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Antibody activates cationic channels via second messenger Ca^{2+}

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Patch-clamp recordings were used to study single channels permeable to multiple cations in a macrophage cell line. At least three conductance levels were found, consistent with the existence of several types of nonselective cation channels or a single channel with multiple open states. The activity of the channels depended very little on voltage but was affected by internal Ca^{2+} concentration. Specific subclasses of immunoglobulins (IgG1 and IgG2b) bound to an Fc receptor on the surface of these macrophages. When an IgG2b was applied to the cell exterior after a patch pipette had been sealed in the cell-attached mode, the nonselective cation channels within the patch were activated. Thus, these channels must be modulated by a second messenger. Since antibodies binding to the Fc receptor have been shown to produce a rise in intracellular Ca^{2+}, this cation must be considered a candidate as a second messenger that amplifies the effect of antibody in gating these channels.

Introduction

The interaction of immune complexes of IgG1 or IgG2b with an Fc receptor on the surface of macrophages induces phagocytosis and release of substances that cause inflammation [1–4]. Concomitantly, the binding of immunoglobulins to this Fc receptor results in membrane depolarization [5]. The depolarization is mediated by an inward, nonselective cation current [6]. When Young et al. [7] incorporated this Fc receptor into a planar lipid bilayer, electrical measurements revealed single-channel currents that were carried by monovalent cations and activated by antibodies to the receptor. The channel had a single open state with a unitary conductance of 60 pS in 1 M KCl. This finding suggested that the Fc receptor can function as an ionophore or pore carrying the cation current and that this current may be involved in triggering phagocytosis and inflammatory responses [7]. In the present study the conductances affected by antibodies binding to the Fc receptor were characterized by single-channel recordings using the patch-clamp technique [8] on native macrophage membranes bathed in physiological solutions. Under these conditions, the antibodies influence the activity of either several types of nonselective cation channels or a single channel with multiple conductance states. None of the various states is strongly voltage dependent. During a cell-attached (on-cell) patch recording, the addition of IgG2b to the bath is associated with an increase in the amount of time channels spend in the open state. Because of the tight seal of the electrode to the membrane, the antibody does not have direct access to channels in the patch [9]. Thus, the effect of immunoglobulin must be mediated by a second messenger. When the same patch of membrane is excised from the cell to form an inside-out patch, as little as 0.2 μM

Abbreviations: Mes, 4-morpholineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*'-tetraacetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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internal Ca^{2+} can activate the channels. Hence, Ca^{2+} must become a candidate as a second messenger in this system.

Materials and Methods

Murine macrophages from the cell line $P388D_1$ were maintained in minimal essential medium with 10% fetal calf serum. Recordings were made within four hours of plating on glass cover slips placed in 35 mm plastic tissue culture dishes. During experimental sessions, the cells were superfused with a mammalian Na-saline solution at 20–23°C. 'Giga-seals' were obtained in the standard manner between the fire-polished tip of the recording pipette and a cell-attached patch of membrane [8]. Seals were typically in the 10–100 GΩ range. If there were no spontaneous openings of nonselective cation channels (using the criteria listed below), a monoclonal antibody of the IgG2b subclass was added to the bath using a microsyringe. Monoclonal antibodies were delivered as ascites or hybridoma supernatant fluid; in each experiment control ascites or supernatant fluid was also applied. If the antibody resulted in the activation of channels, the electrode was pulled away from the cell to form an inside-out patch. Thus, working with the same patch that contained immunoglobulin-activated channels, the effect of various ionic substitutions on the internal face of the membrane could also be evaluated.

The patch pipettes contained Na-saline or K-saline (see figure legends for composition). In some experiments Mes substituted for chloride [10], or Cs^+ and tetraethylammonium (Et_4N^+) replaced Na^+ and K^+. At other times an intracellular/extracellular salt gradient was formed to demonstrate the cation-selectivity of the channel [11,12]. In addition, 5 mM 4-aminopyridine and 20 mM Et_4N^+ were sometimes added to the extracellular solution (in the pipette for cell-attached patches) to block the delayed rectifier as well as the Ca^{2+}- and voltage-activated K^+ channels which were occasionally present in these patches from macrophages [13,14].

Results

Of 88 cell-attached patches, 32 did not display 'spontaneous' channel activity at rest (no potential applied to the pipette, $V_H = 0$ mV), or during voltage steps and ramps from −60 to +60 mV. The lack of single-channel events was determined from examiantion of 10 minutes of membrane current records analyzed on-line with a PDP-11/23 computer. It was important to document the absence of spontaneous channel activity because, as in other cell types [12], openings of the channels often occurred in clusters separated by prolonged closings of minutes duration. For channels with this kind of bursting kinetics, spontaneous events could be misinterpreted as drug-activation unless the patch is totally 'silent' during control periods which are presumably longer than the interburst interval.

When a cell-attached patch without spontaneous activity was encountered, an IgG2b immunoglobulin or immune complex was added to the bath, and an increase in channel openings was observed, as shown in Fig. 1. Similar results were found in 27 of 32 patches. The addition of antibodies to the bath induced channel openings after a relatively constant delay (mean ± S.D. 19 ± 3 s, $n = 62$ trials). Two different IgG2b antibodies were used (the gifts of Dodd, J. and Jessell, T.M.). Immunofluorescence studies have revealed that IgG2b antibodies bind to this macrophage cell line (Lipton, S.A., unpublished observations). Control supernatant and ascites fluid with immunoglobulins of another subclass (the IgG2a anti-rhodopsin antibody of C.J. Barnstable described in Ref. 15) did not activate channels in the same patches in which IgG2b antibodies did. Furthermore, in several patches held for longer than half an hour, the IgG2b effect was reversible after a few minutes, and repeated additions of the antibody re-activated channels with the same cation selectivity and unitary conductances. Fig. 1a shows a small (≈ 3 pA), inward single-channel current. The channel opened for about 10 ms with several rapid transitions to and from another larger state. There may in addition be an even smaller channel (< 1 pA inward current) with multiple openings throughout the sweep, but this conductance was too small to resolve adequately despite efforts to raise the gain and vary the command potential. Shortly after this trace was obtained, the 3 pA channel began to open more frequently (Fig. 1b and left-hand side of Fig. 1c). Actually, close inspection of Fig. 1b

reveals that the 3 pA level may represent the simultaneous open activity of two small channels. For example, just before the 8 ms closure of the channels in the middle of the record, the current level drops to about 1.5 pA. This small conductance will be called the first major state (γ_{m1}). With longer exposure to antibody, it became evident that in addition to γ_{m1} there existed a second and larger major conductance (γ_{m2}). This is seen clearly in the right-hand half of Fig. 1c with the larger current steps, $i_{m2} \cong 11$ pA. In Fig. 1d, i_{m2} was open for the duration of the voltage step and remained open for a few minutes at which time activity in the patch again abated. In addition, i_{m2} occasionally closed to another state (i_s) of approximately half the amplitude (Figs. 1c and e).

The i_{m2} level might arise as merely the superimposition of two i_s channels opening concurrently. At first this explanation seemed likely to be correct since the amplitude of i_{m2} was about twice that of i_s. Alternatively, i_s might represent a substate of the i_{m2} channel. To determine if i_s was possibly a subconductance of i_{m2}, it was necessary to collect thousands of events from stable patches. In Fig. 1f a total amplitude histogram of such an experiment shows that i_{m2} (second peak of the histogram) had a current level of ≈ 12.3 pA at +60 mV. The possible sublevel i_s (first peak at ≈ 6.2 pA) occurred far less frequently. Any theory to explain the various current levels in the histogram must account for this frequency distribution. Thus, if the i_{m2} level were to represent simultaneous openings of two i_s channels rather than a distinct conducting entity, this would require a large degree of cooperativity between i_s units [16]. This explanation remains possible but seems less likely since cooperativity to this extent between channels is without precedent in other systems. The third and fourth peaks of the histogram are approximately integral multiples of i_s and might, therefore, represent openings of multiple units, or double openings of the current levels seen in peaks one and two. For example, peak 3 (at 24.6 pA) might have resulted from the simultaneous opening of four i_s or two i_{m2} units. Similarly, peak 4 (at 31 pA) might be five i_s or one i_s plus two i_{m2} units. The simplest explanation would use the smaller number of units opening simultaneously. The fact that no peak was found corresponding to three i_s units (at ≈ 18–19 pA) is consistent with the notion that i_s is a sublevel of i_{m2} rather than an independent entity. The alternative that i_s units open one, two, four, and five at a time but not three at a time appears less feasible.

In addition, the smaller i_{m1} conductance might represent a separate channel type from that which underlies i_{m2} and i_s but with similar cation selectivity (see below). On the other hand, i_{m1} could be one of multiple conductances of a single channel with other open states, i_{m2} and i_s. At present it is not possible to distinguish definitively between these alternatives. For convenience these conductances will be referred to as separate open states, not necessarily implying, however, the existence of only a single-channel type.

It is important to consider the possibility that a putative sublevel like i_s might represent an artifact of multiple, rapid channel transitions that are inadequately resolved by the recording amplifier or attenuated by filtering. The chance that this error occurred here was made less likely by varying the rate of data acquisition from 8 μs to 1 ms (no external filtering or filtering with a Bessel characteristic at 200 Hz, respectively). This procedure did not appear to influence greatly the number of transitions to or the duration of i_s. Thus, to a first approximation, the i_s state did not appear to be strongly dependent on the frequency resolution of the recording equipment.

When a cell-attached patch that had responded to antibody was excised from the macrophage to the inside-out configuration, the ionic-selectivity and -activation of the channels could be examined. Stable inside-out patches were obtained from 15 of the 27 on-cell patches affected by antibody. The current-voltage (I-V) characteristics were similar for each state in the cell-attached and inside-out patch configurations (Fig. 2). Therefore, in view of the similarities between the properties of these conductances in the same patch of membrane in both the cell-attached and inside-out mode, it is quite feasible that the same channels were studied in each patch configuration. For example, in cell-attached and inside-out patches, the I-V relation of the smaller major state (γ_{m1}) did not vary greatly with ionic substitutions of Na^+, K^+, or Cs^+ and Et_4N^+; the unitary conductance was $\gamma_{m1} = 35$–45 pS (Fig. 2a). The true reversal poten-

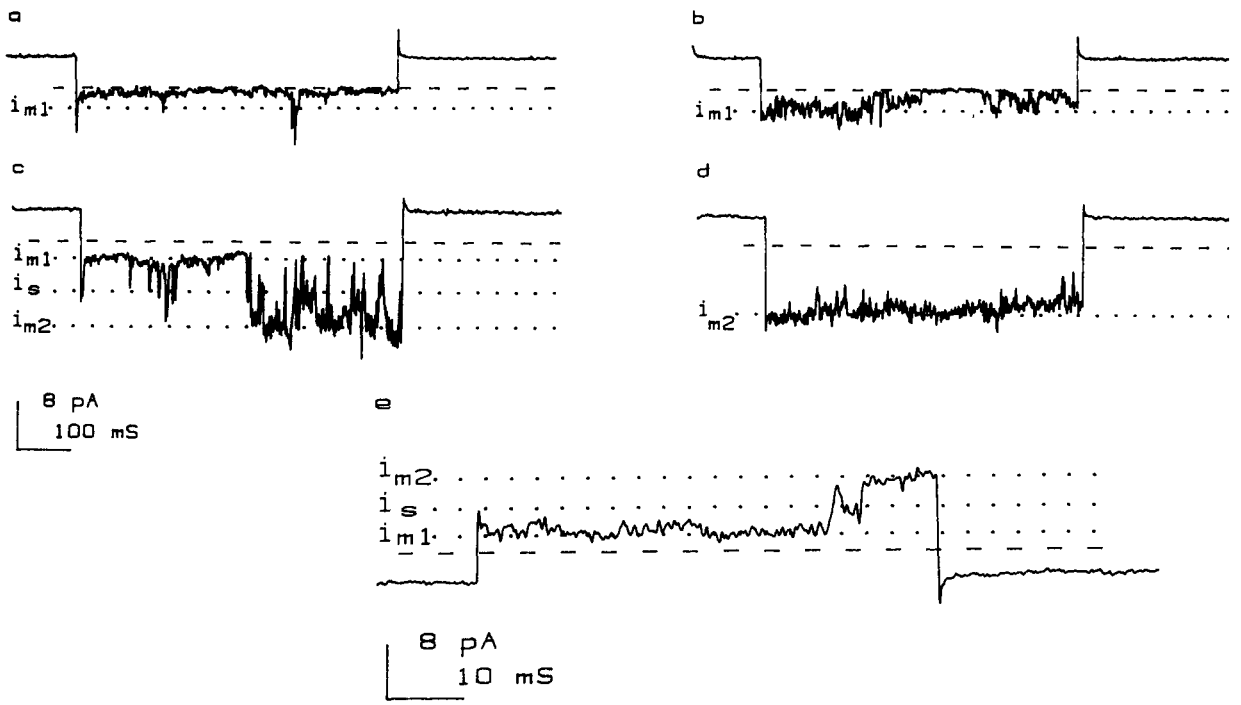
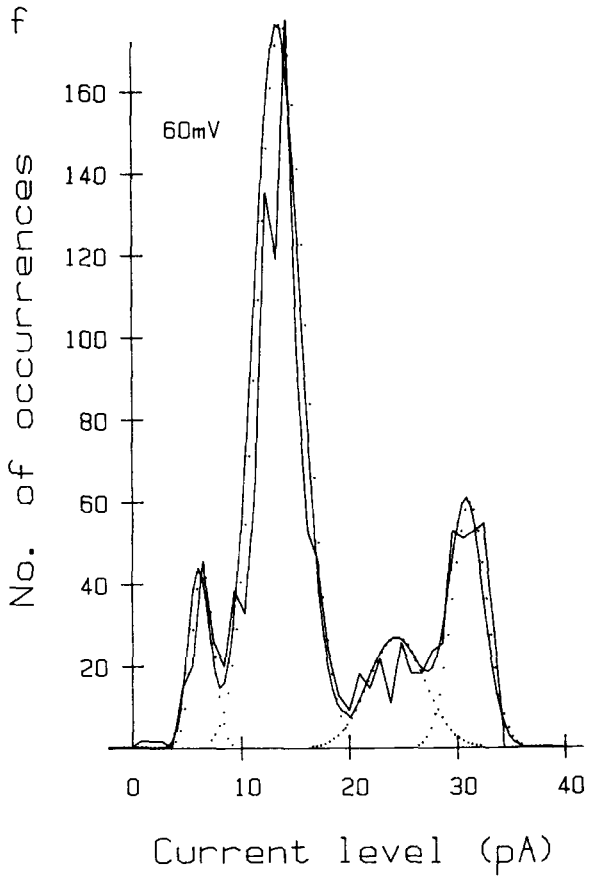


Fig. 1. IgG2b-activated channels in a cell-attached patch. Current flowing into the cell from the pipette is shown downward. After verifying that the patch had no spontaneous channel activity, ascites fluid containing an immunocomplexed IgG2b was added to the bath and superfusion was transiently halted. The traces shown in (a)–(d) were obtained 20, 50, 80, and 110 s later. Membrane potential is specified as the voltage on the intracellular side of the membrane relative to the extracellular. In records (a)–(d) the patch was initially held at rest ($V_H = 0$) and then transiently stepped 40 mV negative ($V_C = -40$) in order to approach the K-equilibrium potential and minimize those outward conductances. The patch was not held continuously at rest because this potential approaches the reversal potential of nonselective cation channels (see Fig. 2). In (e) the potential was modulated from $V_H = 0$ to $V_C = +40$ mV. The dashed line represents the closed state of the channel during the voltage pulse. The dotted lines are labelled with the appropriate open state (see text). Bath and pipette solution (in mM): 140 NaCl, 2 $CaCl_2$, 5 KCl, 4 $MgCl_2$, 10 Hepes, pH 7.2. Temperature 23°C. Sampling frequency 1 kHz (a–c) or 10 kHz (e). Low-pass filtered at 1 kHz (a–d) or 2 kHz (e) with a Bessel frequency cut-off characteristic of 48 dB/octave. (f) Total amplitude histogram (jagged line) from another patch. This histogram was constructed from consecutive traces that had the large conductance (i_{m2}) and its sublevel (i_s), but not the smaller conductance (i_{m1}) or closures. Note, however, that i_{m1} and closures were observed in other traces from this patch, and the lvel of i_{m1} (peak of 2.2 pA from histogram plots of other traces) was clearly smaller than that of i_{m2} or i_s. The patch was held at $V_H = +60$ mV. Single-channel currents were digitized at 10 kHz, filtered at 2 kHz, and corrected for leakage current. The dotted line in the histogram is the single Gaussian fit while the smooth line represents the summed Gaussians.

tial of the single-channel currents under these conditions was zero mV as discussed below. The second major state (γ_{m2}) and its possible sublevel (γ_s) also maintained a constant unitary conductance during ionic substitutions. Fig. 2b show that *I-V* relation of the sublevel which had a conductance of $\gamma_s = 120$–150 pS. The conductance of (γ_{m2}) was twice this size. A fairly broad range of the unitary conductance is given to reflect the large amplitude of the thermal-biological noise [17] that is characteristic of the open state of this channel.

The single-channel reversal potential measured on inside-out patches for the first major conductance (γ_{m1}) was about zero mV. This value was similar to that found in patches attached to cells that had been treated with IgG2b (Fig. 2a). On the other hand, in several cell-attached patches with spontaneous single-channel currents in the absence of an IgG2b, the *I-V* curve had a reversal potential between +15 and 20 mV (X's in Fig. 2a). This variance in the reversal potentials was readily explained by experiments in which a second patch



electrode, filled with K^+-saline, was sealed to the cell. By applying additional suction, a whole-cell current clamp recording was obtained in order to measure the resting membrane potential [8]. In the spherically-shaped macrophages used here within a few hours of plating, the resting potential was -15 to -20 mV (if cultured for longer periods, the resting potential became more hyperpolarized). When an IgG2b was added to the bathing medium, the whole-cell potential rapidly depolarized to between -5 and 0 mV. Thus, for a cell-attached patch in the absence of antibody, the single-channel reversal potential of $+20$ mV actually represented a trans-patch membrane potential of 0 mV when corrected for the cell's resting potential. In addition, this experiment showed that the *I-V* relation of the 'spontaneous' γ_{m1} conductance appeared identical to that of the antibody-induced conductance. The larger 'spontaneous' conductances (γ_{m2}, γ_s) also had similar *I-V* properties when compared to their antibody-activated counterparts (not shown).

The single-channel reversal potentials for the second major conductance (γ_{m2}) and its apparent sublevel (γ_s) were also 0 mV despite variation in the cationic content of the intracellular or extracellular compartments (Fig. 2b). The maintained reversal potentials near 0 mV for the conductances γ_{m1}, γ_{m2}, and γ_s during cationic substitutions are consistent with a channel permeation selectivity for cations, but anion selectivity must be considered. Three experiments mitigate against a significant permeability of anions. First, the reversal potential of antibody-activated channels in cell-attached patches was near rest (Fig. 2), not negative as would be expected for a Cl^- channel. Second, when all but 4 mM of the Cl^- on one or both sides of an inside-out patch of membrane was replaced by the relatively impermeant anion Mes, neither the reversal potential or unit conductances of any of the states changed. Finally, following the procedure of Yellen [12], a 5-fold salt gradient revealed single-channel selectivity for cations rather than anions. At 22°C with an intracellular solution of 150 mM NaCl and extracellular 30 mM NaCl with 240 mM dextrose, purely cation-selective channels would be expected to reverse at -39 mV but purely anion-selective channels at $+39$ mV. In four inside-out patches (that had previously displayed antibody-induced activity while on-cell) and in an additional five inside-out patches (that had had spontaneous activity while on-cell), all of the single-channel currents (i_{m1}, i_{m2}, i_s) reversed within 3 mV of -39 mV.

The voltage- and calcium-dependence of the nonselective cation channels were investigated on inside-out patches that were excised after antibody-activation of these channels had been demonstrated in the cell-attached configuration. When voltage ramps were applied to patches, the channels opened as both positive and negative potentials with approximately equal frequency and were not affected by changing the polarity of the ramp. In order to more rigorously study the relative voltage-independence of the channels, a series of voltage steps lasting 60, 600, or 1200 ms was applied from a holding potential of -20 mV to command potentials of -60 to $+60$ mV in 20 mV intervals. Concomitantly, the internal Ca^{2+} con-

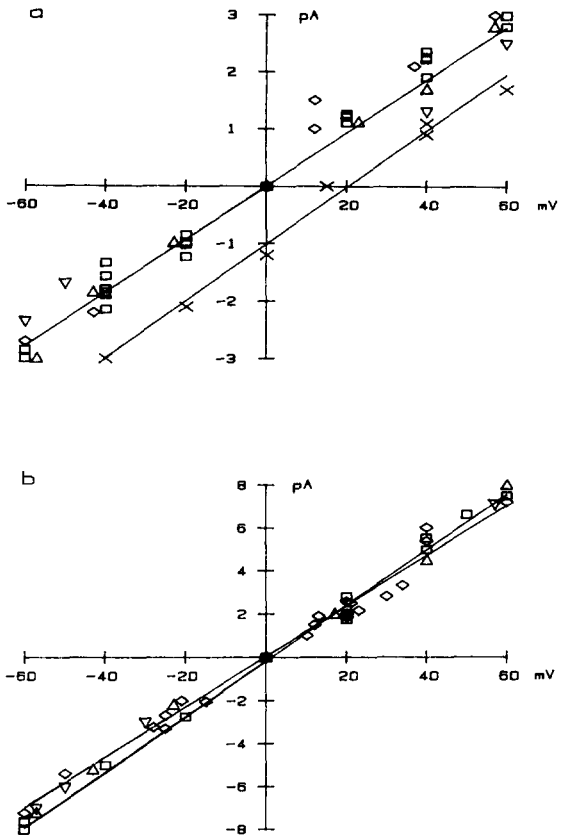


Fig. 2. Relationship between amplitude of single-channel current and membrane potential in symmetrical and asymmetrical cation solutions. Measurements were made at 20 mV intervals, but the symbols have been displaced slighly for clarity in some cases. (a) The single-channel currents associated with the smaller major conductance (γ_{m1}) are plotted. (b) The conductance of the possible sublevel (γ_s) of the second major conductance (γ_{m2}) is illustrated. ◇, Na-saline in pipette, K-saline with 10 μM Cs^{2+} in bath (inside-out patch). △, K-saline with 10 μM Ca^{2+} in pipette and bath (inside-out patch). ▽, K-saline in pipette, Ca^{+}-Et_4N^{+} with 10 μM Ca^{2+} in bath (inside-out patch). □, Na-saline in pipette, IgG2b in Na-saline bath (cell-attached patch). ×, Na-saline in pipette, no antibody in Na-saline bath (cell-attached patch). For cell-attached patches the listed membrane potential is the voltage applied to the patch without correction for the cell's resting potential. Each symbol represents the average amplitude of multiple channel openings in a separate patch. Data for each set were obtained from 16 records with 1024 points per record, digitized at 100 μs per point and filtered at 2 kHz. Straight lines are a least-squares fit. In (b) the lines for cell-attached and inside-out patches are both shown but are virtually the same. K-saline contained 140 mM KCl, 2 mM $MgCl_2$, 10 mM Hepes, and 10 μM free Ca^{2+} (measured by atomic absorption spectrometry), pH 7.2. The Cs^{+}-Et_4N^{+} solution had 120 mM CsCl and 20 mM Et_4NCl substituted for KCl.

centration was varied from 0.2 μM to 2 mM. The patches were allowed to equilibrate at the new Ca^{2+} concentration for at least two minutes before the voltage steps were initiated. When a group of these records is averaged, if the number of channels open were voltage-dependent then the mean

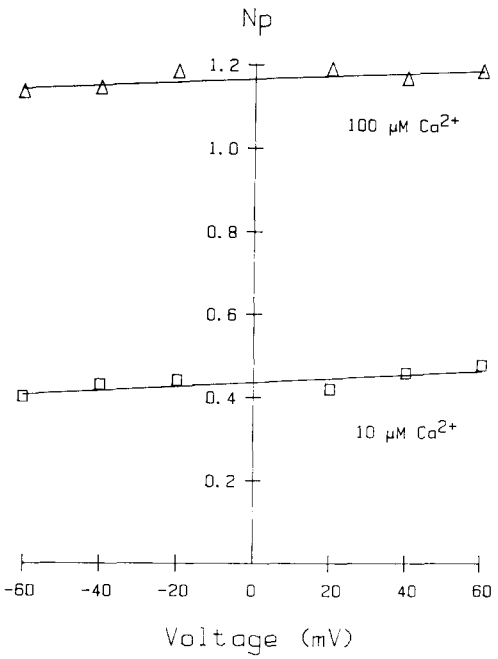


Fig. 3. Channel activity as a function of membrane potential and internal free Ca^{2+} for an inside-out patch. The activity of the largest state (γ_{m2}) is analyzed by integrating its open conductance for a period of time and then dividing by the integral of one open-channel conductance. The resulting measure of channel activity is expressed in units of Np, where N is the number of active channels (of the γ_{m2} amplitude) in the membrane patch and p is the probability of an individual channel of that type opening. Single-channel currents were recorded at a sampling frequency of 100 μs and filtered with a Bessel characteristic at a 2 kHz setting. Each data point represents the average level of channel activity in 64 traces (1024 points each). Channel activity changed only slightly as the voltage was varied from −60 to +60 mV but increased markedly when internal Ca^{2+} was raised from 10 to 100 μM. No data points are shown at 0 mV because this represents the reversal potential of the single-channel current, and therefore no openings were observed. The straight lines are a least squares fit, and the shift with increased Ca^{2+} is typical of results in other experiments. For this particular patch, the amplitude histogram at −60 mV (not shown) revealed peaks corresponding to current levels of a single i_{m1} channel, one and two i_{m2} channels, as well as a sublevel, i_s. Because i_{m1} is significantly smaller than i_{m2} or i_s (see Fig. 1), these various levels could be distinguished.

current would relax towards a new steady-state value following the step in membrane potential [11]. However, when 16 records at each potential were averaged, there was little relaxation of the mean current toward a new level (< 10% relaxation at each command voltage from −60 to +60 mV). This finding indicates that the channels were not strongly voltage dependent under these conditions. Of course these data must be interpreted with the caveat of the settling time of the capactive transients (≤ 100 μs), the rate of digitization (100 μs per point), and the filter settings (2 kHz). Thus, relaxations with a time course of less than 100 μs would not have been detected. Similarly, very slow

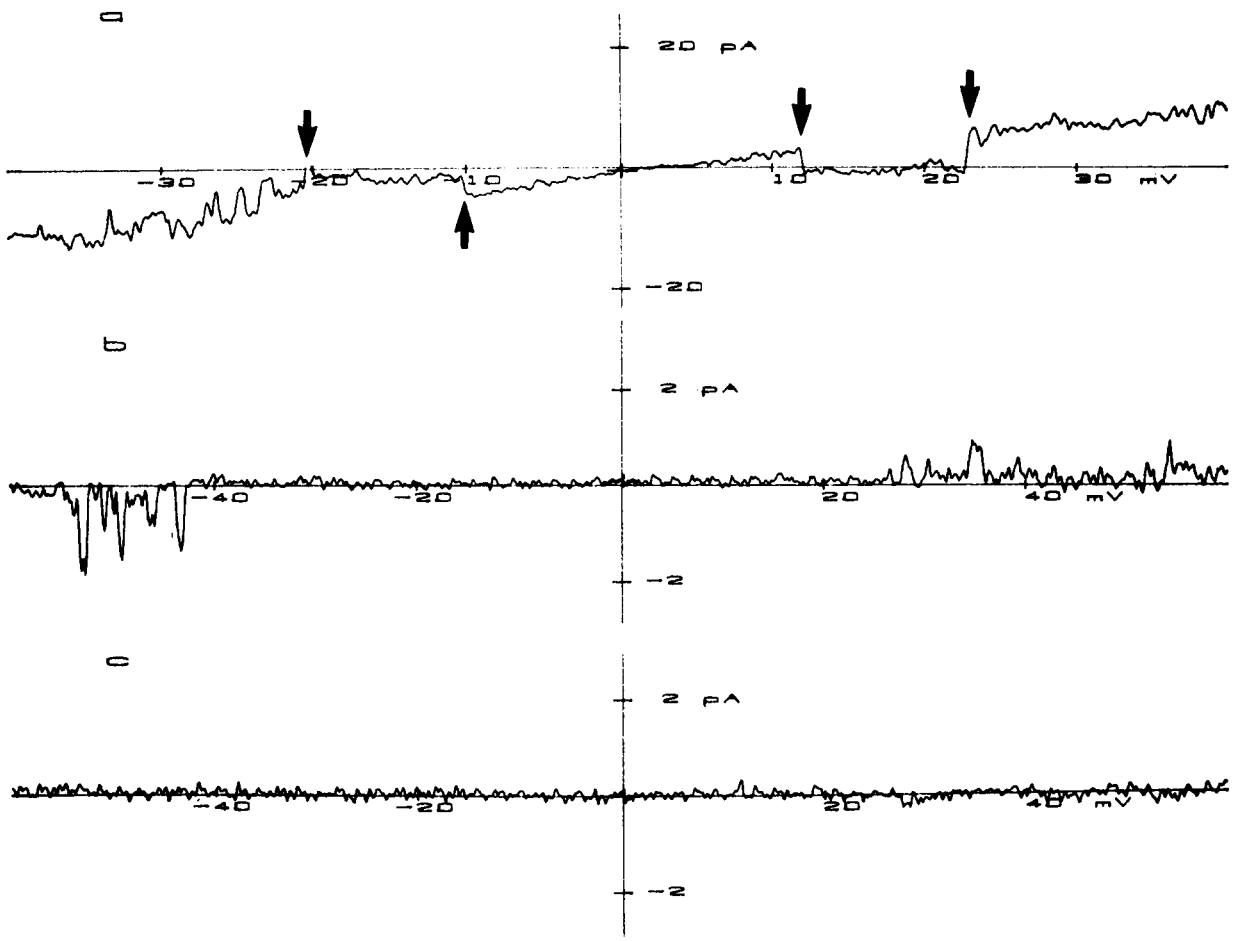


Fig. 4. Calcium-dependence of nonselective cation channels in an inside-out patch. A ramp potential was applied under voltage clamp. Each trace presents a single-channel current during the ramp clamp. When the channel opened, there was a rapid transition from the leakage level to the open channel level. Similarly, when the channel closed, the current quickly returned to the leakage level. Since the average leakage current had been subtracted, the leakage level was zero. (a) An inside-out patch was formed with K-saline plus 0.2 μM Ca^{2+} in the bath and Na-saline in the pipette. Thirty seconds after excision of the patch from the cell this ramp was obtained. Beginning on the left-hand side of the sweep, the open channel current flickered between states γ_{m2} and γ_s several times before closing (first arrow). The current only very briefly remained at zero because of the opening of another small conductance, γ_{m1}. Also, the compensation for leakage was not perfect. At the second arrow, γ_{m2} opened again, passed through zero while open, closed at the third arrow, and opened for the remainder of the record at the final arrow. (b) The same patch 45 s after excision had transitions or partial openings apparently only to γ_{m1} (note change in amplitude scale). (c) Beyond 45 after formation of the patch, the channels were closed and remained closed. This ramp was obtained 1.5 min after excision. Similar ramps lacking any single-channel activity were obtained for the ensuing 20 min. These ramps were performed over one second and filtered at 315 Hz. In traces not shown, ramps were also obtained over 100 ms and had similar characteristics.

relaxations (occurring over a period greater than the maximum length of these records, 1.2 s) would have gone undetected.

Using another approach, Fig. 3 also shows the relative voltage-independence of channel openings at two different Ca^{2+} concentrations. In addition, this figure demonstrates that channel activity is influenced by the level of Ca^{2+} on the internal side of the membrane. Isolated patches in these experiments contained not only more than one size of nonselective conductance but often multiple units of a given size. Thus, channel activity for a conductance of particular amplitude (e.g., γ_{m2}) is expressed in terms of Np, where N is the number of active units of that type in the patch and p is the probability of a single unit opening [18]. Raising the transmembrane potential affected channel activity only very slightly. Raising the level of internal Ca^{2+}, however, increased channel activity for each of the nonselective cation conductances although only the results for γ_{m2} are shown. Nevertheless, the concentration of Ca^{2+} necessary to show this effect is quite high, tens of micromolar, and therefore probably supra-physiological. Thus, further experiments were designed to study the effect of internal Ca^{2+} levels in the physiological range.

The results of one such experiment are shown in Fig. 4. The perfusion chamber was equilibrated in low Ca^{2+} solution for several minutes after the demonstration of antibody-activated channels in a cell-attached patch but before excising the patch from the cell. By using potential ramps it is often possible to identify a channel from its I-V characteristics in a single sweep. Therefore upon excision, voltage ramps were applied to the patch in order to rapidly assess channels that might be transiently active. If an inside-out patch was formed in a bath containing 10^{-8} M Ca^{2+} (1 mM $CaCl_2$, 11 mM EGTA, pH 7.2, calculated as in Refs. 19, 20), the channels in the patch remained silent. On the other hand, immediately after excision of an inside-out patch in 0.2 μM Ca^{2+} (1 mM $CaCl_2$, 1.5 mM EGTA, pH 7.2), openings of nonselective cation channels were present (Fig. 4a), but after 30–45 s they all closed and remained closed for the duration of the recording (Fig. 4c). At this point, however, raising the internal Ca^{2+} (to 10 μM for example) re-activated the channels. If exposed to 2 mM internal Ca^{2+}, the channels displayed vigorous opening and closing activity, and washing the chamber in 10^{-8} M Ca^{2+} for 20 min did not completely quench the openings ($T_{1/2}$ of washout is 20 s $\pm$ 15% from dye studies). These findings after exposure to high Ca^{2+} could possibly indicate that the channels may bind Ca^{2+} very tightly, prolonging the apparent rundown in low Ca^{2+} superfusates. The relatively rapid rundown of the channel openings in patches excised in chambers previously equilibrated with 0.2 μM Ca^{2+} would mitigate against a problem involving inadequate rinsing of the internal face of the membrane. Quite to the contrary, this rundown of activity may have occurred because of the dilution of a component of the natural milieu that is necessary for maximal calcium sensitivity. In any event, these experiments show that under these conditions the nonselective cation channels are active at least transiently in 0.2 μM internal Ca^{2+}.

Discussion

The nonselective cation channels described here have some properties in common with those reported previously by Colquhoun et al. [10] in heart cells, Yellen [12] in neuroblastoma, and Maruyama and Petersen [21] in pancreatic acini. The transient sensitivity of channels in inside-out patches to calcium concentrations of 0.2 μM agrees with results described in a recent review [22]. However, the present study is the first to describe multiple conductances that are permeable to monovalent cations. Whether these conductances represent multiple channel types with similar cation selectivity or substates of a single type of channel remains to be determined. The novel feature of these conductances is their activation by immunoglobulins of the IgG2b subclass. Channels in a cell-attached patch can be activated by antibody applied to the cell outside of the patch. Since these channels are not strongly voltage-dependent, it cannot be the depolarization of the cell by IgG2b that triggers channel openings, suggesting that a second messenger can activate the channels. The activation of channels with similar I-V characteristics in inside-out patches by internal Ca^{2+} makes this ion a candidate for a second messenger in this system. Further support for this idea comes from recent

evidence that immunoglobulin ligands binding to the macrophage Fc receptor for IgG1 and 2b result in a transient elevation in intracellular Ca^{2+}, measured with the fluorescent dye quin-2 [23]. The Ca^{2+} appears to originate in part from internal stores since buffering extracellular Ca^{2+} to low levels with EGTA does not completely block the effect. The increase in Ca^{2+} concentration induced by ligands to the Fc receptor [23] is similar to the minimal level of Ca^{2+} found to activate the cation conductances in inside-out patches in the present study. The rise in intracellular Ca^{2+} could also be important in initiating phagocytosis and the secretory process releasing soluble mediators of inflammation, as well as other macrophage responses. The mechanism(s) for the increase in intracellular Ca^{2+} remains obscure; one possibility is that the enzymatic activity that is associated with the Fc receptor (phospholipase A_2) [24] results in an arachidonic acid congener that mobilizes internal Ca^{2+}. Other possibilities include influx of a small concentration of Ca^{2+} through the nonselective cation channel despite its low permeability to Ca^{2+}, or depolarization-induced Ca^{2+} flow through Ca^{2+} channels [23]. In addition, the findings of the present study do not preclude the possibility that the antibody can also activate nonselective cation channels directly by binding to the Fc receptor or that the Fc receptor constitutes a channel as proposed by Young et al. [7]. Second messenger-activation of channels might represent an additional amplification step in the action of the antibody. Other cells beside macrophages, such as particular subclasses of neurons, possess similar Fc receptors [25]. It will be important to determine if immunoglobulins binding to this receptor on other cells activate nonselective cation channels that are sensitive to internal Ca^{2+}.

Note added in proof (Received February 14th, 1986)

After acceptance of this paper, evidence was reported that other channel types have multiple conductance states that function co-operatively [26]. A similar explanation may underlie the multiple peaks observed in Fig. 1f of the present article.

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