic AMP (Penner, 1988) or because the stimulus was given with a sufficiently long delay after breaking into the cell that wash-out of secretion had occurred (Penner, Pusch & Neher, 1988). Nevertheless, development of the delayed current was full and complete, even with no secretion, indicating that the delayed current is not due to a conductance in vesicle membrane appearing in the whole-cell current as vesicles fuse with the plasma membrane.

Estimates of single-channel conductance

Although the delayed Cl^- current can be large (> 150 pA at +40 mV), it is not associated with large fluctuations or with visible channel events. This can be seen in Fig. 2*A*, which shows a variance of 2·5 pA^2 associated with a mean current of 100 pA. This behaviour can be contrasted, for example, with that of the 50 pS channel described in the previous paper, where much smaller mean currents gave larger variance (e.g. Fig. 2 of preceding paper). The relation between variance and the mean of the Cl^- current during the onset of the current is shown for six cells in Fig. 2*B*. The slope of this relation at a holding potential of +40 mV was 0·034 pA for currents less than half of maximum, where we assume the opening probability to be low. From this slope, we estimated a single-channel conductance of about 0·4 pS, assuming a reversal potential of about −40 mV (see above). The variance used for this estimate was calculated at a bandwidth of 0·5–500 Hz; however, lower frequency fluctuations were visible in the current recordings. To characterize the frequency composition of

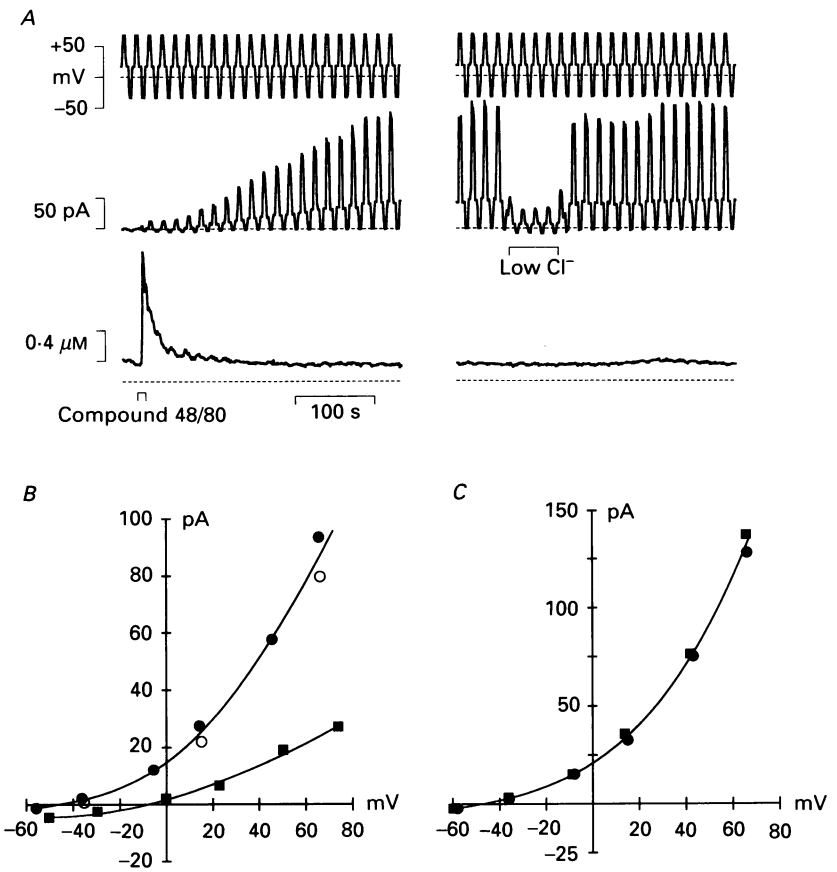

Fig. 1. Compound 48/80 activates a chloride current. *A*, overview of a single experiment, showing effects of compound 48/80 on $[Ca^{2+}]_i$ (bottom trace) and membrane current (middle trace). The upper trace shows the protocol of voltage pulses. Dashed lines indicate zero levels. Compound 48/80 (5 μg ml^{-1}) was applied at the indicated time. The right-hand panel shows the continuation of the left-hand panel after a time break of approximately 100 s. At the indicated time the bathing solution was changed from MCR to low-Cl^- Ringer solution in which all but 14 mM-Cl^- was replaced with glutamate. *B*, current–voltage relation for delayed Cl^- current in MCR (circles) or in low-Cl^- Ringer solution (■; 14 mM-Cl^-). The Cl^- current was in the steady state after activation by compound 48/80. Circles represent before the low-Cl^- solution (●; 156 mM-Cl^-) and after washing out the low-Cl^- (○; 156 mM-Cl^-). Different cell from *A*. *C*, current–voltage relations for delayed Cl^- current in MCR (●; 2·5 mM-KCl) or in MCR with KCl added to a final concentration of 10 mM (■). Same cell as *B*.

the noise associated with the Cl^- current, we calculated the power spectral density of the noise at wider bandwidths. The spectrum seemed to consist of a number of components, approximating $1/f$ form in the frequency range from 0·2–500 Hz. This suggests that the estimated single-channel conductance given above is probably an underestimate because variance components outside the 0·5–500 Hz bandwidth of the calculation were missed.

Further indication that the actual single-channel conductance is larger than 0·4 pS comes from experiments with blockers of the channel. As shown in Fig. 2*A*, the Cl^- current could be blocked by the stilbene derivative DIDS, which has been shown previously to block Cl^- channels from *Torpedo* electroplax (Miller & White, 1984).

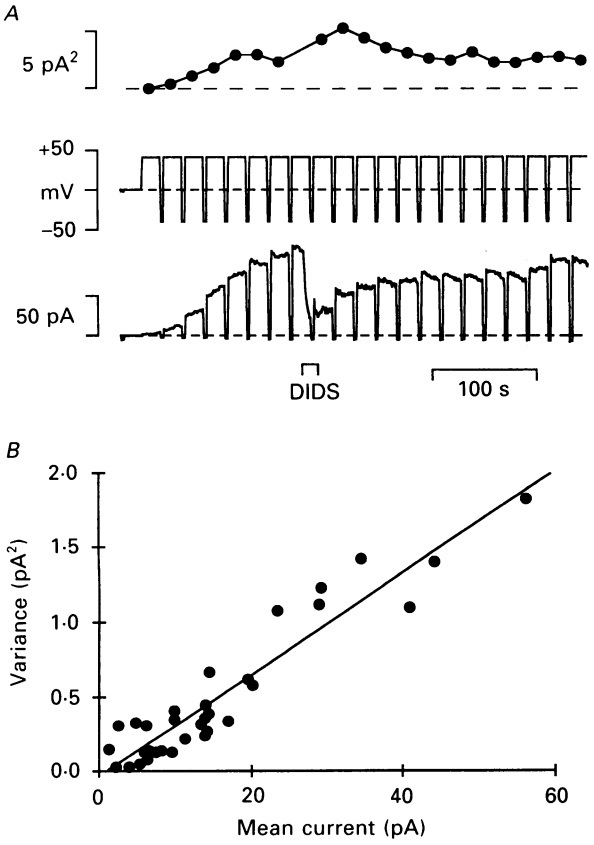

Fig. 2. Relation between variance and mean of delayed Cl^- current and the effect of DIDS on the current. *A*, the bottom trace shows the membrane current, and the middle trace shows the membrane voltage protocol. The upper trace shows the variance of the membrane current calculated at a bandwidth of 500 Hz for 2 s segments each and averaged over 12 s periods during each of the episodes at +40 mV. At the indicated time, 10 μM-DIDS was applied externally. The delayed Cl^- current was activated by 10 μM-8-bromo cyclic AMP in the pipette solution. The dashed lines indicate zero levels. *B*, the relation between variance and mean of membrane current during activation of delayed Cl^- current in six experiments like that of *A*. Only data from mean currents less than 50% of the maximum current are shown. The straight line was fitted to the points by a least-squares criterion and has a slope corresponding to a single-channel current of 0·034 pA. The holding potential was +40 mV.

During DIDS blockade, the variance of the delayed current was larger than before DIDS, even though the mean level of the current was greatly reduced. Thus, the variance/mean ratio increased in the presence of DIDS, to an average of $0{\cdot}13 \pm 0{\cdot}06$ pA (mean $\pm$ S.D.; seven cells) at +40 mV. The variance increase in DIDS is consistent with a model in which DIDS acts as an open-channel blocker, breaking long channel

openings into briefer openings and closings that fall within the 0·5–500 Hz bandwidth of the variance calculation. Assuming that the blocking and unblocking events produced by DIDS represent full-amplitude openings and closings of the channel, the value of variance/mean in DIDS can be used to arrive at an estimate of $1·6 \pm 0·6$ pS (mean $\pm$ S.D.; seven cells) for the single-channel conductance.

The action of DIDS on mast cells differs from that described previously (Miller & White, 1984). For instance, in *Torpedo* electroplax, DIDS irreversibly blocks the Cl^- channel, while the effect of DIDS on Cl^- current in mast cells was partly reversible (see Fig. 2), at least for brief applications. Also, Miller & White (1984) reported that DIDS blocked the channel from only one side, whereas in mast cells internal DIDS seems to block inward current (Cl^- efflux), in addition to the effect of external DIDS on outward current (Cl^- influx) described above. A simple explanation of the effect of DIDS on the delayed Cl^- current is that DIDS enters the channel from either side and blocks it. However, the action of DIDS was more complex than this, because application for more than a few seconds led to an irreversible blockade, which suggests that there is both a rapid, but reversible, and a slower, but irreversible, block of the channel. In addition to DIDS, the Cl^- current could also be blocked by 10 μM-5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB), a blocker of Cl^- conductance in epithelial cells (Wangemann, Wittner, Di Stefano, Englert, Lang, Schlatter & Greger, 1986). Like DIDS, NPPB also produced a variance increase during the blockade of the current.

We also found that external application of the secretagogue compound 48/80, which is an activator of delayed Cl^- current (Fig. 1*A*), produced a reversible blockade of the current stimulated by internal cyclic AMP or by a previous application of compound 48/80. The secretagogue substance P did not have this blocking action on delayed Cl^- current. We do not know the mechanism of blockade by compound 48/80, but it does not seem to involve a reduction in internal cyclic AMP. First, compound 48/80 effectively blocked Cl^- current even with a high concentration (50 μM) of cyclic AMP in the pipette, and second, blockade still occurred when delayed current was stimulated by 50 μM internal 8-bromo cyclic AMP, which is relatively non-hydrolysable. Because compound 48/80 blocks Cl^- current as well as activating it, we considered the possibility that at least part of the delay in onset of the Cl^- current following application of the secretagogue was caused by recovery from block rather than from delayed activation. However, as described in the following section, substance P also activates the delayed Cl^- current (Fig. 3*A*), and the time course of activation of delayed current by substance P, which does not block Cl^- current, was similar to that following compound 48/80. This suggests that the delayed onset may be partly but not solely due to recovery from blockade.

Activation of the delayed current

The delayed Cl^- current could be activated in a number of ways, some of which are illustrated in Fig. 3. In addition to compound 48/80, substance P was an effective trigger for the delayed current when applied externally, as shown in Fig. 3*A*. In cells that were sensitized by prior incubation in IgE directed against DNP–BSA, DNP–BSA also induced the delayed current (not shown). The fact that several external agonists activate the current suggested that the Cl^- conductance might be controlled by an internal messenger. Because G proteins are often involved in the linkage between receptor activation and second messengers, we examined the effect of internal GTP-γ-S on the delayed current. As shown in Fig. 3*B*, GTP-γ-S did

induce the delayed current, suggesting that a G protein plays a role in the regulation of the delayed current.

We tested a number of possible second messengers and found that the delayed current could be activated internally in more than one way. The most rapid and

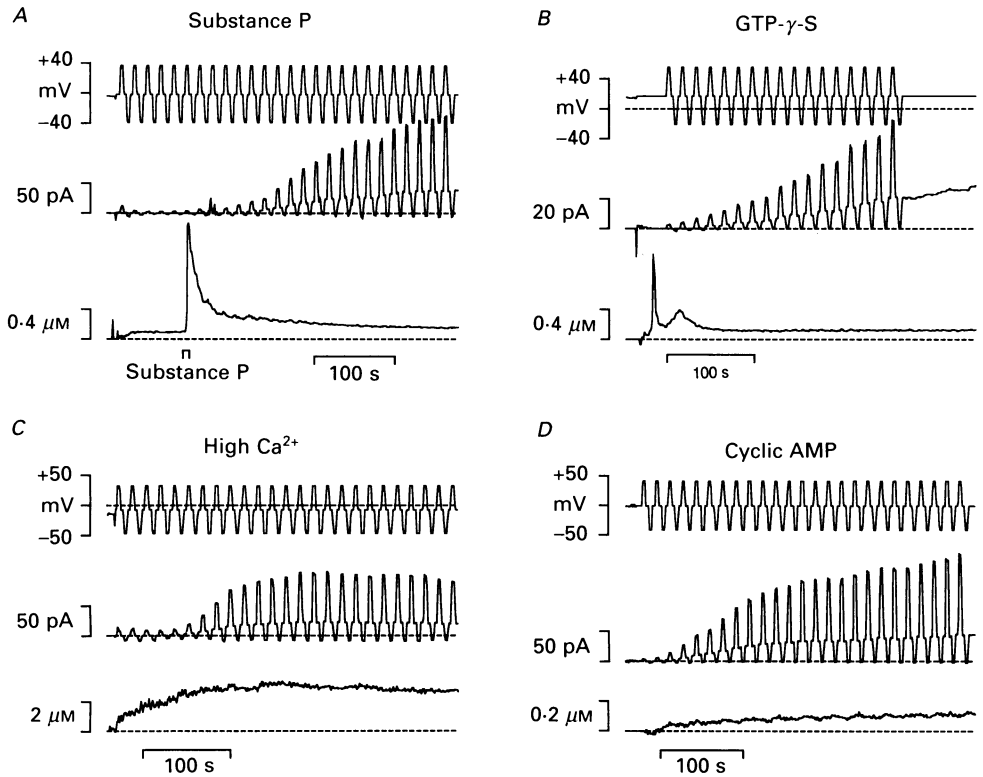

Fig. 3. Activation of delayed Cl^- current by various methods. In each set of traces, the bottom trace is $[Ca^{2+}]_i$, the middle trace is membrane current, and the upper trace shows the voltage pulses. Dashed lines indicate zero levels. *A*, activation by externally applied substance P (50 μg ml^{-1}). *B*, activation by internally applied GTP-γ-S (100 μM). *C*, activation by high $[Ca^{2+}]_i$, which was raised by including 3 mM-Ca^{2+}-dibromo-BAPTA and 1 mM-dibromo-BAPTA in the pipette solution. *D*, activation by internally applied cyclic AMP (50 μM).

reliable activation (84 out of 106 cells; 79·2%) was achieved with cyclic AMP, as illustrated in Fig. 3*D*. Although cyclic AMP was the most rapid stimulus we have found, its effect was not immediate, and the delayed current typically developed with a delay of tens of seconds even though Fura-2 loading and measurements of access resistance demonstrated that the rise of cyclic AMP inside the cell should have been rapid following break-in. This suggests that cyclic AMP's effect on the Cl^- conductance is not direct.

In addition to cyclic AMP, high $[Ca^{2+}]_i$ could also activate the Cl^- current, but only at concentrations exceeding about 1 μM and only with a considerably longer delay than that seen with cyclic AMP. An example of the activation of the current by high $[Ca^{2+}]_i$ is shown in Fig. 3*C*. Internal Ins1,4,5P_3 (10 μM) also induced delayed current

in some cells (thirty-six out of fifty-three), but not when $[Ca^{2+}]_i$ was buffered to low levels with EGTA (one out of seven cells). This indicates that the activation of delayed current by Ins1,4,5P_3 probably occurs via the elevation of internal calcium caused by Ins1,4,5P_3. Because agonist stimulation also triggers an increase in $[Ca^{2+}]_i$,

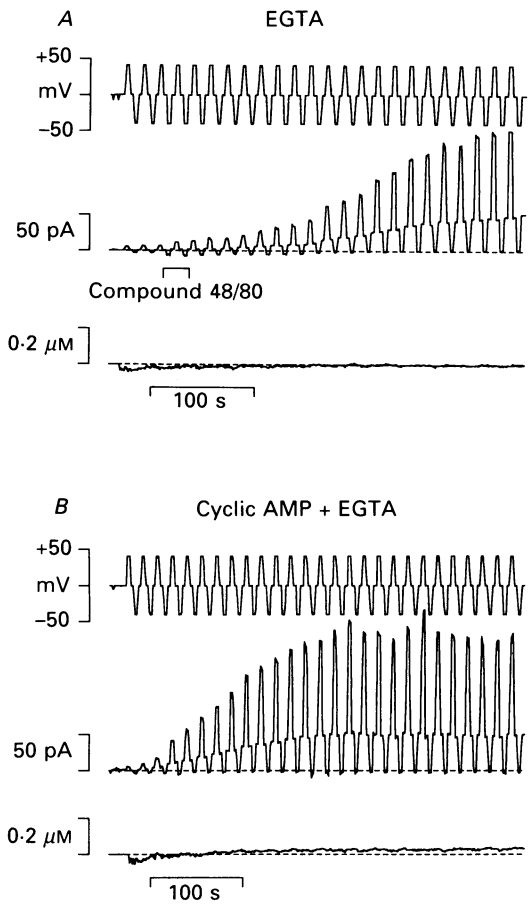

Fig. 4. Buffering $[Ca^{2+}]_i$ to low levels does not affect activation of delayed Cl^- current. Bottom traces are $[Ca^{2+}]_i$, middle traces are membrane current, and top traces show the voltage pulses. In both *A* and *B*, pipette solutions contained 2 mM-K_2-EGTA. *A*, activation of delayed current by externally applied compound 48/80 (5 μg ml^{-1}). *B*, activation of delayed current by internally applied cyclic AMP (50 μM).

it is possible that the activation of delayed current by agonists could also act indirectly via elevated $[Ca^{2+}]_i$. However, the activation of the Cl^- current by compound 48/80 (Fig. 4*A*) or substance P was unaffected by buffering $[Ca^{2+}]_i$ to essentially zero with internal EGTA, and thus increases in $[Ca^{2+}]_i$ were not required for the linkage between agonist stimulation and the delayed current. Similarly, activation of the Cl^- current by internal cyclic AMP was also unaffected by internal EGTA (Fig. 4*B*), demonstrating that cyclic AMP's action is independent of $[Ca^{2+}]_i$.

Other Ca^{2+}-activated conductances

Although the delayed Cl^- current could be stimulated by high $[Ca^{2+}]_i$, it was clearly different from the directly and rapidly Ca^{2+}-activated Cl^- current described in lacrimal gland cells (Marty, Evans, Tan & Trautmann, 1986) and in *Xenopus*

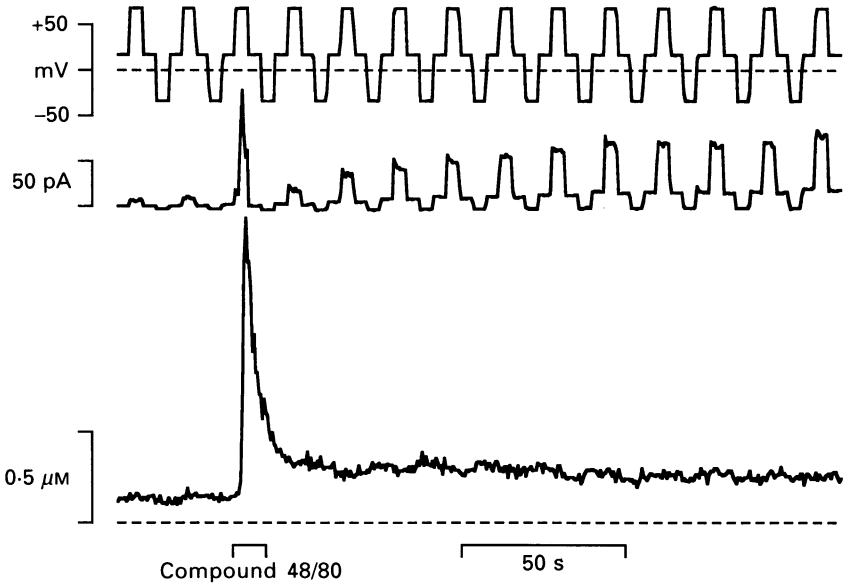

Fig. 5. Rapidly Ca^{2+}-activated current and delayed Cl^- current in the same mast cell. The top trace shows the voltage protocol, the middle trace shows the membrane current, and the bottom trace shows $[Ca^{2+}]_i$. At the indicated time, compound 48/80 (5 μg ml^{-1}) was applied extracellularly, eliciting a rapid Ca^{2+} transient and stimulating delayed Cl^- current. Coincident with the rise in $[Ca^{2+}]_i$ during the Ca^{2+} transient, there was a spike of outward current. Such rapid Ca^{2+}-activated currents were observed in approximately 5% of the preparations.

oocytes (Takahashi, Neher & Sakmann, 1987). The directly Ca^{2+}-activated current immediately tracks changes in $[Ca^{2+}]_i$, but the delayed Cl^- current responded only slowly to increased $[Ca^{2+}]_i$, suggesting that calcium's effect is indirect. In a small fraction ($< 5\%$) of preparations, we encountered a rapidly Ca^{2+}-activated current in addition to the delayed current. This rapidly Ca^{2+}-activated current appeared similar to that of lacrimal gland and oocytes and produced a brief peak of current coinciding with the peak of the Ca^{2+} transient induced by stimulation of mast cells (Fig. 5). In those rare preparations in which this rapid current was observed, it was found in most of the cells of the preparation. The rapidly Ca^{2+}-activated current was not a potassium current, because it was unaffected by increased external potassium concentration in three cells in which the current was activated by high calcium in the pipette solution. As shown in Fig. 5, both the delayed current and the rapidly Ca^{2+}-activated current could be observed in the same cell.

In about 5% of preparations, we also observed large-conductance, Ca^{2+}- and voltage-dependent channels that were similar to the large-conductance Ca^{2+}-activated potassium channels found in a variety of cell types (Meech, 1978; Latorre

& Miller, 1983). The reversal potential of the current through these channels was more negative than that of the delayed Cl^- current and was affected by changes in external potassium concentration, confirming that they were potassium channels. Like the rapidly Ca^{2+}-activated Cl^- current described above, the Ca^{2+}-activated potassium currents were found in the majority of cells in the rare preparations in which they were present.

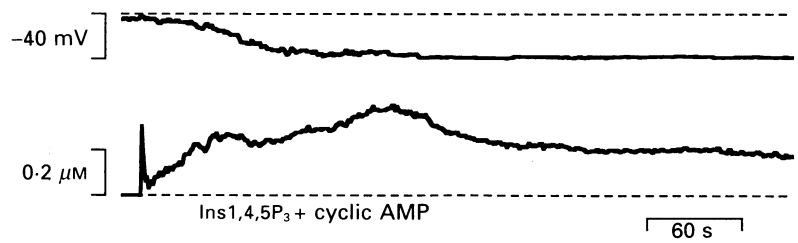

Fig. 6. Internal application of 50 μM-cyclic AMP and 0·5 μM-Ins1,4,5P_3 in a mast cell under current clamp. The top trace shows membrane potential and the bottom trace shows $[Ca^{2+}]_i$.

Effect of volume changes

Chloride currents similar in some respects to the delayed Cl^- current have recently been described in lymphocytes (Cahalan & Lewis, 1988) and in epithelial cells (Hazama & Okada, 1988). These Cl^- currents can be rapidly modulated by volume changes and seem to play a role in volume regulation. We investigated whether similar effects of volume changes on the Cl^- current might occur in mast cells. First, we examined the correlation between the magnitude of delayed current, stimulated either by external agonists or by internal cyclic AMP, and changes in cell volume that sometimes occur during the recordings. Changes in calcium-independent Fura-2 fluorescence (at 360 nm excitation) provided a convenient index of changes in cell volume. Large Cl^- currents could be observed in cells that showed no change in volume during the experiment, particularly after cyclic AMP stimulation, and conversely, cells that swelled did not always show large delayed currents. Thus, there was little apparent correlation between swelling and delayed Cl^- current in mast cells. On the other hand, in most of the experiments at high $[Ca^{2+}]_i$ delayed current developed only after some swelling had occurred. Thus, we also attempted to modulate the delayed current by changes in cell volume after the current was activated by agonists or by cyclic AMP. In lymphocytes, increasing or decreasing cell volume by gentle pressure or suction through the whole-cell recording pipette caused rapid and large reversible changes in the amplitude of the Cl^- current. In mast cells, however, the effects of such volume manipulations were small and slow. Thus, the delayed Cl^- current did not behave like the volume-regulatory Cl^- conductance of other cells, and we conclude that volume regulation is not its principal function in mast cells.

Interaction of delayed Cl^- current with Ins1,4,5P_3-stimulated Ca^{2+} influx

A function for the delayed Cl^- current is suggested by the dependence of Ins1,4,5P_3-induced Ca^{2+} influx on membrane voltage, as described in the preceding

paper. Influx of Ca^{2+} through the Ins1,4,5P_3-activated pathway was shown to require negative membrane potentials. The delayed Cl^- conductance is the dominant membrane conductance of mast cells, and it would be expected to drive membrane potential near its reversal potential. Under our recording conditions, this would be approximately −40 mV, which is sufficiently negative to support increases in $[Ca^{2+}]_i$ via the Ins1,4,5P_3-stimulated pathway. To test for such interactions between delayed Cl^- current and Ins1,4,5P_3-stimulated Ca^{2+} influx, we made current-clamp recordings from mast cells with pipettes containing both cyclic AMP, to activate the delayed Cl^- current, and Ins1,4,5P_3. An example of this type of experiment is shown in Fig. 6. Following break-in, the membrane potential of the cell was initially near 0 mV, but as the delayed Cl^- current activated, the membrane hyperpolarized to about −40 mV, with a time course similar to the expected activation of delayed current by cyclic AMP (e.g. Fig. 3*D*). Concomitant with the hyperpolarization, there was a transient increase in $[Ca^{2+}]_i$, demonstrating that activation of Cl^- current can produce sufficient hyperpolarization to drive increases in internal Ca^{2+}. Thus, we conclude that membrane hyperpolarization, and hence support of Ca^{2+} influx, might be the physiological role of the agonist-stimulated Cl^- current of mast cells.

DISCUSSION

Regulation of delayed chloride current

The delayed chloride current seems to be subject to complex regulation in mast cells. The current can be stimulated by a number of external agonists, and the activation is slow in onset and long outlasts the presence of the agonist. This suggests that the current is activated indirectly via second messengers rather than directly by the agonists. In agreement with this suggestion, internally applied GTP-γ-S could also activate the current, indicating the involvement of G proteins in the linkage between agonist and the Cl^- conductance. In addition to being activated by a number of external agonists and by intracellular GTP-γ-S, the current can be turned on by various other internal stimuli as well, including cyclic AMP, elevated $[Ca^{2+}]_i$, and Ins1,4,5P_3. Of these, cyclic AMP is the most potent and rapid activator of the current. Activation by elevated $[Ca^{2+}]_i$ is slow and weak, indicating that the Cl^- current is not directly activated by internal calcium. Activation by Ins1,4,5P_3 probably occurs via increased $[Ca^{2+}]_i$, because Ins1,4,5P_3's action is attenuated when $[Ca^{2+}]_i$ is buffered to low levels with EGTA. Buffering $[Ca^{2+}]_i$ with EGTA does not affect activation of the Cl^- current by either external agonists or internal cyclic AMP, demonstrating that elevated $[Ca^{2+}]_i$ is not necessary for activation of the current. The effect of cyclic AMP, however, can still require tens of seconds, suggesting that cyclic AMP is not a direct internal agonist for the channels and that other events set in motion by cyclic AMP are required to open the conductance.

Although cyclic AMP is the most potent activator of the Cl^- current that we have found, it is not necessarily the case that an increase in cyclic AMP is the mechanism by which external agonists activate the current. For example, compound 48/80 is a potent activator of the Cl^- current, but Sullivan, Parker, Eisen & Parker (1975) have reported that cyclic AMP levels are not elevated in mast cells by compound 48/80.

Thus, in the intact cell, it is likely that some other factor in addition to cyclic AMP can be of importance in the regulation of the Cl^- current.

Comparison with other types of chloride channel

The delayed chloride current of mast cells appears to be a novel type of Cl^- conductance different from those previously described in other cells. Other chloride channels that are activated by calcium and cyclic AMP have been described (e.g. Hayslett, Gögelein, Kunzelmann & Greger, 1987; McPherson & Dormer, 1988), but the single-channel conductance is larger than that of the delayed Cl^- current of mast cells. Also, although the delayed Cl^- current can be activated by elevated $[Ca^{2+}]_i$, this activation is clearly different from the apparently directly calcium-activated chloride channels of lacrimal gland cells (Marty *et al.* 1986) or oocytes (Takahashi *et al.* 1987), where activation and deactivation closely parallel changes in $[Ca^{2+}]_i$. The volume-sensitive chloride conductances described by Cahalan & Lewis (1988) and Hazama & Okada (1988) are also apparently different from the delayed chloride conductance, because changes in cell volume have only slow and minor effects on the Cl^- current of mast cells.

The blocking action of DIDS on the delayed Cl^- current is also unusual. Miller & White (1984) reported that DIDS produced a sudden and irreversible block of Cl^- channels from *Torpedo* and that blockade occurred only from one side of the channel. However, blockade of delayed Cl^- current by DIDS was at least partly reversible, and DIDS was effective from both sides of the membrane. This suggests that DIDS is an open-channel blocker of the delayed current. The variance increase accompanying DIDS blockade (Fig. 2*A*) is consistent with the idea that DIDS is a simple blocker of the open chloride channel in mast cells.

Physiological role of delayed Cl^- current in secretion

Because the delayed chloride conductance is by far the largest conductance in mast cells, it would be expected to be the dominant determinant of membrane potential when it is activated. Thus, following stimulation, the mast cell membrane potential would be driven to near E_{Cl}, the chloride equilibrium potential. If E_{Cl} of mast cells is similar to that of other cells (e.g. -34 mV in liver cells, Claret & Mazet, 1972) then membrane potential would be held in a range where we observed hyperpolarization-driven Ca^{2+} influx through the Ins1,4,5P_3-induced pathway (e.g. Fig. 4 of Matthews *et al.* 1989). Thus, after stimulation of a mast cell, pathways for Ca^{2+} influx would be opened and the delayed chloride conductance would provide the negative membrane potential necessary for increases in $[Ca^{2+}]_i$ mediated via those pathways. Such a combination of ionic mechanisms could then be responsible for the plateau phase of elevated $[Ca^{2+}]_i$ that can be observed following stimulation in mast cells (Neher & Almers, 1986; Neher & Penner, 1988).

An increase in $[Ca^{2+}]_i$ can dramatically accelerate secretion in mast cells (Beaven, Moore, Smith, Hesketh & Metcalfe, 1987; Neher, 1988). It is interesting to note, however, that the large Ca^{2+} transient due to release of Ca^{2+} from internal stores occurs too early and is over too rapidly to be able to significantly affect secretion in mast cells (Neher & Almers, 1986; Penner, Pusch & Neher, 1987; Neher, 1988; Neher & Penner, 1988). But the sustained rise in $[Ca^{2+}]_i$ due to Ca^{2+} influx after stimulation

has the correct timing to influence secretion, particularly because it occurs during the time when the sensitivity of secretion to increases in $[Ca^{2+}]_i$ is enhanced following a stimulus (Neher, 1988).

The combination of stimulus-activated Ca^{2+} influx pathways, described by Matthews *et al.* (1989), and the chloride conductance described in this paper, provides mast cells with the means to regulate influx of external calcium in the absence of voltage-activated calcium channels. It will be interesting to determine how general this scheme of second messenger-regulated conductances is among non-excitable cells as an alternative to voltage-gated channels.

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