Hydrophobic Ions Amplify the Capacitive Currents Used to Measure Exocytotic Fusion

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ABSTRACT The detection of exocytotic fusion in patch-clamped secretory cells depends on measuring an increase in the cell membrane capacitance as new membrane is added to the plasma membrane. However, in the majority of secretory cells, secretory vesicles are too small (<200 nm in diameter) to cause a detectable signal. We have found that incubations of normal mouse mast cells with the hydrophobic anion dipicrylamine (DPA), increases cell membrane capacitance by about three times. The large capacitive current induced by DPA was voltage-dependent, having a maximum value at −10 mV. The DPA-induced charge movement could be described by a single barrier model in which the DPA molecules move between two stable states in the bulk lipid matrix of the membrane. More importantly, the DPA treatment produced a sevenfold increase in the size of the capacitance steps observed upon the exocytotic fusion of single secretory granules. A similar amplification of DPA on the secretory vesicle capacitance was observed in a cell with larger (≤5 μm in diameter) or with smaller secretory granules (<250 nm in diameter). Additionally, the increased granule membrane capacitance enlarged the transient capacitive discharge measured upon formation of a fusion pore in normal mast cell granules. Our results indicate that hydrophobic ions provide an important tool for high resolution studies of membrane capacitance.

INTRODUCTION

Exocytotic secretion can be studied with the patch-clamp technique by measuring the cell membrane capacitance (Cm) increases caused by the fusion of secretory vesicles with the plasma membrane. Detection of the fusion of single vesicles with the plasma membrane depends on the measurement of changes in the admittance of a patch-clamped secretory cell or in the measurement of a small capacitive current that charges the fusing vesicle to the same potential as that of the plasma membrane. In mast cells, exocytotic fusion can be detected as step increases in the membrane capacitance, each step corresponding to the fusion of a single secretory granule with the plasma membrane. However, most secretory cells have secretory vesicles that are too small to cause a detectable change in the cell membrane admittance. We have sought to amplify these capacitive signals by increasing the specific capacitance of the membrane of a secretory cell.

Hydrophobic ions such as tetraphenylborate or dipicrylamine have been shown to promote capacitive changes in artificial bilayers (for references, see Szabo, 1977) and in cell membranes like the node of Ranvier (Benz and Nonner, 1981), squid giant axon (Fernandez et al., 1983), rat clonal pituitary cells (Fernandez et al., 1984a), and hepatocytes (Turin et al., 1991). These hydrophobic ions strongly adsorb at the membrane-solution interface and translocate across the membrane under the influence of the transmembrane electrical field, producing a net charge movement and consequently contributing to the membrane capacitance. Hydrophobic ions have been used extensively in membrane studies. They have been useful as simple models for the gating currents associated with the opening and closing of ion channels in cell membranes and the translocation of ions across membranes by ion carriers (Szabo, 1977). Because the rate of translocation of hydrophobic ions across the membrane depends on their physical properties (like fluidity or thickness), they have been used as probes of membrane structure (Benz and Lauger, 1977; Benz and Nonner, 1981; Dilger and Benz, 1985; Smejtek and Wang, 1991) and as reporters of the effects of anesthetics on membrane function (Reyes and Latorre, 1979; Fernandez et al., 1982). In addition, hydrophobic ions have been used as probes of membrane surface potentials (Kleijn et al., 1983; Fernandez et al., 1984a).

In this work we studied the effect of the hydrophobic ion dipicrylamine (DPA) on the membrane capacitance of mast cells and chromaffin cells. Our results show that large increases in the plasma membrane capacitance can be promoted by incubating the cells with DPA. We also found that DPA increases the size of the capacitance steps observed upon exocytotic fusion. Surprisingly, the capacitance increase induced by DPA was larger in the secretory granule membrane than in the plasma membrane, resulting in an increase in the signal-to-noise ratio. We interpret this effect as being due to a difference in the lipid composition between the granule membrane and the plasma membrane. Similar results were obtained in chromaffin cells, where a large number of step increases in Cm could be resolved in cells treated with DPA. In addition, DPA amplified the current transients through the nascent fusion pore measured in normal mast cell secretory granules. From these data, the initial conductance of the fusion pore was estimated to be about 260 pS (similar to the value measured in beige mouse

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secretory granules; Spruce et al., 1990). A preliminary report of this work has been presented (Oberhauser and Fernandez, 1993a).

METHODS

Cells

Mast cells from normal mice (C57BL/6J) and mutant beige mice (bg^j/bg^j strain) (Jackson Laboratories, Bar Harbor, ME) were obtained by peritoneal lavage after a procedure described elsewhere (Alvarez de Toldeo and Fernandez, 1990). Chromaffin cells were prepared from bovine adrenal medullae by enzymatic digestion (Burgoyne et al., 1988). Isolated cells were resuspended in Dulbecco's modified Eagle's medium supplemented with 25 mM HEPES and 10% fetal calf serum.

Solutions

The standard external solution used for mast cells was (in mM) 130 NaCl, 1 $MgCl_2$, 2 $CaCl_2$, 1.5 KOH, 10 HEPES at pH 7.3, and the pipette solution contained 140 K-glutamate, 7 $MgCl_2$, 3 KOH, 10 EGTA, 0.2 ATP, 10 HEPES at pH 7.3, and in some experiments 10 μM GTPγS to stimulate secretion. In experiments with chromaffin cells the external solution was 140 NaCl, 20 HEPES, 4 $MgCl_2$, 2.2 $CaCl_2$, 2 mg/ml glucose, and 1 μM tetrodotoxin at pH 7.4, and the internal solution consisted of 125 Cs D-glutamate, 30 HEPES, 8 NaCl, 1 $MgCl_2$, 2 Mg-ATP, 0.3 Cs-EGTA (pH 7.3), and 300 μM of the nonhydrolyzable GTP analog GppNHp to stimulate secretion. DPA (dipicrylamine or hexanitrodiphenilamine; Aldrich, Milwaukee, WI) was dissolved in dimethyl sulfoxide before being diluted 1:10000 to a final concentration of 10 μM. The cells were exposed to external solution containing DPA for not longer than 2 min. We found that larger increases in Cm could be achieved by incubating the cells with higher concentrations of DPA or by extending the incubation time. However, this produced membrane damage because most of the cells degranulated spontaneously, and it was difficult to obtain stable whole-cell recordings.

Patch-clamp recording

All the experiments described in this paper were performed using the whole-cell recording mode of the patch-clamp technique (Hamill et al., 1981). The pipettes (Kimax 51; Kimble, Vineland, NJ) were Sylgard-coated and fire-polished and had a resistance of 1.5–3.0 MΩ when immersed in the bath solution. To study the capacitive currents induced by DPA, voltage pulses of different duration and amplitudes were used. The relaxation time constant of capacitive currents was calculated by fitting a single exponential to the current traces. The total charge moved by the voltage step was calculated from the area under the fitted exponential curve. The current was low-pass filtered at 20 kHz (8-pole Bessel) before sampling it at 66 kHz (15 μs/point).

Changes in membrane capacitance and conductance were followed with a digital phase detector (Joshi and Fernandez, 1988). The correct phase detector angle was obtained by using a phase-tracking method (Fidler and Fernandez, 1989). Every admittance determination was obtained by performing the phase detection over a period of eight sinusoidal cycles (9.6 ms). A calibration signal for the capacitance trace was obtained by unbalancing the C-fast potentiometer of the patch-clamp amplifier (EPC-7, List Medical, Darmstadt, Germany) by 100 fF or 1 pF. The initial and final values of capacitance of the cell membrane and series resistance were estimated using the cancellation circuitry of the patch-clamp amplifier.

To study the first milliseconds of the formation of exocytotic fusion pores in mast cells, we applied a technique that captures changes in pore conductance with microsecond time resolution (Breckenridge and Almers, 1987; Oberhauser and Fernandez, 1993b). Briefly, the patch pipette DC current was continuously monitored upon establishment of a gigaseal, low-pass filtered at 20 kHz and sampled at 100 kHz. A threshold detector was set two times above the noise level. If a point crossed the threshold, the next 924 points and the preceding 100 points were stored (one record of 1024 points).

All experiments were done at room temperature. Measurements given are mean ± SE.

RESULTS

The hydrophobic anion DPA increases the plasma membrane capacitance of mast cells

Fig. 1 *A* shows current transients recorded in the whole-cell configuration elicited by a voltage pulse of 80 mV obtained before (*a*) and after (*b*), exposing the cell to an external solution containing 10 μM DPA for about 1 min. The transient current in (*a*) represents the charging of the Cm to a new voltage level. The relaxation time constant was 55 μs, which is similar to the value predicted by $R_{series}C_m$. DPA induces an additional transient current that relaxes about three times slower (183 μs in (*b*)). This increase in the current is due to an increase of the Cm, since the cell membrane resistance and access resistance remained unchanged. Also, this increase in the Cm was not due to an increase in the cell membrane area caused by exocytotic degranulation and is therefore due to the incorporation of DPA ions into the plasma membrane of mast cells. Interestingly, as in the squid giant axon (Fernandez et al., 1983) and hepatocytes (Turin et al., 1991), this short-term DPA treatment is practically irreversible because the Cm decreases very slowly in DPA-free solution. This decrease was estimated to be about 1%/min ($n = 4$) or 150 fF/min for the experiment shown in Fig. 1 *A*.

If hydrophobic ions are incorporated into the membrane, they should respond to changes in electrical potential across the membrane; i.e., the enhancement of the capacitive current induced by DPA should be voltage sensitive. We therefore analyzed the currents induced by DPA that were elicited by voltage pulses of different magnitudes. After initiating the whole-cell recording in a mast cell preincubated with DPA, the membrane was depolarized to different voltage values from a holding of −90 mV. Typical whole-cell DPA currents are shown in the inset of Fig. 1 *B*. From the time integral of the current traces, we calculated the number of charges that moved in the membrane. Cells preincubated with DPA had a sigmoidal *Q-V* relationship (Fig. 1 *B*). The slope of the *Q-V* curve was voltage-dependent with a maximum of 12 pF at −10 mV and was 4.5 pF at extreme positive or negative voltages. The *Q-V* relationship for the cell before DPA treatment was linear, with a slope of 4.5 pF (not shown). We also studied the rate of DPA movement in the membrane as a function of the transmembrane voltage. Fig. 1 *C* shows a plot of the time constant of the current generated by DPA upon stepping the membrane voltage from the holding potential of −90 mV to different voltages. The relaxation time constant shows a maximum at −10 mV, and at extreme voltages the time

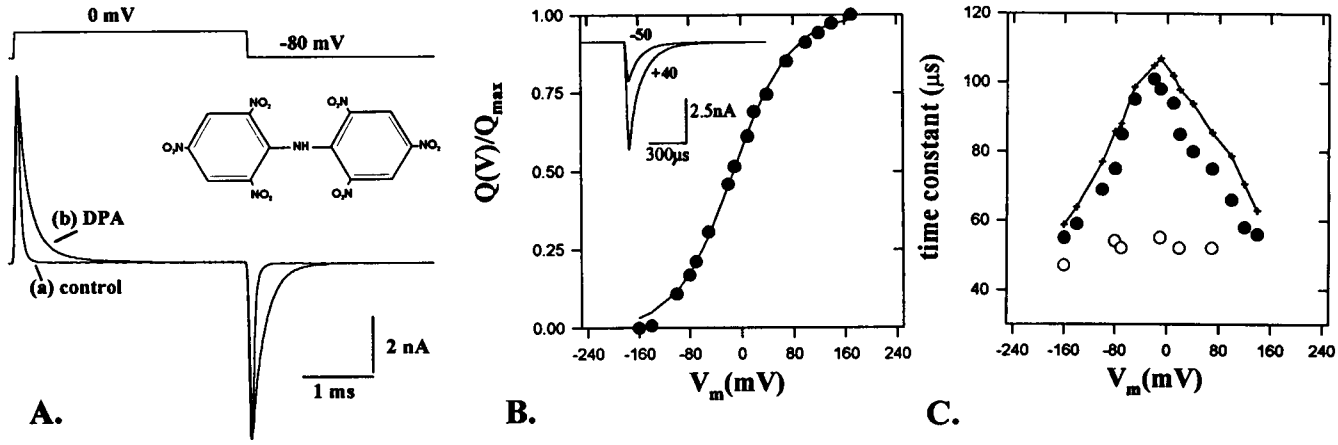

FIGURE 1 The hydrophobic anion DPA increases the mast cell Cm. (*A*) Trace (*a*) is a recording of the whole-cell membrane capacitive current elicited by a voltage pulse of 80 mV (from a holding of −80 mV to 0 mV for 3.3 ms). Trace (*b*) is the current recorded after the cell was exposed to an external solution containing 10 μM DPA for about 1 min. DPA induced an increase in the Cm from 5.4 pF (*a*) to 19.2 pF (*b*). The series resistance and cell membrane resistance remained unchanged, their respective values being 10.2 MΩ and >5 GΩ. The cell did not degranulate in this experiment. The inset shows the molecular structure of dipicrylamine; with a pK of 2.6, DPA is fully deprotonated at pH 7.2 (Ketterer et al., 1971). (*B*) Charge movement induced by DPA as a function of the membrane voltage. The inset shows the currents obtained after the cell was repolarized to the holding potential of −90 mV after voltage steps to +50 mV and −40 mV. These traces were fitted to a single exponential whose area was integrated to obtain the charge moved. The charge was normalized and plotted as a function of the test voltage. Q_{max} was estimated from the charge moved at large positive voltages (410 fC in this cell). The continuous lines represent the prediction of a single-barrier model using $V_h = -10$ mV and $\alpha = 0.57$ in Eq. 1. (*C*) Time constant of the DPA-induced displacement current decay as a function of the voltage (●). The open symbols are data obtained before treating the cell with DPA. The solid line represents the values predicted by R_{series}Cm, where Cm was calculated from Fig. 1 *B*. The series resistance in this cell was 9.3 MΩ.

constant is not different from the values obtained from the cell before being exposed to DPA (*open circles*).

These results are consistent with the view that DPA ions are trapped in the membrane of these cells and that their distribution across the membrane is voltage-dependent (Ketterer et al., 1971; Szabo, 1977; Benz and Nonner, 1981; Fernandez et al., 1983). Then a voltage step from a strongly negative voltage toward a more negative voltage should produce very little translocation (nearly all DPA ions being already on one membrane surface), whereas a positive step should translocate a fraction of (or all of) the DPA ions to the other surface. This behavior can be simply described by a single-barrier model, in which DPA ions move between two stable states across the membrane with a first-order transition (Ketterer et al., 1971; Reyes and Latorre, 1979; Benz and Nonner, 1981; Fernandez et al., 1983). The total anion charge moved at any given membrane voltage, $Q(V)$, is given by

$$Q(V) = \frac{Q_{max}}{1 + \exp\dfrac{-e\,\alpha\,(V - V_h)}{kT}},$$

where Q_{max} is the total charge available to move (in this cell it was 410 fC), V_h is the voltage at which half of the total charge is in each stable state, α represents the fraction of the electrical field through which the DPA ions translocate ($0 < \alpha < 1$), and e, k, and T have their usual meanings. The fit of the model to the data (continuous curve in Fig. 1 *B*) shows that the effect of DPA on the Cm has the properties expected for the translocation of hydrophobic ions across the lipid membrane.

DPA improves the resolution of capacitive currents generated by single exocytotic fusion events

DPA also increases the size of the capacitance steps observed in degranulating mast cells (Fig. 2). In these experiments, the Cm was measured with a software-based phase detector by superimposing a sine wave of 54 mV peak-to-peak and 830 Hz onto the holding potential (0 mV). Fig. 2 *A* shows the time course of the Cm increase caused by the exocytotic fusion of about 200 granules in a control mast cell. Secretion was stimulated by including GTPγS in the patch pipette solution. Cm increased from 4.3 pF to 11.5 pF. An expansion of this trace shows that single fusion events can be clearly resolved (seen as step increases in Cm; Fernandez et al., 1984b). The mean size of the steps recorded in this cell was 23.4 ± 1.2 fF (n = 195; range: 10–85), and the mean peak-to-peak noise value was 6.0 fF, giving a signal-to-noise ratio of 3.9 for this cell. Fig. 2 *B* shows the secretory response of a cell that was pretreated with DPA. An expansion of this trace shows that the capacitance steps are bigger, the mean step size was 143.1 ± 9.3 fF (n = 170; range: 15–1100 fF), and the signal-to-noise ratio increased to 8.5 (the noise level was 16.7 fF). The initial and final membrane capacitance values were 14.5 pF and 50.4 pF, respectively. As expected, the capacitive noise increases by a factor similar to the increase in plasma membrane capacitance (about threefold). This is due to the

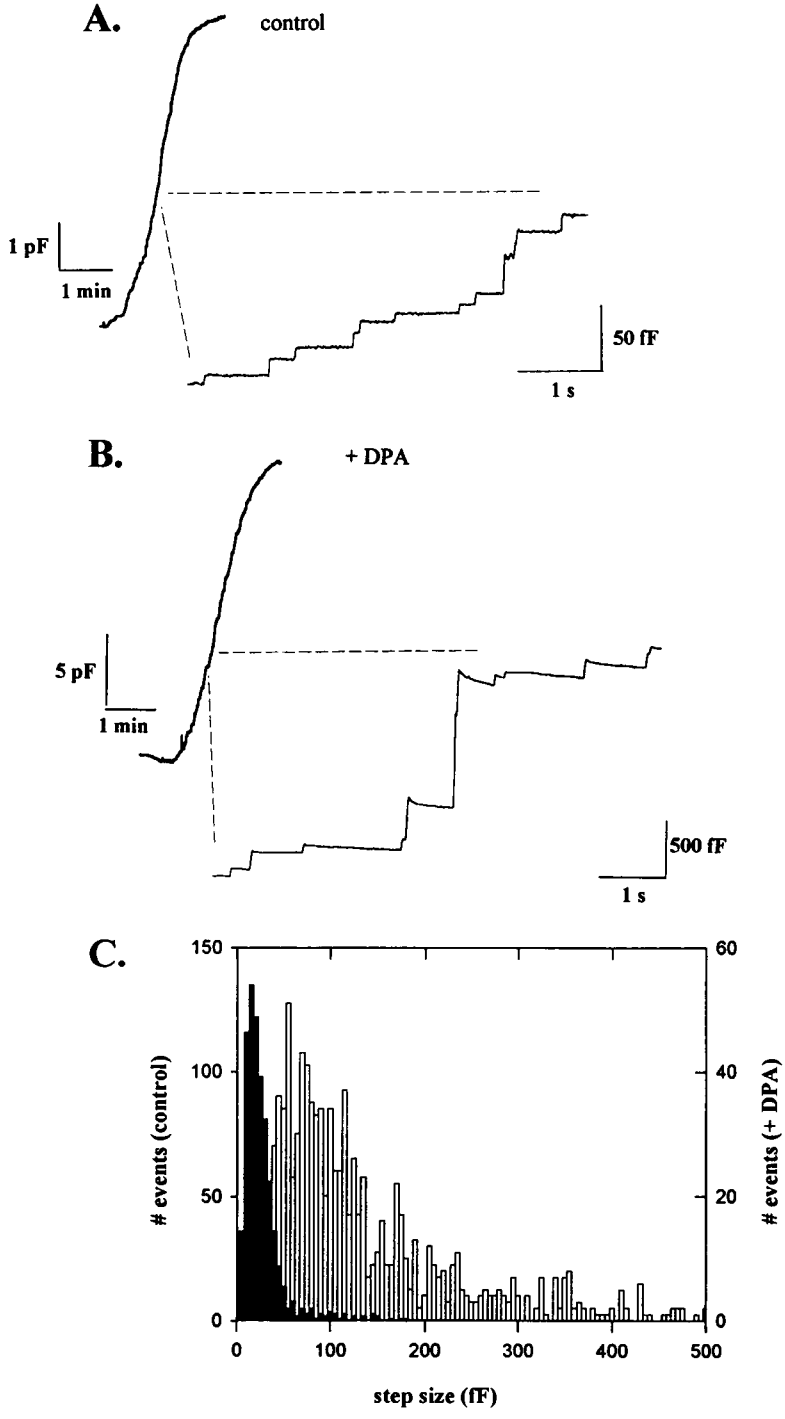

FIGURE 2 DPA amplifies the size of the capacitance steps measured in degranulating mast cells. (*A*) Recording of the Cm as a function of time in a control cell (not treated with DPA). Shortly after obtaining the whole-cell configuration, the Cm starts to increase in a stepwise manner (see expansion of the trace), each step representing the exocytotic fusion of a single secretory granule. The patch pipette solution included 10 μM GTPγS. Cm increased from 4.3 pF to 11.5 pF. The rate of degranulation, calculated between 20% and 80% of the final capacitance, was about three fusion events per second. (*B*) Time course of the degranulation in a cell pretreated with DPA. Cm increased from 14.5 pF to 50.4 pF, with a rate of about two fusion events per second. The holding voltage was 0 mV. (*C*) Amplitude histogram of capacitance steps measured in control mast cells (*solid bars*) and DPA-treated mast cells (*open bars*). The mean values were 27.1 $\pm$ 0.7 fF (n = 760; 5 cells; range: 8–180 fF) in control cells and 195.5 $\pm$ 11.2 fF (n = 1053; 8 cells; range: 18–6350 fF) in DPA-treated cells. The bin-width used was 5 fF. Because the amplification effect of DPA on the capacitance steps was voltage dependent, the histogram was constructed using experiments performed at a holding of 0 mV (where a maximum amplification factor was obtained).

incorporation of mobile charges that contribute to the capacitive noise (Kolb and Lauger, 1977). We found that the amplification effect of DPA on the secretory granule membrane capacitance was voltage-dependent, as it was in the plasma membrane. When the holding potential was changed from the normal value of 0 mV to large positive or negative values, the amplitude of the capacitance steps was smaller (not shown).

The amplification effect of DPA on the amplitude of the capacitance steps is better shown by the histogram in Fig. 2 *C*. This histogram shows the amplitude distribution of membrane capacitance step increases collected from control mast cells (*solid bars*) and those treated with DPA (*open bars*). In control cells, the mean step size was 27.1 $\pm$ 0.7 fF (n = 760; 5 cells), whereas in DPA-treated cells the mean step size was 195.5 $\pm$ 11.2 fF (n = 1053; eight cells). The mean value for the peak-to-peak capacitance noise was 6.3 $\pm$ 1.2 fF (n = 5 cells) in control cells and 21.5 $\pm$ 3.4 fF (n = 8 cells) in DPA-treated cells. Thus, in normal mast cells, DPA amplifies the size of the capacitance steps by approximately sevenfold, about two times

more than the effect on the plasma membrane. The improvement of the signal-to-noise ratio on the capacitance recording by DPA is likely to be due to a difference in the lipid composition between the granule membrane and the plasma membrane (see Discussion).

The improvement of the signal-to-noise ratio of the capacitance step sizes induced by DPA was also observed in other secretory cells. Fig. 3 *A* shows capacitance traces obtained from beige mouse mast cells, a mutant with giant secretory granules ($\leq$5 μm in diameter). The traces represent complete degranulations of a control cell (*upper trace*) and a cell treated with 10 μM DPA for 90 s (*lower trace*). The mean step size in the control cells was 211 $\pm$ 32 fF (n = 49; four cells) and 1258 $\pm$ 156 fF in the DPA-treated cells (n = 28; three cells). The mean value for the peak-to-peak capacitance noise increased from 5.0 fF (control) to 19.5 fF (with DPA). Therefore, the signal-to-noise ratio of the capacitance recording is about 1.5 times better in DPA-treated cells than in controls. Fig. 4 *B* shows examples of Cm recordings obtained from bovine adrenal chromaffin cells. Exocytosis was triggered by including 300 μM GppNHp in the patch pipette solution. The control recording (*upper trace*) shows that the Cm increases smoothly, with occasional steps of 3–10 fF (4.6 $\pm$ 0.7 fF, n = 18; noise level: 4.0 fF) (Robinson et al., 1994). Because of the size of the secretory granules (< 250 nm in diameter), the fusion of a single secretory granule with the plasma membrane does not cause a detectable increase in the membrane capacitance. So these large steps in capacitance presumably reflect the fusion of large secretory vesicles (~500 nm in diameter). The bottom trace shows a similar recording of a chromaffin cell treated with 10 μM DPA for 30 s. Superimposed on the gradual increase in Cm, many step increases are evident (31.8 $\pm$ 2.3 fF, n = 48, two cells; noise level: 16.5 fF). This experiment was difficult because as in mast cells the amplification effect of DPA was voltage-dependent with a maximum at about 0 mV. Unfortunately, in the majority of the cells tested, at this holding voltage the capacitance recording was very noisy due to the activation of ion channels. This problem limits the usefulness of the method because most secretory cells have active ion channels at this voltage.

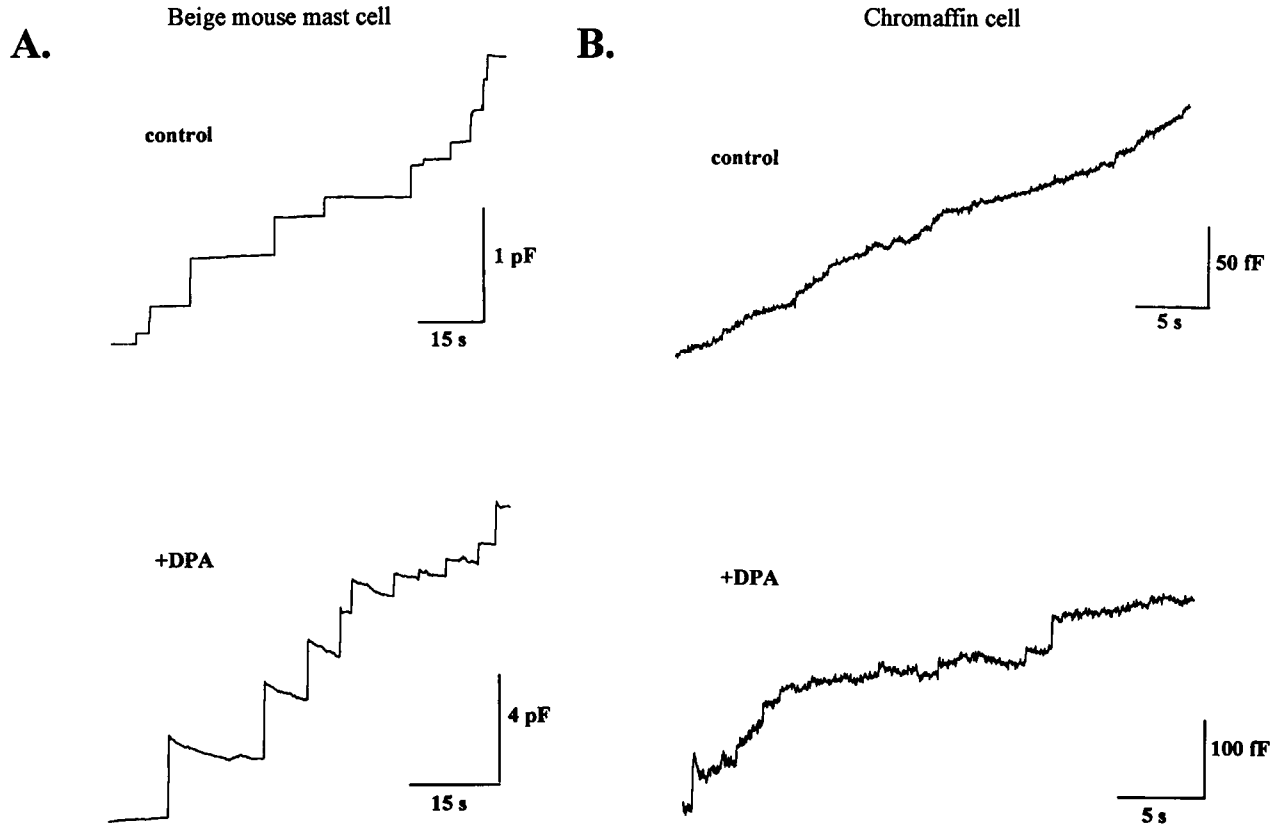

FIGURE 3 DPA improves the resolution of capacitive current increases resulting from exocytotic fusion events in beige mouse mast cells (*A*) and bovine adrenal chromaffin cells (*B*). (*A*) The upper trace corresponds to a complete degranulation recorded from a control beige mouse mast cell. The initial and final values of Cm were 5.1 pF and 9.3 pF, respectively. The bottom trace was recorded from a DPA-treated cell, where Cm increased from 22 pF to 40 pF. Exocytosis was stimulated by including 10 μM GTPγS in the pipette solution. (*B*) The upper trace is a recording of the increase in membrane capacitance of a control chromaffin cell undergoing exocytotic secretion (stimulated by GppNHp). Cm increased from an initial value of 3.5 pF to the final value of 4.1 pF in 8 min. Only a short section (35 s) of the secretory response is shown here. The holding potential was −70 mV. The bottom trace is a Cm recording obtained from a DPA-treated chromaffin cell. The initial capacitance was 17 pF, and the final value was 55 pF. The holding voltage was 0 mV.

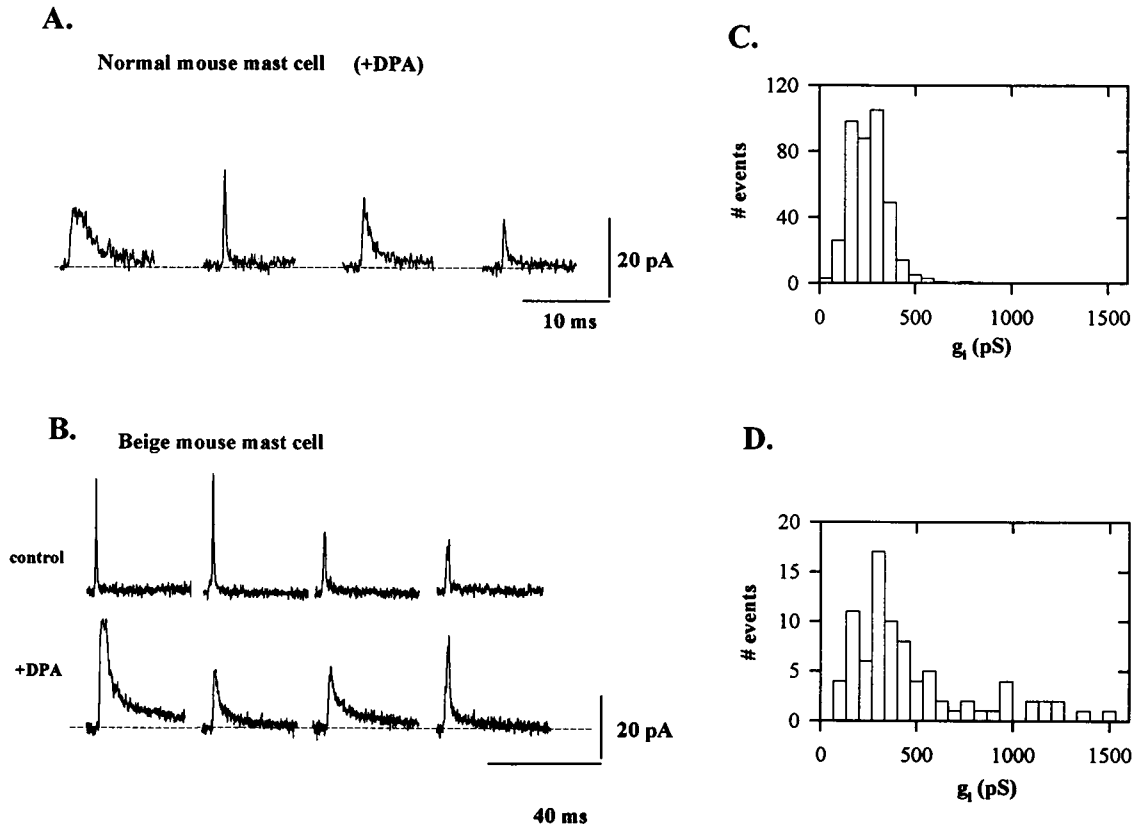

FIGURE 4 DPA amplifies the current transients that mark the opening of the fusion pore. (*A*) Membrane current recordings obtained from a degranulating normal mouse mast cell treated with DPA. Each spike represents the capacitive current that flows through the expanding fusion pore due to the different plasma membrane and granule membrane voltages; 211 current transients were recorded from this cell. (*B*) Current transients generated by individual fusion events recorded in a beige mouse mast cell. In control cells the current transients lasted <2 ms, whereas in DPA-treated cells they lasted for >20 ms. (*C*) Amplitude histogram of the initial fusion pore conductance (g_i) in normal mouse mast cells. g_i was calculated from the mean value of the first three points of the current transient (30 μs) divided by the total voltage across the fusion pore ($E_c - E_v$). The mean value was 256 ± 11 pS (n = 242; three cells). The bin-width was 66 pS. (*D*) Distribution of g_i in beige mouse mast cells calculated as in (*C*). The mean value was 390 ± 36 pS (n = 81; 12 cells).

In summary, DPA increases the secretory granule membrane capacitance more than the plasma membrane capacitance in three different secretory cells. DPA increased the signal-to-noise ratio of the capacitance recordings by 2.1 in normal mouse mast cells, 1.5 in beige mouse mast cells, and 1.7 in chromaffin cells.

Effect of DPA on the first milliseconds of the formation of the exocytotic fusion pore

During exocytosis, secretory vesicles of beige mouse mast cells generate a current transient that marks the opening of the exocytotic fusion pore. This transient is the result of the movement of charges necessary to make the granule membrane equipotential with the applied plasma membrane voltage. From this current transient it is possible to reconstruct the time course of the fusion pore conductance during its first milliseconds (Breckenridge and Almers, 1987). It was observed that the fusion pore grows in two phases: it opens very fast (<100 μs) to an initial conductance of about 300 pS and then the conductance increases more slowly (Spruce et al., 1990). Normal mast cell secretory vesicles are too small (<1 μm in diameter) to generate a detectable current transient, so the initial fusion pore conductance is unknown. Because DPA increases the granule membrane capacitance more than the plasma membrane capacitance in these cells, it should be possible to estimate the initial conductance of the fusion pore in DPA-treated mast cells.

After treatment of normal mouse mast cells with DPA, almost every fusion event generated a current transient (that is, more than 200 current transients per cell). In contrast, about 1% of the fusion events generated a current transient in a control cell (only four current transients, after the fusion of about 300 granules), which could not be analyzed because they were too fast (<50 μs or five data points). Fig. 4 *A* shows current transients generated by individual fusion events recorded in a degranulating mast cell. The increased granule membrane capacitance enlarged the transient capacitive discharge measured upon formation of a fusion pore (13 ± 2 fC; n = 16) in DPA-treated cells compared with 3.3 ± 0.4 fC (n = 4) in control cells.

This amplification effect of DPA on the transient capacitive discharge through the fusion pore was also observed in mast cells from the beige mouse. After preincubating beige

mouse mast cells with DPA, we found that the initial current amplitude is not significantly affected, but that the current decays with an additional slow component (Fig. 4 *B*). Without DPA the current transients had a highly variable decay rate that in most cases could not be described by a single exponential because of the changing fusion pore conductance (Spruce et al., 1990). The large majority of the current transients decayed to the baseline within 2 ms, with a time constant of about 500 μs (530 ± 79 μs; $n = 22$; range: 100 μs to 2 ms) (see also Spruce et al., 1990). After DPA treatment, the current transients lasted more than 40 ms and showed an extra time constant of 4.6 ± 1.1 ms ($n = 28$; range: 0.5–13.0 ms). This additional current is generated by the movement of DPA molecules across the membrane due to the redistribution to a new transmembrane voltage.

In order to reconstruct the time course of the fusion pore conductance from these data, the time course of the electrical potential across the fusion pore has to be calculated first from the time integral of the current divided by the capacitance steps that follow (Breckenridge and Almers, 1987). However, it is difficult to estimate the contribution of DPA charge movement to the current transient because it is voltage-dependent. The time course and amplitude of the voltage across the fusion pore will depend on several factors such as the fusion pore conductance, the vesicle size, and the intrinsic transmembrane voltage of the vesicle. These problems make the reconstruction of time course of the fusion pore conductance in DPA-treated secretory vesicles very difficult. However, as Fig. 4 *B* shows, DPA ions do not affect the initial amplitude of the current transient. Therefore, the initial conductance of the fusion pore was simply estimated from the amplitude of the current transient divided by the voltage across the fusion pore (potential across the cell membrane (E_c) minus the potential of the vesicle membrane (E_v)). We assumed a value of −75 mV for the granule potential (E_v, cytosol relative to lumen) because it was shown previously that beige mouse secretory granules and other secretory granules have a transmembrane potential of about −75 mV generated by a proton pump (Breckenridge and Almers, 1987; Johnson and Scarpa, 1984).

The distribution of the initial values of the fusion pore conductance for normal mast cells and beige mouse mast cells are shown in Fig. 4 *C* and *D*, respectively. The mean value of the initial fusion pore conductance in normal mast cell granules (256 ± 11 pS, $n = 242$) is not significantly different from the value in beige mouse mast cell granules (390 ± 36 pS, $n = 81$; or 329 ± 8 pS, $n = 620$) (Spruce et al., 1990).

Effect of tetraphenylborate on the membrane capacitance of mast cells

We also tested the effect of a different hydrophobic ion, tetraphenylborate, on the membrane capacitance of mast cells. We found that this hydrophobic cation increases the capacitive currents elicited by voltage pulses similar to DPA (Fig. 1), but the induced capacitive current decays were slower, with a time constant of about 5 ms at 0 mV (Ketterer et al., 1971; Fernandez et al., 1984a). Tetraphenylborate did not induce a significant increase in the Cm measured with the admittance technique. This is because the translocation rate of tetraphenylborate in the membrane is too slow to induce an increase in the capacitance current at the applied stimulus voltage frequency (830 Hz).

DISCUSSION

We have studied the effect of a hydrophobic ion, dipicrylamine, on the membrane capacitance of three different secretory cells: normal mouse mast cells, beige mouse mast cells, and bovine chromaffin cells.

We found that cells pretreated with an external solution containing DPA had an increased Cm. This effect on the membrane capacitance is expected for a hydrophobic ion. Hydrophobic ions such as DPA are able to spontaneously penetrate the hydrophobic core of the membrane, as the coulombic energy of the ion is compensated by the hydrophobic interaction with the membrane. The potential energy of a hydrophobic ion has a deep minimum in either membrane solution interface, the two minima being separated by a dielectric energy barrier. The charged groups of this ion increase the electric capacitance of the membrane as the hydrophobic portions of the ions become incorporated into the membrane core. As shown in Fig. 1, a simple one-barrier model adequately describes the translocation of DPA ions across the plasma membrane. The estimated values for the parameters used in the model (V_h, α) are in the range with the values reported in different preparations (Benz and Nonner, 1981; Fernandez et al., 1983). The value of V_h (about −10 mV) indicates that there is a small electrical asymmetry in the mast cell plasma membrane when the external voltage is zero, perhaps reflecting a different lipid composition between the membrane leaflets.

We also found that the DPA treatment produced an increase in the secretory granule membrane capacitance (Figs. 2 and 3). Surprisingly, however, DPA ions increase the capacitance of the secretory granules more than the capacitance of the plasma membrane, resulting in an increase in the signal-to-noise ratio. This increase was 2.1 in normal mast cells, 1.5 in beige mouse mast cells, and 1.7 in chromaffin cells. There are some simple explanations for the increase in the signal-to-noise of the capacitance recordings by DPA. 1) DPA works as a fusogenic molecule by inducing fusion between secretory granules. This is unlikely because the number of step increases in capacitance per cell were comparable in DPA-treated and in control cells (131 and 170, respectively) (Fig. 2). Moreover, in beige mouse mast cells, where the granules can be counted under the microscope, DPA does not cause a reduction in the number of the anticipated capacitance steps. 2) DPA increases the probability of fusion so that a single capacitance step could be made of two or more simultaneous granules fusing. This

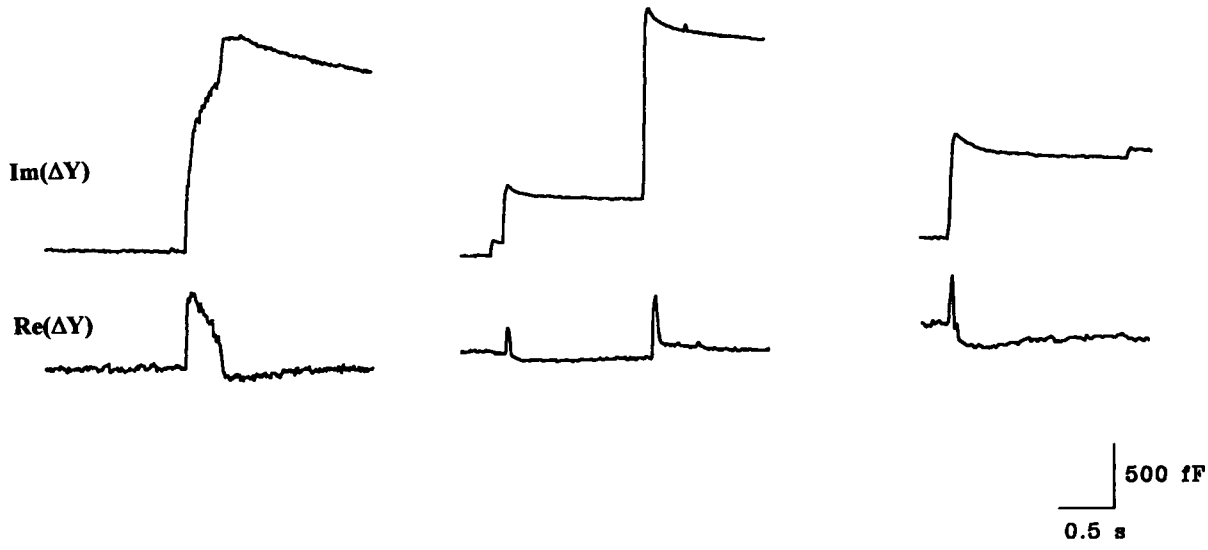

FIGURE 5 Relaxation of the Cm after single fusion events recorded in DPA-treated normal mouse mast cells. Different examples of the changes in admittance associated with the exocytotic fusion of single secretory granules are shown. Im(ΔY) is proportional to changes in membrane capacitance, and Re(ΔY) is proportional to changes in AC conductance. The calibration for Re(ΔY) is 2 nS.

is also unlikely because DPA ions do not significantly increase the rate of degranulation in normal mast cells (Fig. 2), beige mouse mast cells (Fig. 3 *A*), or adrenal chromaffin cells (Fig. 3 *B*). Also this would not explain the amplification effect of DPA on the current transients that mark the opening of the fusion pore (Fig. 4), since overlapping fusion events would increase the amplitude of the current transient but would not change the decay time constant.

We favor the idea that the increase in signal-to-noise ratio in the capacitance recordings induced by DPA is due to a difference in the physicochemical properties between plasma and granule membrane, such that DPA ions in the granule membrane would have a higher probability to respond to a change in voltage. The parameters that determine the contribution of a hydrophobic ion to the membrane electrical capacitance, V_h, α, and the translocation rate are strongly sensitive to the structural parameters such as thickness, dielectric constant, dipole potential, and the degree of order of the lipid hydrocarbon chains (Ketterer et al., 1971; Benz and Lauger, 1977; Reyes and Latorre, 1979; Fernandez et al., 1982). For instance, a faster rate of translocation of DPA in the secretory granule membrane would increase the signal-to-noise ratio of the capacitance recordings because this would result in more charges moved per unit of time, increasing the contribution to the capacitive current. This model predicts that upon fusion of the granule with the plasma membrane, the capacitance added by the granule should decrease with time as both membranes mix. Fig. 5 shows single fusion events recorded in mast cells treated with DPA, like the steps shown in Fig. 2, but with an enlarged amplitude and time scale. It is evident that after the step increase in capacitance, Cm decreases to about 90% of its initial value within 1 s. This relaxation of the Cm after a fusion event was also observed in beige mouse mast cells and chromaffin cells (see Fig. 3). We interpret the decline in the membrane capacitance after exocytotic fusion as the equilibration of the lipids of the granule membrane with the lipids of the plasma membrane. Another interpretation for the decay of the capacitance after exocytotic fusion would be that DPA molecules escape from the cell membrane, therefore reducing their contribution to the membrane capacitance. However, as mentioned before, the DPA-induced change in the capacitance is practically irreversible, as demonstrated by the stable baseline before the capacitance step (the washout of DPA from the cell membrane corresponds to a decrease in capacitance of only 1–5 fF/s).

The increased granule membrane capacitance enlarged the transient capacitive discharge measured upon formation of a fusion pore in normal mast cell granules (13 ± 2 fC in DPA-treated cells compared with 3.3 ± 0.4 fC in control cells). The amplification effect of DPA on the secretory granule membrane capacitance allowed us to estimate, for the first time, the initial conductance of the fusion pore in normal mast cells. We calculated that the conductance of the fusion pore, during its first 30 μs of existence, is about 260 pS, a value that is not significantly different from the one measured in beige mouse mast cell granules (Breckenridge and Almers, 1987; Spruce et al., 1990).

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