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Patch-Clamp Profile of Ion Channels in Resting Murine B Lymphocytes

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Summary. Patch-clamp studies of single ion channel currents in freshly isolated murine B lymphocytes are characterized here according to their respective unitary conductances, ion selectivities, regulatory factors, distributions and kinetic behavior. The most prevalent ion channel in murine B lymphocytes is a large conductance (348 pS) nonselective anion channel. This report characterizes additional conductances including: two chloride channels (40 and 128 pS), a calcium-activated potassium channel (93 pS), and an outwardly rectifying potassium channel which displays two distinct conductances (18 and 30 pS). Like the anion channel, both chloride channels exhibit little activity in the cell-attached patch configuration. The kinetic behavior of all of these channels is complex, with variable periods of bursting and flickering activity interspersed between prolonged closed/open intervals (dwell times). It is likely that some of these channels play an important role in the signal transduction of B cell activation.

Key Words anion channel · potassium channel · chloride channel · patch clamp · single-channel recording · murine B lymphocytes

Introduction

Crosslinking of membrane immunoglobulin (m)Ig on B lymphocytes stimulates B lymphocytes to enter the cell cycle. Recent studies have also implicated a number of second messenger systems in the transduction of the growth-promoting signal. Using mIg crosslinking to activate B cells, diacylglycerol and 1,4,5-trisinositolphosphate have been identified as primary second messengers responsible for an increase in intracellular Ca^{2+}, and activation of phosphokinase C which is, in turn, thought to be responsible for the transition of B cells from G_o into S phase (Bailey & Siu, 1987). Moreover, rapid membrane depolarization occurs after crosslinking of mIg (Monroe & Cambier, 1983). These observations suggest a role for ion channels as important components of initial signal transduction events. Only a few reports at this time have characterized ion channels in resting B cells and have explored their functional roles. Among the channels currently reported are a large conductance nonselective anion channel (Bosma, 1989; McCann et al., 1989*a*) and a cyclic AMP modulated potassium channel (Choquet et al., 1987). Voltage-gated calcium channels have been identified in neoplastic lymphocytes (Fukushima & Hagiwara, 1983; Fukushima, Hagiwara & Saxton, 1984), where they are involved in regulation of the cell growth cycle (Fukushima & Hagiwara, 1985). In human tonsilar B lymphocytes, a chloride conductance has been related to volume regulation (Grinstein et al., 1983). The significance of differences observed in the expression of ion channels in lymphocytes obtained from various sources (i.e., human lymphoid tissue, murine splenic cells, hybridomas derived from fusion with myeloma cells) remains to be determined. We have selected normal B lymphocytes freshly isolated from murine spleens in order to establish a data base from which to evaluate changes in ion channel conductances induced by mitogens, ligands for membrane immunoglobulin, and lymphokines. We have thus, constructed this profile of ion channel conductances and their respective biophysical characteristics.

Materials and Methods

Isolation of B Lymphocytes

B cells were prepared from the spleens of 8–12 week-old DBA/2J and C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME). Spleens were dissociated into a single cell suspension and erythrocytes were lysed by treatment with Tris-buffered ammonium chloride. T cells were cytotoxically eliminated by treatment of the spleen cells with anti-Thy 1.1 (HO-13.2; American Type Tissue Culture Assoc., Rocklyn Pike, MD) and a complement (Accurate Scientific, Westbury, NY). Viable cells were isolated by flotation on Ficoll-Hypaque (Sigma Chemical, St. Louis, MO). Purity of the cell preparation was verified by cytofluorimetry and found to be 95% B lymphocytes.

Preparation

Electrodes were pulled from cleaned Corning #7052 glass capillary tube and coated with Sylgard 184 (Dow Corning). Tips were fire polished to resistances of 5 to 8 MΩ. Suction was applied by way of a syringe coupled to a manometer and the electrode holder. Freshly isolated B lymphocytes were placed in a chamber (0.15 ml volume) which was equipped with multiple pipettes that made individual connections with an array of test solutions. This method provided rapid exchange of the bath. Junction potentials were balanced out by way of a bath amplifier (Dagan) coupled through a 3 M KCl-filled conventional microelectrode. Bath ground was a silver-chloride wire coupled to the bath solution via an agar-bridge. A volume of 0.15 ml of B lymphocytes (10^6) was placed in the chamber and the cells were then washed very gently with physiological saline in the perfusion system. The chamber was placed on the stage of a Zeiss inverted phase microscope where cells were observed with a 40× water immersion objective in conjunction with 16× oculars. Inside-out patches were achieved by rapidly pulling the small section of membrane sealed to the pipette away from the cell. All experiments were carried out at room temperature (22–24°C).

Solutions

All solutions were made with reagent grade chemicals and distilled de-ionized water. Stock solutions were filtered through 0.45 μm nitrocellulose (Millipore, MA) and stored at 4°C. Solutions for filling the bath and patch electrodes were again filtered through 0.22-μm syringe filters immediately prior to use. The pH of the solutions was adjusted to 7.4 using a minimum of the base whose counter ion was already present in high concentration. Both EGTA and HEPES were used to buffer Ca^{2+} and pH, respectively. Except where noted, patch experiments were conducted in normal physiological gradients. Extracellular solution for these gradients consisted of (in mM): 140 NaCl, 4.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES buffer. Intracellular solution consisted of (in mM): 140 KCl, 4.5 NaCl, 0.10 $CaCl_2$, 1 $MgCl_2$, 1.1 EGTA, and 10 HEPES buffer. Ion substitution experiments were conducted as previously described (McCann et al., 1989*a*).

Data Acquisition

Single-channel currents were measured in either a cell-attached or inside-out (*i/o*) patch configuration using the model 8900 patch clamp/whole cell clamp head stage and amplifier circuit (Dagan, Minneapolis, MN). Only seal resistances greater than 1 GΩ, measured with a 20-mV square wave test pulse, were used for recording.

Data were recorded on an IBM AT equipped with a hard disk via a 12 bit a/d converter (Data Translation, DT2801a). The program used for data collection was as previously described (McCann, Stibitz & Keller, 1987*b*). Cells were held at a predetermined membrane voltage close to the resting potential, then the command potential was stepped by 10-mV increments and channel activity was recorded at each membrane potential for 20 sec. To allow recovery of the membrane from each command pulse, the voltage was returned to the holding voltage for a period of 20 sec. In all experiments data were filtered at 300 Hz and digitized at 1 kHz.

Analysis of Single-Channel Data

Data were analyzed for conductance, selectivity, and kinetics with a computer program as previously described (McCann et al., 1987*b*). All measurements cited in the results section of this paper are reported with the number of observations (n) and standard error in the 95% confidence range. Reversal potentials were derived for each experiment by extrapolating I *vs.* V curve to zero current. Conductances were calculated from the slope of the linear regression line of the I *vs.* V curve at points closest to E_R. For nonrectifying conductances, only I *vs.* V curves with regression coefficients >0.95 for the root mean squared test were included in this report. Relative permeabilities of recorded channels to ions in the bath and pipette solutions were determined using the Goldman-Hodgkin-Katz equation as described by Hille (1984):

$$E_{rev} = \left(\frac{RT}{ZF}\right) \ln \left(\frac{(P_K[K]_o + P_{Na}[Na]_o + P_{Cl}[Cl]_i)}{(P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_o)}\right).$$

Kinetic measurements were calculated from half-amplitude crossings of single-channel currents to baseline current. If more than one channel was present, the number of channels in the patch was estimated by finding the maximum number of discrete current steps of the appropriate amplitude for all recordings in a given patch. This method does not negate the possibility of the existence of additional channel(s) in the patch. The probability of a channel being open (percent open time) was determined by: $\sum_1^N (nP_n)/N$ (Palmer & Frindt, 1986), where N is the number of channels estimated to be in the patch and nP_n refers to the probability of n of the N channels in the patch being open as determined by percent open time for n channels. Similarly, frequency of opening was defined as the number of half-amplitude crossings to the open current level, divided by the number of channels in the patch. All voltages presented follow the convention of referencing the inside of the cell membrane with respect to the outside of the cell membrane, and inward currents are downward, outward currents upward.

Significant differences arising from experimental variables in conductance, reversal potential, and percent open time were determined by the unpaired Student's t test. Experimental data with p values less than 0.05 were considered significantly different.

Results

Previous studies of inside-out patches, obtained from freshly isolated untreated B lymphocytes, characterized a large conductance (348 pS) anion channel which was nonselective to aspartate (McCann et al., 1989*a*). This channel has also been characterized in a mouse cell line (Bosma, 1989). Through additional experiments, we now characterize four additional channels according to their respective single-channel conductance, ion selectivity, regulatory factors and kinetics. These channels include: a 40-pS chloride channel, a 128-S chloride channel, a 93-pS calcium-activated potassium channel, and an outward rectifier potassium channel with two predominant conductances (18 and 30 pS).

Table. Summary of channel characteristics

Channel	Conductance (pS)	Selectivity	Kinetic effectors			Incidence (n = 221)
			Voltage	$[Ca^{2+}]_i$	Excision	
Anion	348 ± 4.4	$Cl^- > Asp > Na^+ > K^+ > TEA$	yes	no	yes	87
Outward-rectifying K^+	18.5 ± 1.4	$K^+ > Na^+ > Cl^- > Asp > TEA$	yes	slight	no	20
	30.1 ± 2.7	$K^+ > Na^+ > Cl^- > Asp > TEA$	yes	no	no	6
40-pS Cl^-	40.2 ± 3.2	$Cl^- > Asp > K^+ > Na^+ > TEA$	yes	no	yes	23
128-pS Cl^-	128.0 ± 14.6	$Cl^- > Asp > K^+ > Na^+ > TEA$	slight	no	yes	9
$[Ca^{2+}]_i$-activated K^+	93.3 ± 6.2	$K^+ > Cl^- > Na^+ > Asp > TEA$	yes	yes	yes	7

We present individual characterizations for each of these channels and describe a composite profile of ion channels in murine B lymphocytes. A summary of these data is shown in the Table.

Outwardly Rectifying K^+ Channel

An outwardly rectifying K^+ channel exhibiting two distinct conductances, 18.5 ± 1.4 pS (n = 14) and 30.1 ± 2.7 pS (n = 6), was found in both cell-attached and *i/o* patches. No significant change in kinetic behavior occurred as a result of patch excision. In the majority of our experiments where this channel was observed, only one conductance was observed in the current recordings. The 18-pS conductance was usually found as a single unitary conductance with no evidence for an associated 30-pS conductance (n = 14). Conversely, opening and closing of the 30-pS conductance frequently exhibited fast transitions (flickering) to/from the 18-pS conductance (n = 6). Because these experiments displayed only occasional dwell times in this apparent 18-pS subconductance state, amplitude histograms were used to elucidate both conductances. Figure 1*A* shows an experiment in which both K^+ conductances are clearly visible. In these current recordings, the 18-pS conductance appears hidden in the fast transitions to the 30-pS open state from −40 to −20 mV. At −10 mV the 30-pS conductance partially inactivates down to the 18-pS conductance and prevails at all subsequent depolarizing voltages. The *I/V* curve (Fig. 1*B*) shows that both conductances have the same E_{rev} and display the outward rectification characteristic of these channels. Furthermore, the conductances are exclusively selective to K^+ (P_K/P_{Na} = 28 : 1, P_K/P_{Cl} = 13 : 1) and are blocked by both TEA and Ba^{2+}. Ca^{2+} added intracellularly induced a slight decrease in the percent open time of the 18-pS conductance. Both conductances display classic voltage-gated kinetics but are active in distinct voltage ranges. In particular, greater depolarizing pulses are required to activate the 18-pS conductance than the 30-pS conductance. (Fig. 1*C* and *D*). The 30-pS conductance generally exhibits much faster kinetics than the 18-pS conductance. Both conductances increase with depolarizing voltages but do so at rates which bear no similarity. The possibility that both K^+ conductances are independent channels with the same ionic selectivity is negated by the deviation of the amplitude histogram from the predicted binomial distribution for two independent channels (Fig. 1*E*). Similar deviations were observed for the majority of current traces exhibiting one or both of these K^+ channel conductances. Although cooperativity between these two conductances is evident, they each display occasional long dwell times in their respective open states and thus do not fall into the category of conventional subconductance states.

40-pS Cl^- Channel

A 40.2 ± 3.2 pS (n = 7) chloride channel was measured in *i-o* patches. This channel, like the large conductance anion channel, was frequently inactive in the cell-attached patch configuration, becoming active with excision of the patch. The channel was voltage sensitive and exhibited greater kinetic activity at positive than at negative command potentials. Occasionally, large depolarizing pulses induced channel activity in the cell-attached configuration. Figure 2*A* shows typical current recordings of Cl^- influx at positive voltages in symmetrical Cl^- gradients. The corresponding *I/V* curve for this experiment displayed outward rectification characteristic of this channel (Fig. 2*B*). The channels were highly selective for Cl^- (P_{Cl}/P_{Na} = 12, P_{Cl}/P_K = 16, P_{Cl}/P_{Asp} = 16), which is illustrated by the resulting shift of E_{rev} to E_{Cl} with aspartate substituted for Cl^- in the bath (Fig. 2*C*). Although percent open times for

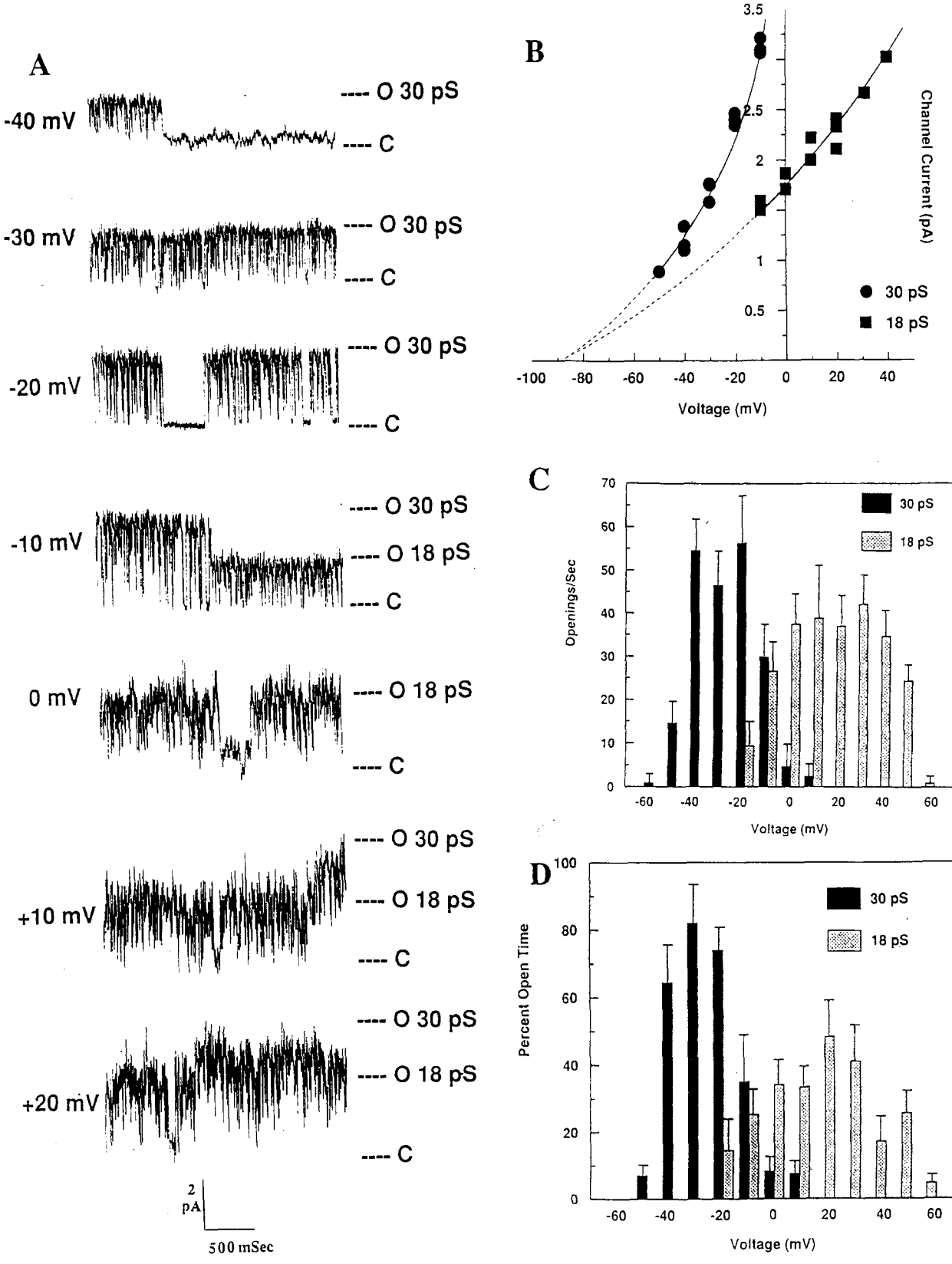

Fig. 1. *For caption to Fig. 1 see next page*

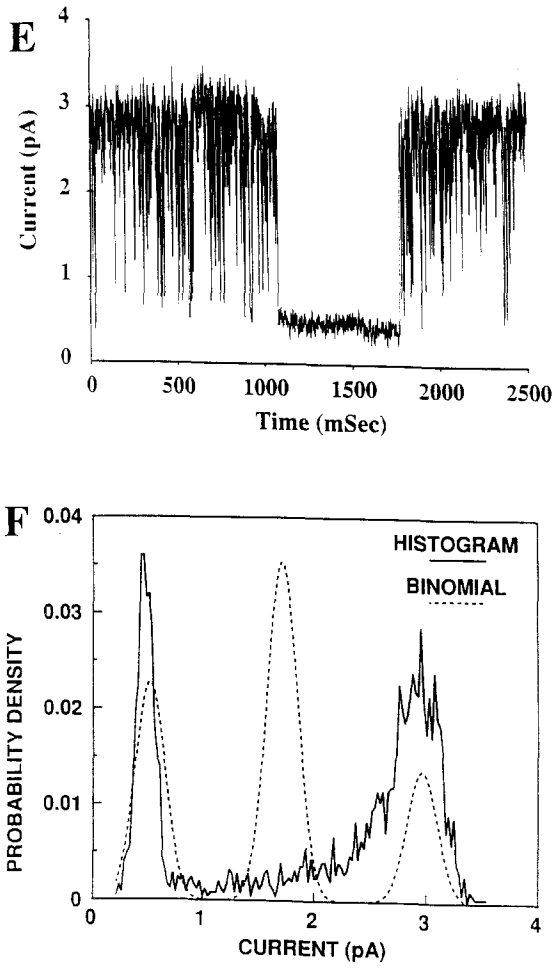

Fig. 1. Characterization of outward-rectifier K^+ conductances. (*A*) Current recordings in an *i/o* patch in normal physiological gradients displaying both 18- and 30-pS conductances. In the first three recordings, only the 30-pS conductance prevails. Transient flickering to the 18-pS conductance can be resolved only via amplitude histograms (*cf.* methods). At −10 mV the 30-pS conductance partially closes to the 18-pS conductance which predominates over subsequent depolarizing voltages. (*B*) *I/V* curves showing the K^+ selectivity and characteristic outward rectification of both conductances (E_K = −86 mV). (*C, D*) Histograms of frequency of channel opening and percent open time, respectively, for both conductances. Each conductance displays kinetics which are classically voltage gated in respectively distinct ranges of voltage (n = 4). (*E, F*) Cooperativity between conductances. (*E*) A 2.5-sec current trace from which analysis in *F* was used. (*F*) Amplitude histogram (solid line), elucidating distinct current amplitudes in the recording. The binomial distribution (broken line) indicates the predicted current amplitudes for two independent channels in the patch, each with a probability of opening derived from the largest current amplitude measured in the amplitude histogram. The binomial was fitted to a normal distribution with variance determined from baseline current in *E*. The deviation between the two curves negates the possibility of independent conductances and suggests cooperativity between two subconductances which collectively define the 30-pS K^+ conductance

this chloride channel (Fig. 2*D*) resembled those of the other channels described in this paper, it exhibited kinetics which were typically slower (Fig. 2*E*) with erratic dwell times (Fig. 2*A*). Our studies show that $[Ca^{2+}]_i$ elicited no effect on this chloride channel.

128-pS Cl^- Channel

A 128.0 ± 14.6 pS (n = 3) chloride channel was also found which, like the 40-pS chloride channel and the anion channel, was inactive in the cell-attached patch but activated with patch excision. Unlike the 40-pS chloride channel, this chloride channel was only slightly sensitive to voltage and was active at more negative potentials. This conductance was usually composed of transient spikes between the open and closed states. These transitions were more sporadic than the frequent transitions with activation of the outward rectifying K^+ channel. Distinct dwell times in the open state were rarely measured. Both the infrequent dwell time openings and the more prevalent bursting of this channel are illustrated in Fig. 3*A*. This channel was selective for Cl^- over cations in the same manner as the 40-pS chloride channel (P_{Cl}/P_{Na} = 11, P_{Cl}/P_K = 8), but was more permeable to the large aspartate anion (P_{Cl}/P_{Asp} = 4). *I/V* plots showed a linear relationship (Fig. 3*B*) in contrast to the outward rectification characteristic of the 40-pS Cl^- channel. The kinetics of this channel resemble those of the 40-pS Cl^- channels but are less voltage sensitive by comparison (Fig. 3*C* and *D*).

Ca^{2+}-Activated K^+ Channel

A 93.3 ± 6.2 pS (n = 4) Ca^{2+}-activated K^+ channel was recorded in only a small percentage of our experiments. The permeability of this channel was highly selective towards K^+ (P_K/P_{Na} = 21, P_K/P_{Cl} = 20, *cf.* Fig. 4*B*). In cell-attached patches channel activity was observed only after applying large depolarizing voltages (+50 mV) to elicit outward K^+ currents. Increasing $[Ca^{2+}]_o$ has no effect on channel activity in the cell-attached configuration. Excision of the patch into the *i/o* configuration induced spontaneous channel activity in normal physiological gradient (10^{-8} $[Ca^{2+}]_i$) at both negative and positive potentials. Upon increasing $[Ca^{2+}]_i$, the kinetic activity of this channel increased in a dose-dependent manner. Adding EGTA to the bath (intracellular side) reversibly inactivated the channel. The kinetics of this channel were composed of both bursting activity and amplitudes with variable dwell

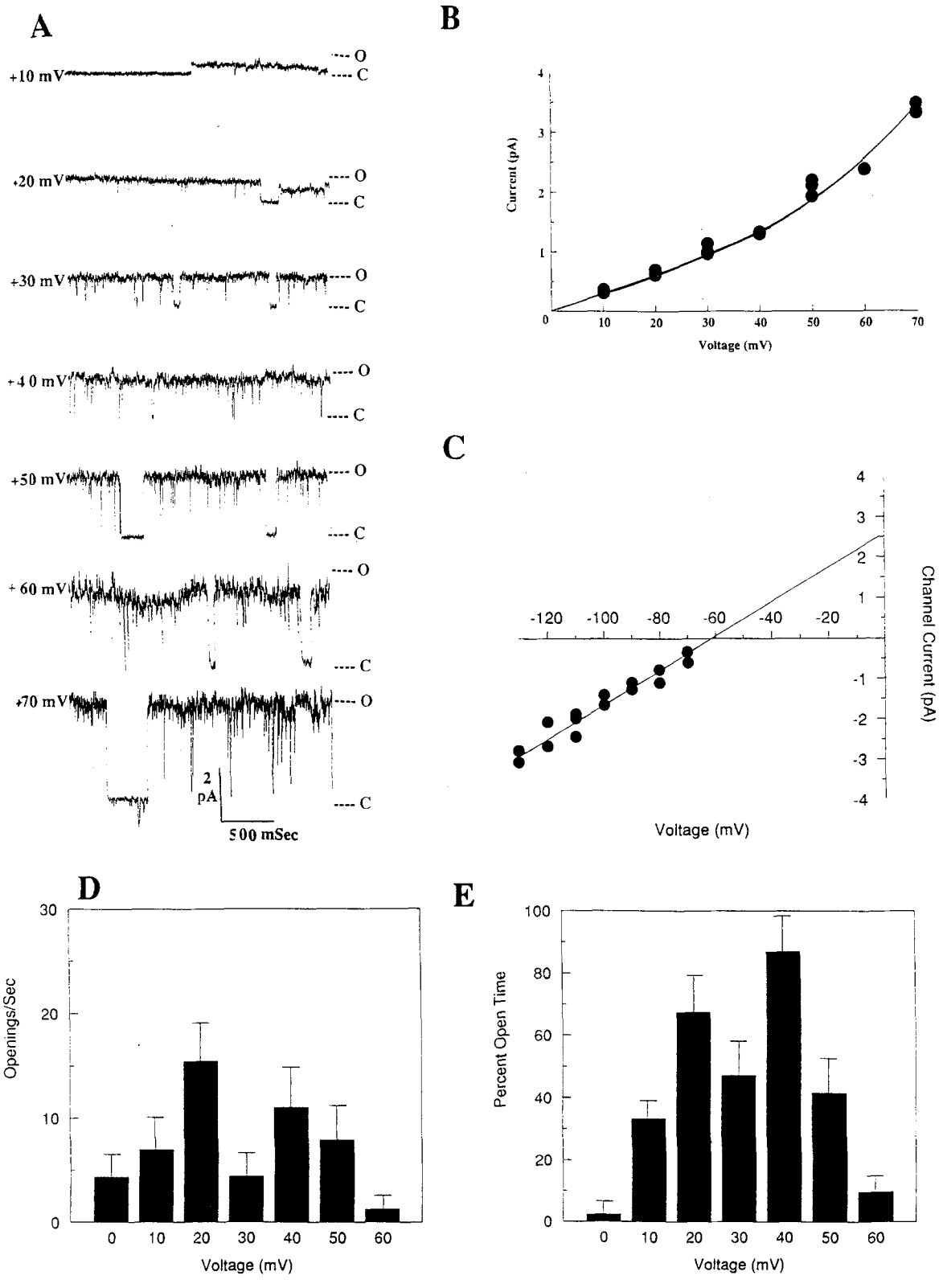

Fig. 2. Characterization of a 40-pS chloride channel. (*A*) Current recordings from an *i/o* patch in symmetrical chloride gradients (140 mM Cl^-) showing single-channel chloride currents at positive voltages. Both bursting and dwell times in the open state are displayed. (*B*) *I/V* curve plotted from the current records in *A* displaying outward rectification. (*C*) *I/V* curve of same channel with aspartate substituted for chloride in the bath. Note shift of E_{rev} towards E_{Cl} (−86 mV). (*D*, *E*) Histograms for both frequency of channel opening and channel percent open time display voltage-gated kinetics at positive voltages ($n = 5$)

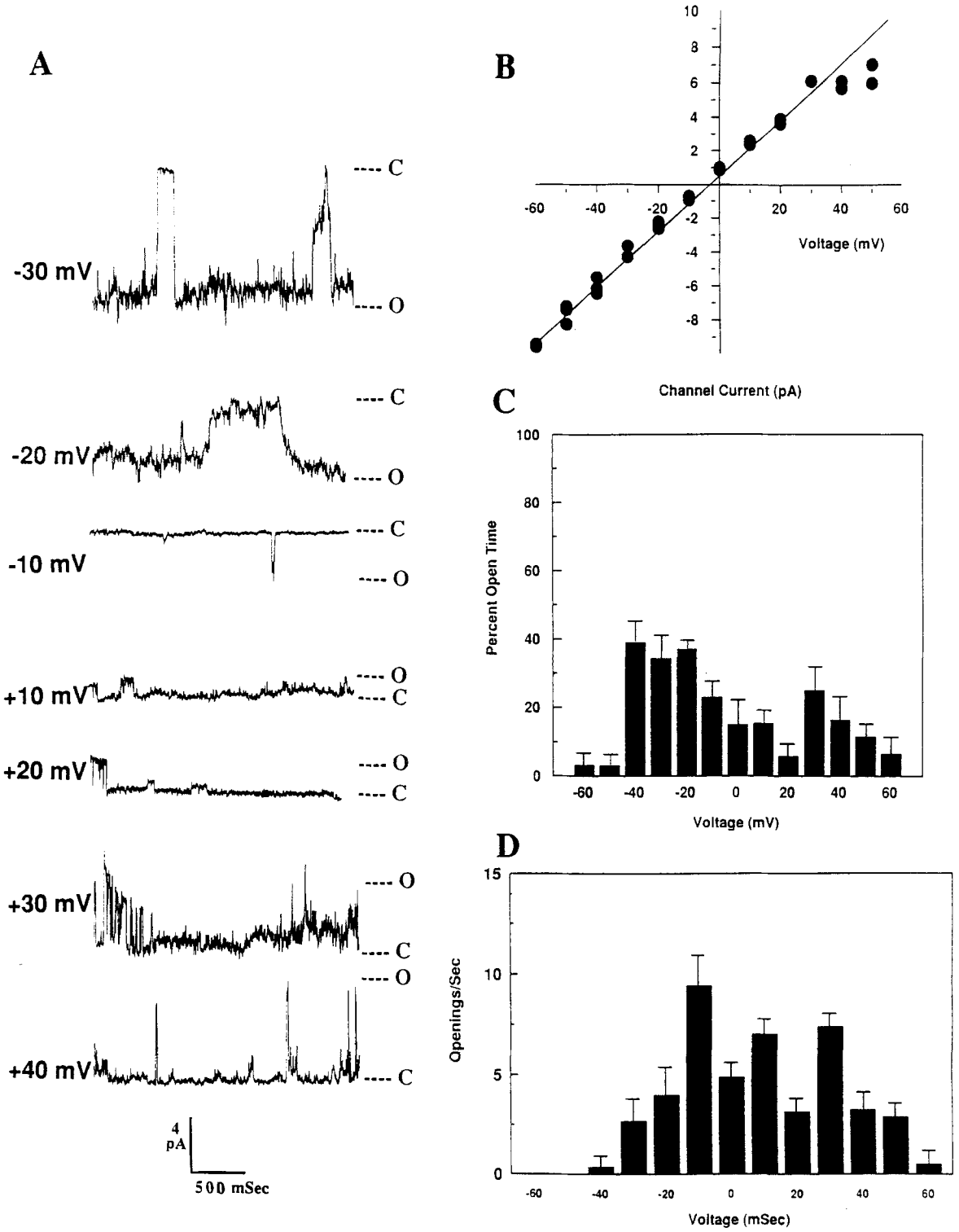

Fig. 3. Characterization of a 128-pS chloride channel. (*A*) Current recordings from an *i/o* patch in symmetrical chloride gradients. This channel displays more bursting activity and fewer distinct dwell times in the open state than does the 40-pS Cl^- channel. (*B*) *I/V* plot for same experiment showing linear relationship. (*C, D*) Histograms of kinetic activity in symmetrical Cl^- gradients ($n = 3$). This channel is less sensitive to voltage than the 40-pS Cl^- channel, exhibiting more activity at negative potentials

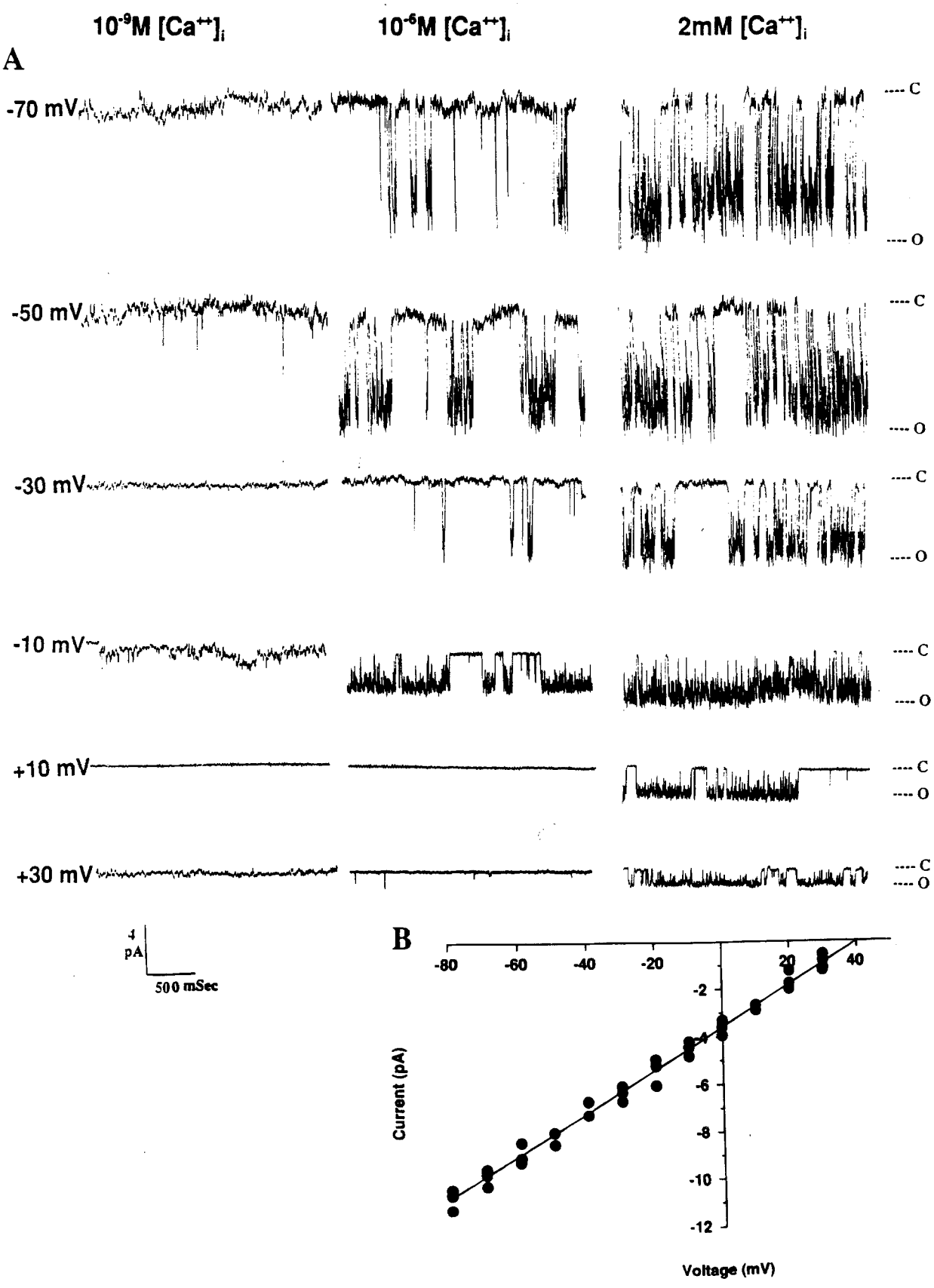

Fig. 4. *For caption to Fig. 4 see next page*

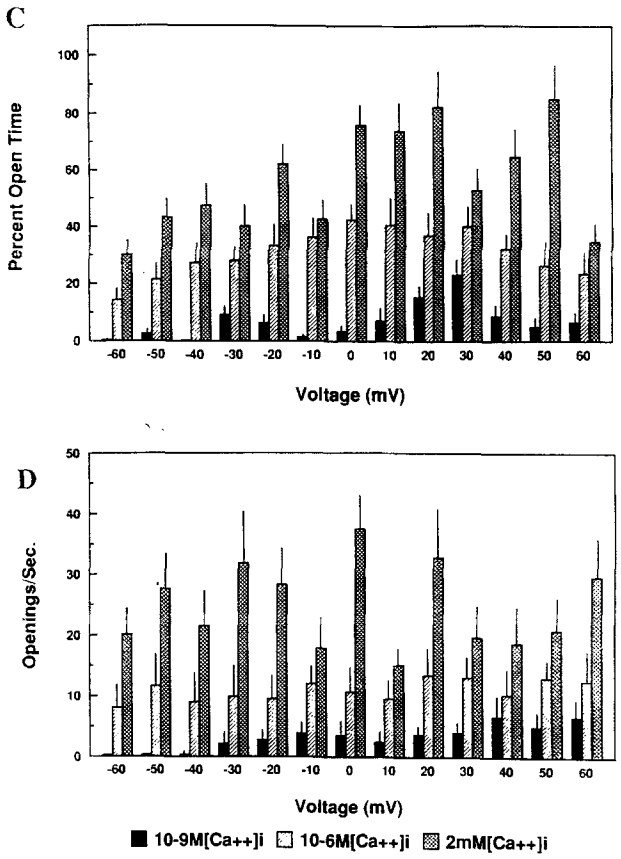

Fig. 4. Characterization of a 93-pS calcium-activated potassium channel. (*A*) Current recordings of three consecutive experiments from the same *i/o* patch in different $[Ca^{2+}]_i$. Current recordings were obtained in 140 mM $[K^+]_o$ and 5 mM $[K^+]_i$ gradients to elucidate inward K^+ currents. (*B*) *I/V* curve for same patch, in same gradients, demonstrating shift of E_{rev} towards E_K (+80 mV). (*C*, *D*) Histograms displaying dose-dependent effect of $[Ca^{2+}]_i$ on both channel percent open time and frequency of opening ($n = 3$)

times. At least two open states were apparent. Both the rate of channel opening and the aggregate dwell time of the channel increased in direct proportion to $[Ca^{2+}]_i$ (Fig. 4*C* and *D*). $[Ca^{2+}]_i$ induced both inward and outward K^+ currents at various voltages in *i/o* patches. Figure 4*A* illustrates Ca^{2+}-activated inward current of this voltage-insensitive channel. Although not previously characterized in B lymphocytes, indirect evidence has suggested the presence of this channel (Macdougall, Grinstein & Gelfand, 1988*a*).

Other Conductances

Additional single-channel currents were observed which we have not completely characterized due to their infrequent occurrence and lability. At least two different channels with single-channel conductanes <10 pS were recorded. One of these may correspond to the Ca^{2+} conductance recorded in several lymphocyte cell lines (Fukushima & Hagiwara, 1983; Kuno et al., 1986). Similarly, we observed a 40-pS channel, distinct from the 40-pS chloride channel with properties that suggest Na^+ selectivity. In addition to the ion channels characterized in the resting B lymphocytes, there probably are others that are revealed only upon activation. The large conductance anion channel is the predominant channel observed in *i/o* patches but remains inactive in the cell-attached patch configuration (McCann et al., 1989*a*; Bosma, 1989). Similarly, an inward-rectifying K^+ channel is found only in murine B cells cultured with lymphokine Interleukin-4 (McCann et al., 1989*b*).

Discussion

Single-channel currents measured in more than 200 patches of murine B lymphocyte membranes has provided data for the construction of a composite profile of the predominant ion channels present and their respective biophysical properties. In addition to the large conductance nonselective anion channel (348 pS) (McCann et al., 1989*a*), we now characterize four smaller conductance channels which include: an outwardly rectifying potassium channel, two chloride channels (40 and 128 pS), and a calcium-activated potassium channel (93 pS). The outwardly rectifying K^+ channel exhibited two distinct but cooperative conductances (18 and 30 pS). The anion channel and both chloride channels were frequently inactivated in the cell-attached configuration, becoming active with excision to an *i-o* patch. Similarly, the calcium-activated potassium channel displayed a twofold increase in percent open time with excision of the patch. All channels displayed some degree of voltage sensitivity. Similar to the anion channel, the 40-pS chloride channel required extensive depolarizing pulses to activate. Both outwardly rectifying K^+ conductances displayed classic voltage-gated kinetics. Although both conductances of this channel required depolarization to activate, each remained active in relatively distinct ranges of voltage. All voltage-sensitive channels displayed some plasticity, activating at more negative voltages following successive depolarizing pulses. Extreme depolarizing pulses (>+80 mV) often inactivated these channels. A summary of the types of ion channels we have characterized is presented in Fig. 5.

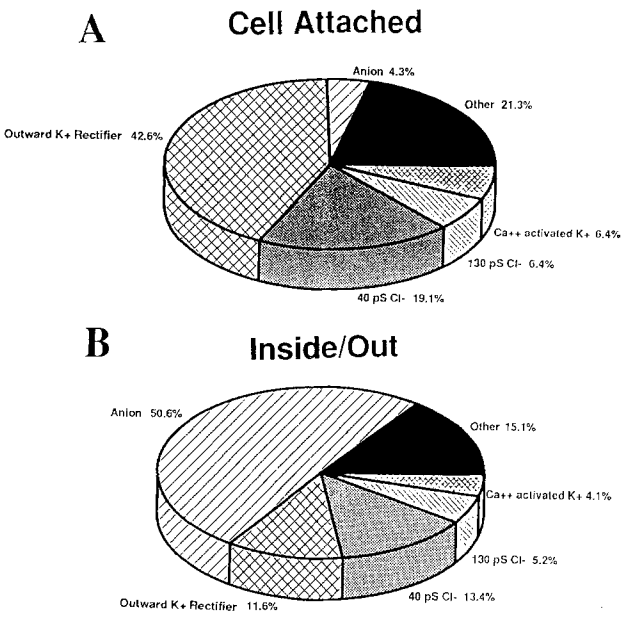

Fig. 5. Pie graphs summarizing and comparing the relative presence of channel conductances in murine B lymphocytes in both (*A*) the cell-attached patch configuration and (*B*) the inside-out patch configuration. Graphs reflect frequency of channel occurrence in the total number of recorded patches ($n = 221$). Percentage values were normalized to the total number of distinct patches that expressed channel activity. Collectively, the graphs illustrate a significant difference in the composition of channel activity with respect to patch configuration. The large conductance anion channel, predominant in *i/o* patches, remains virtually inactive in the cell-attached configuration. Similarly, the chloride channels are frequently inactive in the cell-attached configuration. The delayed rectifier K^+ channel appears to predominate in the cell-attached configuration in the absence of the large conductance anion channel

Ion Channels in B Lymphocytes

Past electrophysiological studies on cells of the immune system have suggested that ion channels play a role in the immune response perhaps by initiating a biochemical cascade of enzymatically controlled events. Ion channels apparently mediate many immunological functions including: IgE crosslinking in mast cells (Kanner & Metzger, 1984), phagocytosis and activation in macrophages (Ding-E Young et al., 1983*a*,*b*; Ince et al., 1988), and cytotoxicity of both killer T cells (Ding-E Young et al., 1986; Mikael, Janet & Maj-Britt, 1987; Sharma, 1988) and natural killer cells (Ng, Fredholm & Jondal, 1987). Moreover, some immunological cells behave as excitable cells (Gallin & Livengood, 1980; McCann et al., 1983). T lymphocytes, which arise from the same hematopoetic progenitor as B lymphocytes, have been the target for numerous ion channel studies, but relatively few studies have focused on B cells. Both K^+ channels and Ca^{2+} channels have been characterized in T cells and linked to cellular function (Chandy et al., 1984; Chandy et al., 1985; Gray et al., 1989). Cahalan et al. (1985) found delayed rectifying K^+ channels predominant in T cells and have recently confirmed their presence in B lymphocytes (Sutro et al., 1988). A Ca^{2+}-induced hyperpolarization was measured in human B lymphocytes (Macdougall et al., 1988*a*), but single Ca^{2+}-activated K^+ channels have not been previously characterized in B lymphocytes. A large conductance anion channel, characterized in both murine B lymphocytes (McCann et al., 1989*a*) and B cell hybridomas (Bosma, 1989), has not been identified in T lymphocytes. Chloride channels have been identified in neoplastic B lymphocytes (Bosma, 1989), Jurkat T cells, and B lymphoblasts (Chen, Schulman & Gardner, 1989). Calcium currents have been recorded in both B cell hybridomas (Fukushima & Hagiwara, 1983; Fukushima et al., 1984; Fukushima & Hagiwara, 1985) and human B cells (Macdougall et al., 1988*a*; Macdougall, Grinstein & Gelfand, 1988*b*), but single Ca^{2+} channels have not yet been effectively characterized in B lymphocytes as they have in T lymphocytes (Kuno et al., 1986). Na^+ channels have not been found in B lymphocytes, but an anti-IgM induced depolarizing Na^+ current has been observed in human tonsillar B cells (Macdougall et al., 1988*b*), and Na^+ can be conducted through Ca^{2+} channels in mouse neoplastic B lymphocytes (Fukushima & Hagiwara, 1985).

The Outward Rectifying Potassium Channel

Our studies define two subclasses of this channel by virtue of their respective conductances and voltage sensitivities (*cf.* Fig. 1). The 18-pS conductance resembles the delayed rectifier potassium channel described as the predominant ion channel in T lymphocytes (Cahalan et al., 1985; Lewis & Cahalan, 1988). We suggest that these conductances correspond to two of the three subclasses of outward rectifier K^+ channels (n, n', l) characterized in T lymphocytes (Lewis & Cahalan, 1988). Specifically, the 30-pS conductance appears similar to the l-type K^+ channel and the 18-pS to the n or n'-type K^+ channel. In patches where the 30-pS conductance was observed, the 18-pS conductance appeared as a subconductance state for the 30-pS unitary conductance. However, the 18-pS conductance was most often observed as its own unitary conductance in

patches where no 30-pS conductance was found. Thus the 18-pS conductance does not fall into the category of conventional subconductance states. One possible structural model for this cooperativity is the physical association of two 18-pS K^+ channels in the membrane to form a dimer complex which acts synnergistically to create a 30-pS conductance. Similar models have been used to explain the cooperative increases in ligand binding affinity of various receptors (e.g., the IL2 receptor (Smith, 1988)). In channels which display numerous subconductance states, multibarrel models have been created to describe the complex gating mechanisms (Krouse, Schneider & Gage, 1986; Hunter & Giebisch, 1987). Voltage-gated K^+ channels, clearly present in lymphocytes, suggests that an outward rectifier K^+ channel can have a functional role in cells even though they are generally regarded as nonexcitable. Voltage-gated K^+ currents (whole cell) in T lymphocytes (DeCoursey et al., 1984; Deutsch, Krause & Lee, 1986; DeCoursey et al., 1987) and in B lymphocytes (Fukushima & Hagiwara, 1985) are generated by individual K^+ channels that have similar electrical and pharmacological properties (Cahalan et al., 1985; Choquet et al., 1987). The observed expression of this channel in the early developmental stages of both cell types (McKinnon & Ceredig, 1986; Schlichter, Sidell & Hagiwara, 1986) suggests that the channel is common to both T lymphocyte and B lymphocyte lineages and may share similar functional roles. In T cells three subclasses of the delayed rectifying K^+ channel have been characterized and correlated to T cell proliferation, differentiation, and mitogenic activation via whole cell recording measurements (Lewis & Cahalan, 1988). Conversely, Choquet et al. (1987) have shown that little change occurs in B lymphocyte whole cell K^+ currents induced by activation, and suggest that the occurrence of this channel is unrelated to immunocompetency. Serotonin, a well known modulator of voltage-gated K^+ conductances in the nervous system, regulates outward rectifier K^+ channels in a pre-B lymphocyte cell line (Choquet & Korn, 1988). Cyclic AMP also modulates the channel, decreasing outward K^+ currents and increasing the rate of inactivation (Choquet et al., 1987).

Calcium-Activated Potassium Channels

We also present evidence for the presence of a 93-pS conductance Ca^{2+} and voltage-activated K^+ channel which has not been previously observed in B lymphocytes. Ca^{2+}-activated K^+ channels have been found in macrophages (Gallin, 1984; McCann, Keller & Guyre, 1987*a*), T lymphocytes (Tsien, Pozzan & Rink, 1982), rat muscle (Blatz & Magleby, 1984), pituitary cells (Wong, Lecar & Adler, 1982), sympathetic nuerons (Adams et al., 1982), *Necturus* enterocytes (Sheppard, Giraldez & Sepulveda, 1988), and other cell types. $[Ca^{2+}]_i$-sensitive whole cell K^+ currents, described in B lymphocytes, have been identified as a component in lymphocyte activation (Tsien et al., 1982; Macdougall et al., 1988*a*,*b*). Because we observed that $[Ca^{2+}]_i$ induced both inward and outward K^+ currents depending on the command potential, we suggest that the binding of calcium rather than the depolarization associated with a rise in $[Ca^{2+}]_i$ modulates this channel in the intact cell. These channels may act in concert with anion channels to regulate secretion in secretory vesicles (Stanley, Ehrenstein & Russell, 1988). In macrophages, the Ca^{2+}-activated K^+ channel has been well defined and implicated in macrophage function (Persechini, Araiyo & Oliviera-Castro, 1980; Gallin, 1984; McCann et al., 1987*a*). However, these channels are not found in freshly isolated monocytes and thus are not likely involved in phagocytosis or chemotaxis, functions which are observed in freshly isolated monocytes (Gallin, 1984; McCann et al., 1987*a*). Other studies have attributed regulation of both volume and $[K^+]_i$ to this channel (Deutsch et al., 1986).

Chloride Channels

We have presented evidence for the presence of two distinctly different types of Cl^- channels. They differ primarily in their conductances, sensitivities to transmembrane voltage and to a lesser degree, their ionic selectivities. Cl^- channels have been described in a variety of cells with conductances that range from <10 pS (Greger, Schlatter & Gogelein, 1985) to >400 pS (Nelson, Tang & Palmer, 1984; Blatz & Magleby, 1985) indicating that multiple types exist and that each may express different functions. Our characterization of a 40-pS chloride channel correlates with other studies performed on this channel that suggest it to be the same chloride channel that is defective in cystic fibrosis patients (Chen et al., 1989). The 40-pS channel displays slower kinetics and operates in a voltage range more positive to the larger 128-pS channel. The outward rectification and activation at more positive voltages of the smaller channel indicates a role in membrane repolarization similar to the delayed rectification common to certain K^+ channels. The outward rectifier K^+ channel (*cf.* Fig. 1) may act in concert with this Cl^- channel as in T lymphocytes

(Lee et al., 1988) to effectively regulate cell volume. We suggest that the small conductance rectifying Cl^- channel, the outward-rectifying K^+ channel, and the large conductance anion channel, characterized earlier (McCann et al., 1989*a*; Bosma et al., 1989), all interact to endow the B lymphocyte with a volume regulatory mechanism required of a cell that must exercise vigorous secretory activity. Although these cells are resting and therefore presumably not secreting antibodies, the machinery is nevertheless present awaiting activation by appropriate ligands. The larger conductance Cl^- channel, in view of its activation at more negative voltages, may function in the control of the resting potential as proposed by Bosma (1989).

Other Channels

The large conductance anion channel is the most predominant conductance present in *i/o* patches in B lymphocytes but it remains inactive in the cell-attached patch configuration (McCann et al., 1989*a*; Bosma, 1989). Thus, it is plausible that this channel is dormant in the intact resting B lymphocyte, requiring specific stimuli for activation. Indeed, a recent study on resting murine B lymphocytes reports whole cell currents that are primarily mediated by K^+ channels of the delayed rectifier type and not by the large conductance anion channel predominant in *i/o* patches (Sutro et al., 1988). Although specific functions attributable to anion channels have not been clearly defined, several studies have suggested a role for anion channels in osmoregulation (Bosma, 1989) and in secretion (Stanley et al., 1988).

Neither Ca^{2+} nor Na^+ channels have been clearly defined in B lymphocytes. We have not observed distinct Ca^{2+} channels in murine B cells, but have measured small unitary currents (<10 pS) which may be mediated via Ca^{2+} channels. Although single Ca^{2+} channels have not been clearly characterized in B lymphocytes, several studies have elucidated whole cell Ca^{2+} currents. Furthermore, distinct Ca^{2+} channels in T lymphocytes have previously been described (Kuno et al., 1986; Pecht et al., 1987) as have Ca^{2+} channels in a mouse B cell hybridoma cell line (Fukushima & Hagiwara, 1983; Fukushima & Hagiwara, 1985). We have also observed a 40-pS channel with properties distinct from the 40-pS chloride channel which displays Na^+ selectivity. While distinct sodium channels have also not yet been characterized in B lymphocytes, Na^+ currents have been recorded and a Na^+/H^+ exchange has been demonstrated in T lymphocytes (Dornand, Moatassim & Mani, 1987; Gelfand et al., 1987). Na^+ can induce depolarization by conduction through Ca^{2+} channels in lymphocytes (Fukushima & Hagiwara, 1985). Furthermore, voltage-gated Na^+ currents have been associated with drug-resistant human leukemia cells (Yamashita et al., 1987). Thus, although sodium and calcium fluxes have been demonstrated, the presence of sodium and calcium channels in B cells remains unsettled. Although no inward-rectifying K^+ channels have been characterized in lymphocytes as they have in macrophages (Gallin & McKinney, 1988), we have recently recorded them in cultured murine B lymphocytes treated with the lymphokine Interleukin-4 (McCann, McCarthy & Noelle, 1989*b*).

Ion channels have now been shown to mediate specific physiological functions of cells involved with the immune response. Some of the channels characterized in this study are similar to those described in different cell types and may thus share similar functional roles in cellular physiology. Other channels are likely to be present in B cells and are likely to mediate cellular functions unique to their specific physiology. Further experimentation will elucidate their functional roles.

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References

Adams, P.R., Constanti, A., Brown, D.A., Clark, R.B. 1982. Intracellular Ca^{2+} activates a fast voltage-sensitive K^+ current in vertebrate sympathetic neurones. *Nature (London)* **296:**746–749

Bailey, J.S., Siu, C. 1987. Purification and characterization of a murine cellular retinoic acid-binding protein. *Biochem. Cell Biol.* **66:**750–757

Blatz, A.L., Magleby, K.L. 1984. Ion conductance and selectivity of single calcium-activated potassium channels in cultured rat muscle. *J. Gen. Physiol.* **84:**1–23

Blatz, A.L., Magleby, K.L. 1985. Single chloride selective channels active at resting membrane potentials in cultured rat skeletal muscle. *Biophys. J.* **47:**119–123

Bosma, M. 1989. Anion channels with multiple conductance levels in a mouse B lymphocyte cell line. *J. Physiol. (London)* **410:**67–90

Cahalan, M.D., Chandy, K.G., Decoursey, T.E., Gupta, S. 1985. A voltage-gated potassium channel in human T lymphocytes. *J. Physiol. (London)* **358:**197–237

Chandy, K.G., DeCoursey, T.E., Cahalan, M.D., Gupta, S. 1985. Ion channels in lymphocytes. *J. Clin. Immunol.* **5:**1–5

Chandy, K.G., DeCoursey, T.E., Cahalan, M.D., McLaughlin, C., Gupta, S. 1984. Voltage-gated potassium channels are required for human T lymphocyte activation. *J. Exp. Med.* **160:**369–385

Chen, J.H., Schulman, H., Gardner, P. 1989. A cAMP-regulated chloride channel in lymphocytes that is affected in cystic fibrosis. *Science* **243:**657–660

Choquet, D., Korn, H. 1988. Dual effects of serotonin of a voltage-gated conductance in lymphocytes. *Proc. Natl. Acad. Sci. USA* **85:**4557–4561

Choquet, D., Sarthou, P., Primi, D., Cazenave, P.-A., Korn, H. 1987. Cyclic AMP-modulated potassium channels in murine B cells and their precursors. *Science* **235:**1211–1214

DeCoursey, T.E., Chandy, K.G., Gupta, S., Cahalan, M.D. 1984. Voltage-gated K^+ channels in human T lymphocytes: A role in mitogenesis? *Nature (London)* **307:**465–468

DeCoursey, T.E., Chandy, K.G., Gupta, S., Cahalan, M.D. 1987. Mitogen induction of ion channels in murine T lymphocytes. *J. Gen. Physiol.* **89:**405–420

Deutsch, C., Krause, D., Lee, S.C. 1986. Voltage-gated potassium conductance in human T lymphocytes stimulated with phorbol ester. *J. Physiol. (London)* **372:**405–423

Ding-E Young, J., Nathan, C.F., Podack, E.R., Palladino, M.A., Cohen, Z.A. 1986. Functional channel formation associated with cytotoxic T-cell granules. *Proc. Natl. Acad. Sci. USA* 83:150–154

Ding-E Young, J., Unkeless, J.C., Kaback, H.R., Cohn, Z.A. 1983*a*. Macrophage membrane potential changes associated with 2b/1Fc receptor-ligand binding. *Proc. Natl. Acad. Sci. USA* **80:**1357–1361

Ding-E Young, J., Unkeless, J.C., Young, T.M., Mauro, A., Cohn, Z. 1983*b*. Role for mouse macrophage IgG Fc receptor as ligand-dependent ion channel. *Nature (London)* **306:**186–189

Dornand, J., Moatassim, C., Mani, J.C. 1987. Amiloride-induced suppression of lymphocyte proliferation: Inhibition of IL2 receptor expression after blockade of early sodium influx. *Immunobiology* **174:**365–379

Fukushima, Y., Hagiwara, S. 1983. Voltage gated calcium channel in mouse myeloma cells. *Proc. Natl. Acad. Sci. USA* **80:**2240–2242

Fukushima, Y., Hagiwara, S. 1985. Currents carried by monovalent cations through calcium channels in mouse neoplastic B lymphocytes. *J. Physiol. (London)* **358:**255–284

Fukushima, Y., Hagiwara, S., Saxton, R.E. 1984. Variation of calcium current during cell growth cycle in mouse hybridoma lines secreting immunoglobulins. *J. Physiol. (London)* **355:**313–321

Gallin, E.K. 1984. Calcium- and voltage-activated potassium channels in human macrophages. *Biophys. J.* **46:**821–825

Gallin, E.K., Livengood, D.R. 1980. Nonlinear current-voltage relationships in cultured macrophages. *J. Cell Biol.* **85:**160–165

Gallin, E.K., McKinney, L.C. 1988. Patch-clamp studies in human macrophages: Single-channel and whole cell characterization of two K^+ conductances. *J. Membrane Biol.* **103:**55–66

Gelfand, E.W., Mills, G.B., Cheung, R.K., Lee, J.W.W., Grinstein, S. 1987. Transmembrane ion fluxes during activation of human T lymphocytes: Role of Ca^{2+}, Na^+/H^+ exchange and phospholipid turnover. *Immunol. Rev.* **95:**59–87

Gray, L.S., Gnarra, J.R., Russell, J.H., Engelhard, V.H. 1989. The role of K^+ in the regulation of the increase in intracellular Ca^+ mediated by the T lymphocyte antigen receptor. *Cell* **50:**119–127

Greger, R., Schlatter, E., Gogelein, H. 1985. Cl-channels in the apical cell membrane of the rectal gland "induced" by cAMP. *Pfluegers Arch.* **403:**446–448

Grinstein, S., Clarke, C.A., Rothstein, A., Gelfand, E.W. 1983. Volume-induced anion conductance in human B lymphocytes is cation independent. *Am. J. Physiol.* **245:**c160–c163

Hille, B. 1984. Ionic Channels of Excitable Membranes. p. 18. Sinauer, Sunderland

Hunter, M., Giebisch, G. 1987. Multi-barrelled K channels in renal tubules. *Nature (London)* **327:**522–524

Ince, C., Coremans, J.M.C.C., Ypey, D.L., Leijh, P.C.J., Verveen, A.A., van Furth, R. 1988. Phagocytosis by human macrophages is accompanied by changes in ionic channel currents. *J. Cell Biol.* **106:**1873–1878

Kanner, B.I., Metzger, H. 1984. Initial characterization of the calcium channel activated by the cross-linking of the receptors for immunoglobulin E. *J. Biol. Chem.* **259:**10188–10193

Krouse, M., Schneider, G.T., Gage, P.W. 1986. A large anion-selective channel has seven conductance levels. *Nature (London)* **319:**58–60

Kuno, M., Goronzy, J., Weyand, C.M., Gardner, P. 1986. Single-channel and whole-cell recordings of mitogen-regulated inward currents in human cloned helper T lymphocytes. *Nature (London)* **323:**269–304

Lee, S.C., Price, M., Prystowsky, M.B., Deutsch, C. 1988. Volume response of quiescent and interleukin 2 stimulated T-lymphocytes to hypotonicity. *Am. J. Physiol.* **254:**C286–C296

Lewis, R.S., Cahalan, M.D. 1988. Subset-specific expression of potassium channels in developing murine T lymphocytes. *Science* **239:**771–775

Macdougall, S.L., Grinstein, S., Gelfand, E.W. 1988*a*. Activation of Ca^{2+} dependent K^+ channels in human B lymphocyes by anti-immunoglobulin. *J. Clin. Invest.* **81:**449–454

Macdougall, S.L., Grinstein, S., Gelfand, E.W. 1988*b*. Detection of ligand-activated conductive Ca^{2+} channels in human B lymphocytes. *Cell* **54:**229–234

McCann, F.V., Cole, J.J., Guyre, P.M., Russell, J.A.G. 1983. Action potentials in macrophages derived from human monocytes. *Science* **219:**991–993

McCann, F.V., Keller, T.M., Guyre, P.M. 1987*a*. Ion channels in human macrophages compared with the U-937 cell line. *J. Membrane Biol.* **96:**57–64

McCann, F.V., McCarthy, D.C., Keller, T.M., Noelle, R.J. 1989*a*. Characterization of a large conductance non-selective anion channel in B lymphocytes. *Cell. Signal.* **1:**31–44

McCann, F.V., McCarthy, D.C., Noelle, R.J. 1988*b*. Ion channel activity in B lymphocytes mediated by interleukin-4. *J. Gen. Physiol.* **94:**39*a*–40*a*

McCann, F.V., Stibitz, G.R., Keller, T.M. 1987*b*. A computer method for the acquisition and analysis of patch clamp single channel currents. *J. Neurosci. Methods* **20:**45–55

McKinnon, D., Ceredig, R. 1986. Changes in the expression of potassium channels during mouse T cell development. *J. Exp. Med.* **164:**1846–1861

Mikael, J., Janet, N., Maj-Britt, A. 1987. Direct induction of lymphocyte killing by calcium ionophore and phorbol ester treatment. *Cell. Immunol.* **105:**23–32

Monroe, J.G., Cambier, J.C. 1983. B cell activation. I. Anti-immunoglobulin-induced receptor cross-linking results in a decrease in the plasma membrane potential of murine B lymphocytes. *J. Exp. Med.* **157:**2073–2086

Nelson, D.J., Tang, J.M., Palmer, L.G. 1984. Single-channel recordings of apical membrane chloride conductance in A6 epithelial cells. *J. Membrane Biol.* **80:**81–89

Ng, J., Fredholm, B.B., Jondal, M. 1987. Studies on the calcium dependence of human NK cell killing. *Biochem. Pharmacol.* **36:**3953–3949

Palmer, L.G., Frindt, G. 1986. Amiloride-sensitive Na^+ channels

from the apical membrane of the rat cortical collecting tubule. *Proc. Natl. Acad. Sci. USA* **83:**2767–2770

Pecht, I., Corcia, A., Liuzzi, M.P.T., Alcover, A., Reinherz, E.L. 1987. Ion channels activated by specific Ti or T3 antibodies in plasma membranes of human T cells. *EMBO. J.* **6:**1935–1939

Persechini, P.M., Araujo, E.G., Oliveira-Castro, G. 1980. Electrophysiology of phagocytic membranes: Induction of slow membrane hyperpolarizations in macrophages and macrophage polykaryons by intracellular calcium injection. *J. Membrane Biol.* **61:**81–90

Schlichter, L., Sidell, N., Hagiwara, S. 1986. K^+ channels are expressed early in human T-cell development. *Proc. Natl. Acad. Sci. USA* **83:**5625–5629

Sharma, B. 1988. Inhibition of the generation of cytoxic lymphocytes by potassium ion channel blockers. *Immunology* **65:**101–105

Sheppard, D.N., Giraldez, F., Sepúlveda, F.V. 1988. Kinetics of voltage and Ca^{2+} activation and Ba^{2+} blockade of a large-conductance K^+ channel from *Necturus* enterocytes. *J. Membrane Biol.* **105:**65–75

Smith, K.A. 1988. The interleukin 2 receptor. *Adv. Immunol.* **42:**165–179

Stanley, E.F., Ehrenstein, G., Russell, J.T. 1988. Evidence for anion channels in secretory vesicles. *Neuroscience* **25:**1035–1039

Sutro, J.B., Vayuvegula, B., Gupta, S., Cahalan, M. 1988. Up-regulation of voltage-sensitive K^+ channels in mitogen-stimulated B lymphocytes. *Biophys. J.* **53:**460*a*

Tsien, R.Y., Pozzan, T., Rink, T.J. 1982. T-cell mitogens cause early changes in cytoplasmic free Ca^{2+} and membrane potential in lymphocytes. *Science* **295:**68–70

Wong, B.S., Lecar, H., Adler, M. 1982. Single calcium-dependent potassium channels in clonal anterior pituitary cells. *Biophys. J.* **39:**313–317

Yamashita, N., Hamada, H., Tsuruo, T., Ogata, O. 1987. Enhancement of voltage-gated Na^+ channel current associated with multidrug resistance in human leukemia cells. *Cancer Res.* **47:**3736–3741

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