Voltage-dependent and Ca^{2+}-activated Ion Channels in Human Neutrophils

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Abstract

To investigate the regulation of membrane voltage and transmembrane ion fluxes in human neutrophils, we studied plasma membrane currents using the whole-cell patch-clamp method. We observed three distinct ion channel currents: (*a*) a voltage-dependent K^+ current, (*b*) a Ca^{2+}-activated K^+ current, and (*c*) a Ca^{2+}-activated Cl^- current. The voltage-dependent K^+ current was found in cells at rest. Its conductive properties suggested an inwardly rectifying channel. The channel was activated at membrane potentials more positive than −60 mV, suggesting that it may determine the resting membrane potential of neutrophils. Activation of neutrophils by the Ca^{2+} ionophore ionomycin led to an increase in whole-cell K^+ and Cl^- currents. The Ca^{2+}-activated K^+ channel differed from the voltage-dependent K^+ channel because it was insensitive to voltage, because it rectified outwardly, and because the voltage-sensitive K^+ channel was Ca^{2+}-independent. The Ca^{2+}-activated Cl^- channel showed outward rectification and no apparent voltage dependency. The Ca^{2+}-activated K^+ and Cl^- channels may play a role in cell volume homeostasis and/or cellular activation. (*J. Clin. Invest.* 1990. 85:491–498.) chloride channels • potassium channels • neutrophils

Introduction

Neutrophils are mobile, phagocytic cells, crucial in the host defense against bacterial and fungal infections (1). To exert their functions, neutrophils must be able to respond to extracellular stimuli. The role of intracellular messengers, such as Ca^{2+} and protein kinases, in the coupling of extracellular stimuli to neutrophil responses has been intensively studied (for review see references 2 and 3). Although plasma membrane ion channels play an important role in stimulus-response coupling in other cell types (4), very little is known about ion channels in neutrophils. One previous study described nonselective cation channels (5). Some indirect information has been obtained using membrane potential–sensitive dyes or radiotracers (6–12). Most of those studies suggest that the membrane potential of resting human neutrophils is around −60 mV (7–10, 12), although values as low as −100 (11) and as high as −25 mV (6) were reported. In response to chemoattractants, membrane voltage depolarizes and subsequently repolarizes (7, 8). In contrast to excitable cells, where depolarization causes Ca^{2+} influx and cellular activation (4), depolarization in neutrophils may be an inhibitory signal (10, 13). It may provide a negative feedback mechanism that limits the release of granules and oxygen radicals during activation.

To understand the regulation of membrane voltage and ion currents, we applied the whole-cell patch-clamp technique to human neutrophils. We were able to demonstrate the existence of a voltage-dependent K^+ channel and of Ca^{2+}-activated Cl^- channels and K^+ channels.

Methods

Human neutrophils were prepared from blood of healthy volunteers as described (10) and used for experiments within 10 h after preparation. In most experiments we used neutrophils adherent to collagen-coated plastic coverslips. Neutrophils were allowed to adhere by incubating the coverslip in a suspension of 0.5×10^6 neutrophils in buffer B1 (Table 1), containing 0.2% glucose and 0.1% bovine serum albumin. Neutrophils were used for experiments within 60 min after adhering to the coverslip. In a few experiments, neutrophils adherent to uncoated plastic coverslips were used; no differences in membrane currents were detected.

Whole-cell patch-clamp recordings were performed as described (14). All experiments were done at room temperature. Pipette resistance varied between 2.5 and 5 MΩ, seal resistance between 5 and 100 GΩ. Pipettes were fire polished and the tip coated with Sylgard 184 (Dow Corning Corp., Midland, MI). Bursts of suction on the pipette caused the appearance of capacitive currents, indicating that the whole-cell configuration had been attained. Cell capacitance was between 2 and 5 pF. Values for whole-cell resistance were only slightly lower than seal resistance (2.5–50 GΩ). As series resistance was consistently more than 100-fold lower than whole-cell resistance, it was not compensated. Cell capacitance was compensated with the analog circuit of the patch-clamp amplifier. For voltage clamping and current amplification we used a EPC 7 patch-clamp amplifier (List Electronics, Darmstadt, Federal Republic of Germany) and for data acquisition and analysis a laboratory computer (Indec, Sunnyvale, CA). Currents were filtered at 2,000 Hz and data acquired at 250-μs intervals. Voltages are referenced to the bath; i.e., a positive voltage is depolarizing and a negative voltage is hyperpolarizing. Outward current (= positive current) refers to the flow of cations from the cell interior to the bath or anion flow from the bath to the cell interior.

The buffers we used in this study and their designation are shown in Table I. The pipette $[Ca^{2+}]$ was lightly buffered (0.2 mM EGTA) to allow an increase in intracellular Ca^{2+} after addition of ionomycin.

To add ionomycin to the bath or to change the ionic composition of the bath, the bath solution was exchanged. In control experiments exchange of the bath solution by itself did not lead to detectable changes in whole-cell conductance.

Leak-currents were only subtracted where indicated in the figure legends. Change of the bath solution to Na^+-Isethionate (Figs. 4 and 6) led to a junction potential of 3–4 mV (determined as described in reference 15). This potential change is negligibly small when compared with the effect of Na-isethionate observed in cells exposed to ionomycin. In addition, changing the bath solution to Na-isethionate in cells not exposed to ionomycin produced no significant current changes. We therefore did not correct these data for junction potentials.

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Table I. Composition of Bath and Pipette Solutions

Solution	NaCl	KCl	K-Asp	Na-Ise	$MgCl_2$	$CaCl_2$	EGTA	ATP	GTP
					mM				
P1	5	30	110		2		0.2	1	0.02
P2	5	140			2		0.2	1	0.02
P3	5		140		2		0.2	1	0.02
P4	140	5			2		0.2	1	0.02
B1	140	5			2	2			
B2	115	30			2	2			
B3	5	140			2	2			
B4	5		140		2	2			
B5		5		140	2	2			

Composition of pipette (P1–P4) and bath solutions (B1–B5) used in this study. All solutions contained 10 mM Hepes and were buffered to pH 7.2.

Results

Voltage-dependent whole-cell currents. Our first experiments examined currents using solutions with ionic compositions similar to "physiological" values. The pipette solution contained KCl and K-aspartate, and NaCl was the predominant salt in the bath (B1, P1). The currents observed under these conditions (Fig. 1 *A* and open squares in Fig. 1 *B*) showed two prominent features: (*a*) they reversed at around −25 mV[1]; and (*b*) they activated at positive voltages.

With an external NaCl and an internal KCl and K-aspartate solution, a current reversing at −25 mV could be due to K^+ efflux or Cl^- influx. To test for these possibilities, we measured the whole-cell current–voltage relationship and varied the ionic composition of the pipette solution (Fig. 1 *B*). The size of the outward current and the reversal potential was independent of Cl^- concentration (35 or 145 mM). In contrast, when the K^+ concentration in the pipette was 5 mM (P4) instead of 140 mM K^+, the outward current was greatly reduced and the reversal potential shifted to 0 mV.

These results are best explained by a K^+-selective ion channel. To demonstrate the K^+ selectivity of the channel, we activated the current by applying depolarizing pulses to +90 mV and then analyzed the reversal of the tail currents when the voltage was returned to more hyperpolarizing test potentials (Fig. 2, *A–C*). From the current-voltage relationship of the tail currents, we determined reversal potentials, which are shown in Fig. 2 *D* as a function of the bath $[K^+]$. The dependency of the reversal potential on the bath $[K^+]$ showed a slope of 20.4; the ideal Nernstian slope for a K^+-selective channel would be 25.4. These results show that the channel is K^+ selective; the slight deviation from the ideal Nernstian slope might be due to a second, quantitatively minor conductance or to imperfect K^+ selectivity of the channel.

With the ionic conditions described above, we observed a relatively rapid loss of channel activity (rundown) with time (voltage-dependent current was entirely lost within ~ 10 min after achieving the whole-cell configuration). In contrast, when symmetrical K-aspartate solutions (B4, P3) were used, channel activity was constant for the duration of the experiment. We have not yet investigated the underlying mechanism of this phenomenon. However, the use of symmetrical K-aspartate solutions in further experiments was useful to assure that only K^+ currents were monitored and to be able to perform studies that required longer time periods. We also noticed that the number of cells that showed a spontaneous K^+ current was increased in symmetrical K^+-aspartate solutions (> 90%).

Three parameters determine the whole-cell current–voltage relationship shown in Fig. 1 *B:* the electrochemical gradient for ion movement, the conductive properties of the channel, and the probability that the channel is in the open state. To assess the conductive properties of the channel independent of its open probability, we obtained an instantaneous current–voltage relationship in symmetrical K-aspartate solutions. Membrane voltage was held at +90 mV for 400 ms to achieve maximal activation of the channel and then instantaneous currents were determined after stepping to various voltages. These experiments revealed that the voltage-dependent K^+ current flows through an ion channel with an inwardly-rectifying current-voltage relationship (Fig. 2 *E*).

The negative reversal potential of the whole-cell current, shown in Fig. 1, suggested that the threshold of voltage activation for the voltage-dependent K^+ channel was more negative than 0 mV. In order to determine the voltage-dependence of activation, we applied the following protocol. We used a membrane holding voltage of −90 mV, because no voltage-activated current was observed at this potential (i.e., no tail currents could be observed when stepping from −90 mV to a more negative voltage). Voltage was then stepped for 400 ms to various target voltages. The tail currents produced when the voltage was returned to −90 mV were analyzed (Fig. 3). The tail currents reflect the fact that channels are activated during the step to the respective target voltage and, since the channels do not close instantaneously on stepping back to the holding voltage (−90 mV), the tail current amplitude gives a measure of the degree of activation. Because all tail currents were measured at −90 mV, the electrochemical gradient and conductive

1. The reversal potential in whole-cell recordings is determined by both leak currents and currents through channels; thus it is always smaller than expected for the reversal potential of current through the channel alone. The effect of the leak current is more important in determining reversal potential in small high-resistance cells like neutrophils, where channel currents are small. In experiments in which seal resistance was very high (50–100 GΩ) reversal potential was in the range of −50 to −60 mV, suggesting an approximate value of resting membrane voltage.

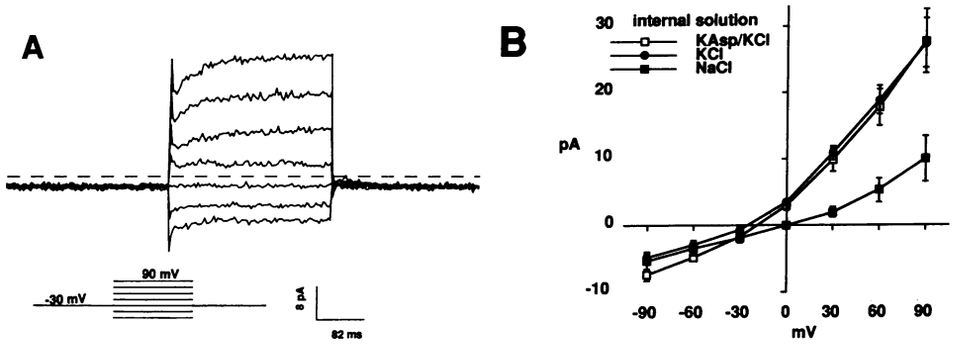

Figure 1. Membrane currents of unstimulated, adherent neutrophils. (*A*) Whole-cell currents in response to various target voltage steps (−90 to 90 mV) from a holding voltage of −30 mV. *Insets* show the voltage protocol and current and time scale. The dash line indicates the zero-current level. Solutions: bath NaCl/pipette KCl, K-aspartate (B1, P1). (*B*) Whole-cell current–voltage relationship with various ionic conditions of the pipette solution. The bath solution was NaCl (B1) for all conditions. Pipette solution: (□) KCl, K-aspartate (P1; n = 3); (●) KCl (P2; n = 5); (■) NaCl (P4; n = 5). Values are mean±SEM.

properties of the channel were constant. Fig. 3 shows that the threshold of voltage activation of the channel is around −60 mV. The size of the tail currents increased with the target voltage and maximal tail currents, i.e., maximal probability of the channel to be in the open state, was obtained at around +80 to +90 mV. The tail currents at −90 mV could be fit by a single exponential suggesting that only one K^+ current is activated by voltage in this range. However, because the currents are small, particularly at negative voltages, we cannot exclude other, quantitatively minor currents.

Macrophages possess a K^+ channel[2] that activates and then inactives when membrane potential is held at depolarizing voltages (time constant around 500 ms, [16, 17]). We observed no voltage-dependent inactivation of the neutrophil channel within 400 ms (e.g., Fig. 1 *A*) or during longer depolarization pulses up to 4 s (not shown).

In macrophages, an inwardly rectifying, hyperpolarization-activated K^+ channel has also been described (17–19). That channel is activated at voltages below −60 mV. To determine if neutrophils possess a similar type of channel we performed experiments at various holding voltages (0 to −60 mV) and hyperpolarized to various target voltages (−90 to −240 mV). No hypolarization-activated currents could be detected, in either symmetrical K^+-aspartate solutions or in symmetrical KCl solutions.

Ca^{2+}-activated whole-cell currents. With KCl in the pipette solution and NaCl in the bath solution, ~ 60% of the cells showed voltage-dependent K^+ channels that disappeared within ~ 10 min. To test for Ca^{2+}-activated currents, we studied cells that did not exhibit voltage-dependent K^+ currents or cells in which the current had run down. To increase the intracellular [Ca^{2+}] we added 1 μM ionomycin to an external solution which contained 2 mM Ca^{2+} (Fig. 4, *A* and *B*). Addition of ionomycin produced a large increase in outward current (at +90 mV the current increased from 10.6±3.4 to 51.2±9.7 pA; n = 5, mean±SEM) and a smaller increase in inward current (at −90 mV from −4.3±0.9 to −10.8±1.6 pA). Addition of ionomycin at a low extracellular [Ca^{2+}] (< 40 nM Ca^{2+}, solution B1 containing 10 mM EGTA) did not significantly increase current: current at +90 mV before ionomycin was 14.5±6.6 pA and after ionomycin was 18.3±7.5 pA (n = 4). During steps to depolarizing voltages there was no evidence of current activation, suggesting that the Ca^{2+}-activated outward current was not due to activation of the voltage-dependent K^+ channel described above. In fact, there was sometimes a small and variable decrease in current amplitude during depolarizing voltage steps.

The Ca^{2+}-activated outward current could be due to K^+ efflux or Cl^- influx. Therefore, we changed the bath solution to Na-isethionate (Fig. 4 *C*). This procedure led to a decrease of outward currents at all positive voltages and a shift of the reversal potential towards positive voltages (Fig. 4, *C* and *D*). This result suggested the presence of Ca^{2+}-activated Cl^- channels in human neutrophils. However, because an outward current persisted in all experiments, the results also suggested the presence of a Ca^{2+}-activated K^+ current.

To isolate the Ca^{2+}-activated Cl^--current, we performed experiments in symmetrical NaCl-solutions. After exposure to ionomycin, the cells exhibited an outward current (Fig. 5, *A* and *B*). However, the ionomycin-induced increase in current (at +90 mV the current increased from 12.9±3.0 to 26.7±7.4 pA; n = 6) was much less than that observed when the pipette solution contained K^+ (see above). With NaCl in the pipette, the Ca^{2+}-induced current was largely inhibited by changing the bath solution to Na-isethionate (at +90 mV the current was inhibited by > 80%, Fig. 6 *B*), demonstrating that the current was due to Cl^- influx and not to Na^+ efflux. The current-voltage relationship of the Ca^{2+}-activated Cl^- current (currents before ionomycin were subtracted from currents after ionomycin, Fig. 5 *C*) showed outward rectification. As there was no apparent voltage activation of the Cl^- conductance, this current-voltage relationship probably reflects the conductive properties of the Cl^- channel.

Fig. 6 shows cumulative results of studies on Ca^{2+} activation performed with either (*A*) KCl or (*B*) NaCl in the pipette. The outward current at +90 mV increased four to fivefold with KCl in the pipette, but only twofold with NaCl in the pipette. With NaCl in the pipette, changing the bath solution to Na-isethionate abolished the Ca^{2+}-induced outward current, while with KCl in the pipette, only a partial decrease was observed. These data suggest that, in addition to Ca^{2+}-acti-

2. That channel is also referred to as "delayed outward rectifier." This refers to activation of the channel by positive voltages; the single channel current–voltage relationship of the channel was linear (16).

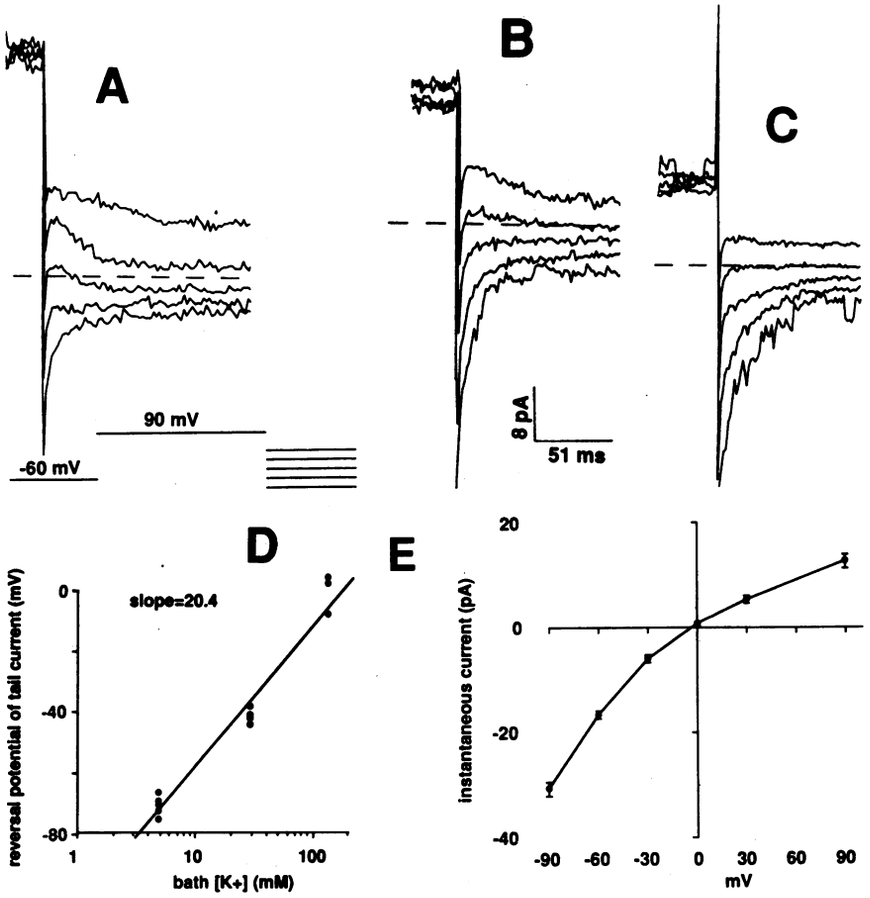

Figure 2. Analysis of tail currents with different external [K^+] Tail currents at various voltages (+30 to −90 mV) were measured after stepping to +90 mV for 400 ms. Panels *A*, *B*, and *C* show representative experiments. The dashed line indicates the zero-current level. The *inset* in *A* shows the voltage protocol for *A*, *B*, *C*, and *E*. Solutions: (*A*) bath NaCl, pipette KCl (B1, P2); (*B*) bath KCl/NaCl, pipette KCl B2, P2; (*C*) bath KCl, pipette KCl (B3, P2). (*D*) Reversal potential of tail currents plotted vs. log $[K^+]_0$. Line is linear regression through data, slope = 20.4. Each point represents a separate cell. (*E*) Whole-cell current–voltage relationship with maximally activated channel. Current was measured 15 ms after stepping from a holding voltage of +90 mV (400-ms duration) to the indicated voltage. Values are mean±SEM of three determinations in the same cell; similar results were obtained on more than 10 occasions. Solutions: bath K-aspartate, pipette K-aspartate (B4, P3). Leak currents were subtracted for values shown in *D* and *E*. Sweeps with a holding voltage of −60 mV and a target voltage of −90 mV were used as leak sweaps.

vated Cl^- channels, neutrophils possess Ca^{2+}-activated K^+ channels.

To isolate the Ca^{2+}-activated K^+ current, we performed experiments in symmetrical K-aspartate solutions. As mentioned above, we observed the spontaneously active K^+ current in most cells under these conditions (Fig. 7 *A*). After exposure to ionomycin (Fig. 7 *B*), an increase in outward current was observed (at +90 mV current increased from 18.2±3.2 to 36±9.7 pA; $n = 6$). The current–voltage relationship of the Ca^{2+}-activated K^+ channel (currents before ionomycin were subtracted from currents after ionomycin, Fig. 7 *C*) showed outward rectification. Again note that there was no appreciable voltage-dependent activation of the Ca^{2+}-activated current.

The apparent voltage insensitivity of both the Ca^{2+}-activated Cl^- channel and the Ca^{2+}-activated K^+ channel might theoretically be explained by a very rapid change of channel open probability. Thus, when stepping to an activating voltage, the channel might alter its open probability nearly instantaneously and therefore appear to be outwardly rectifying. To investigate this possibility we measured tail currents after addition of ionomycin (experimental conditions as described in Fig. 4). On stepping from positive voltages (+60 to +150 mV) to various negative voltages (−210 to −30 mV), we were unable to detect tail currents, even under recording conditions that allow resolution in the low msec range. It thus seemed that the Ca^{2+}-activated currents in neutrophils were indeed voltage-independent over this voltage range.

We also tested the effect of various K^+ channel blockers. Both, the voltage-dependent and the Ca^{2+}-activated K^+ current were blocked by 10–20 mM $BaCl_2$ in the bath, however this inhibition was difficult to evaluate, because addition of $BaCl_2$ also caused a K^+-independent outward current (not shown; we have not yet defined the ionic basis of this current). No inhibition of K^+ currents was observed by charybdotoxin (1,000 nM), apamin (20 nM), quinine (200 μM), or 4-aminopyridine (10 mM) (values in parentheses indicate the maximal concentrations tested). Thus, we have not yet been able to distinguish the voltage-dependent and the Ca^{2+}-activated K^+-currents by using blockers.

Discussion

In this study, we analyzed the conductive properties of the plasma membrane of neutrophil granulocytes using the whole-cell patch-clamp method. Our results demonstrate three types of plasma membrane ion channels in neutrophils: (*a*) a voltage-dependent K^+ channel, (*b*) a Ca^{2+}-activated K^+ channel, and (*c*) a Ca^{2+}-activated Cl^- channel. To our knowledge, this is the first study of whole-cell currents in neutrophils.

A variety of K^+ channels have been shown to be sensitive to both Ca^{2+} and voltage (20). Therefore, we considered the possibility that the voltage-dependent K^+ conductance and the Ca^{2+}-activated K^+ conductance might reflect two different modes of activation of the same channel. The K^+ channel blockers used in this study did not distinguish between the two conductances. However, several observations indicate that they are two different K^+ channels. The Ca^{2+}-activated K^+

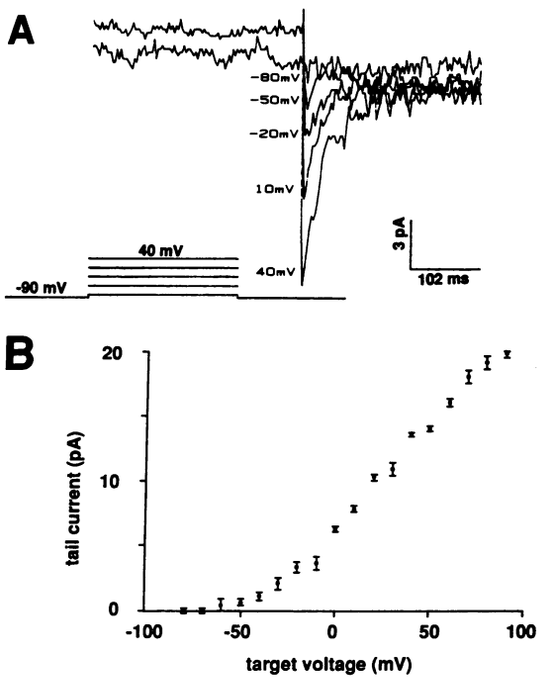

Figure 3. Voltage dependence of K^+-channel activation. Activation of the K^+ channel by a voltage was assessed as the tail current observed when stepping to −90 mV after holding for 400 ms at the respective voltage. Tail currents at −90 mV after holding at −80, −50, −20, 10, and 40 mV, respectively, are shown in *A*. *Insets* show the voltage protocol and current and time scale. In *B* the size of the tail current (capacitance currents measured after stepping from the holding voltage to the target voltage were subtracted from the tail current observed in the same sweep) is plotted vs the respective holding voltage (mean±SEM, four measurements in one cell; similar results were obtained in five different cells). Solutions: bath K-aspartate, pipette K-aspartate (B4, P3).

channel was dependent on an increase in intracellular $[Ca^{2+}]$, voltage-insensitive, and outwardly rectifying. In contrast, the voltage-dependent K^+-channel was Ca^{2+}-independent,[3] voltage-dependent and inwardly rectifying.

One previous single-channel study (5) demonstrated the existence of nonselective cation channels in neutrophils. In our whole-cell recordings we occasionally observed channels of similar appearance (see Fig. 2 *C* for example). As these channels were rare in whole-cell recordings (found in ~ 1 out of 30 recordings), their contribution to the whole-cell conductance of neutrophils appeared to be minor.

The resting membrane potential of neutrophils has been estimated to be around −60 mV in most studies (7–10, 12). Functional studies (13) show that bathing cells in high K^+ leads to rapid depolarization, indicating that the potential is maintained by K^+ channels. The voltage-dependent channel described in this study has properties that suggest that it may determine resting membrane potential: (*a*) it is observed in unstimulated cells; (*b*) it is K^+-selective; and (*c*) with a threshold of voltage activation around −60 mV, it would hyperpolarize cells only to this voltage (which is more positive than the expected K^+ reversal potential of approximately −80 mV). Thus, we speculate that the physiological function of this

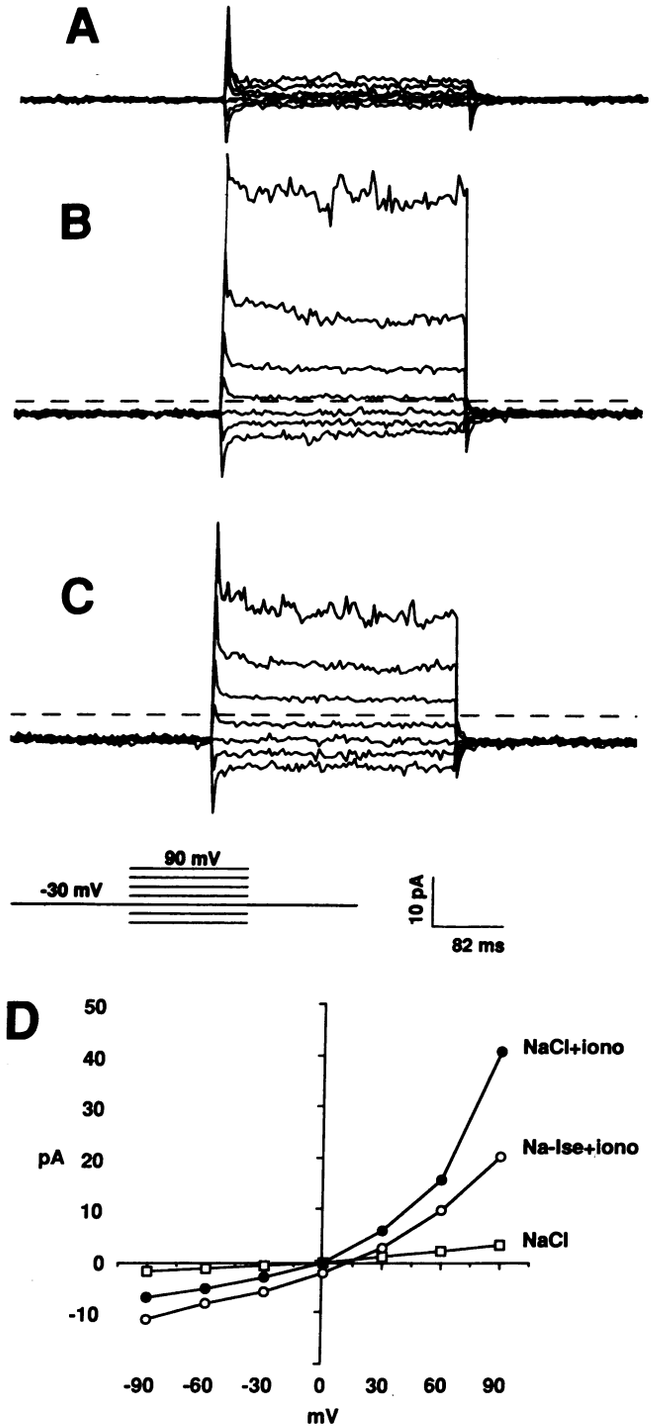

Figure 4. Effect of increasing intracellular $[Ca^{2+}]$ on membrane current. (*A*) An unstimulated neutrophil that did not exhibit voltage-dependent currents (*B*) was exposed to 1 μM ionomycin. Solutions: bath NaCl, pipette KCl (B1, P2). (*C*) Bath solution was changed to Na-isethionate containing 1 μM ionomycin (*Na-Ise + iono*). *Insets* show the voltage protocol and current and time scale for *A*, *B*, and *C*. (*D*) Whole-cell current–voltage relationship of the traces shown in *A–C*. Similar results were obtained in five different cells.

3. We also observed the voltage-dependent K^+ current in the presence of a bath solution containing ionomycin and 10 mM EGTA (data not shown).

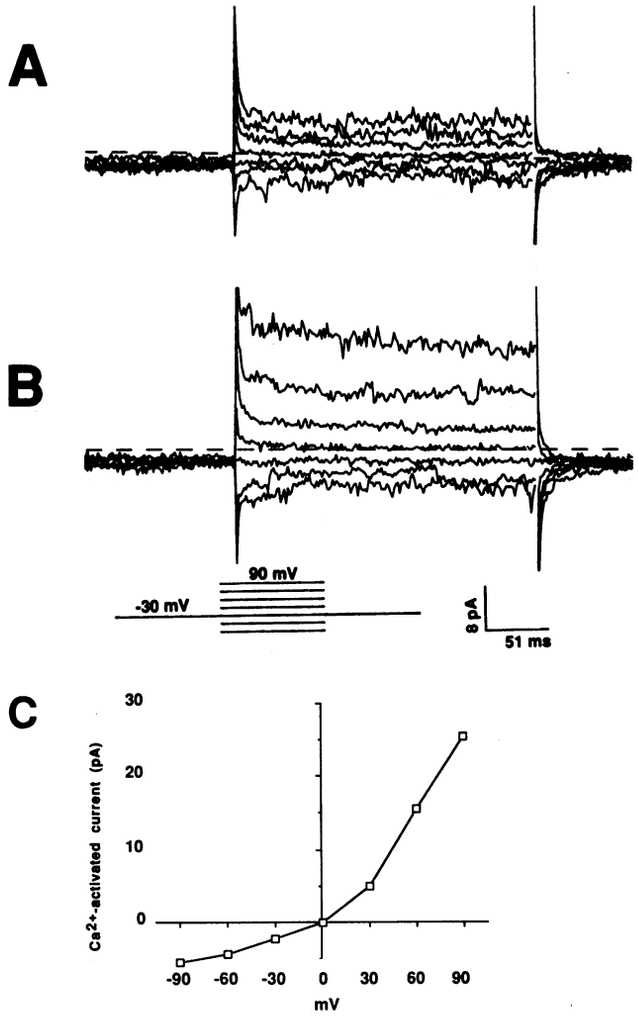

Figure 5. Ca^{2+}-activated current in the absence of K^+. (*A*) An unstimulated neutrophil that did not exhibit spontaneous whole-cell currents (*B*) was exposed to 1 μM ionomycin. Solutions: bath NaCl, pipette NaCl (B1, P4). *Insets* show the voltage protocol and current and time scale. (*C*) Current–voltage relationship of the Ca^{2+}-activated current obtained by subtraction of currents in *A* from those in *B* (similar results were obtained in six different cells).

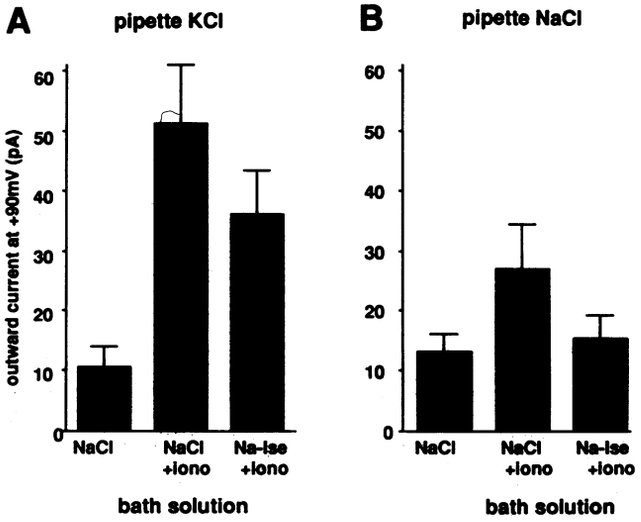

Figure 6. Effect of pipette and bath solution on Ca^{2+}-activated currents. Experiments were performed as described in Figs. 4 and 5. Pipette solutions contained KCl (P2, panel *A*) or NaCl (P4, panel *B*). Size of outward current at +90 mV is shown in unstimulated cells (*NaCl*), after addition of 1 μM ionomycin (*NaCl + iono*) and after exchange of bath solution to Na-isethionate containing 1 μM ionomycin (*Na-Ise + iono*) (mean±SEM, n = 4–6).

channel in neutrophils is the maintenance of the resting potential.

It is of interest to compare the voltage-dependent ion channels in neutrophils with those observed in another phagocytic cell, the macrophage. As shown in Table II, three types of voltage-dependent K^+ channels have been described in macrophages: (*a*) a hyperpolarization-activated, inwardly rectifying K^+ channel; (*b*) a depolarization-activated K^+ channel, referred to as a delayed rectifier; and (*c*) a voltage-dependent and Ca^{2+}-activated K^+ channel. The first is clearly different from the neutrophil channel because it is activated by hyperpolarization. The second is activated by depolarization like the neutrophil voltage-dependent K^+ channel, however it differs from the neutrophil channel in (*a*) its conductive properties (the macrophage channel has a linear current-voltage relationship [16]), (*b*) its relatively rapid deactivation (16, 17), and (*c*) its

Table II. Comparison of Electrophysiological Properties of Macrophages and Neutrophils

Property	Neutrophils	Macrophages
Resting membrane voltage	Ca. −60 mV (6–12)	−40 to −60 mV (21)
Addition of chemotactic peptide	Depolarization (7, 8)	Hyperpolarization (21)
Addition of Ca^{2+} ionophore	Depolarization (22)	Hyperpolarization (21)
Depolarization activated, "delayed rectifier" K^+ channel	No*	Yes (16, 17)
Depolarization activated, inwardly rectifying K^+ channel	Yes*	No
Hyperpolarization activated, inwardly rectifying, K^+ channel	No*	Yes (17–19)
Ca^{2+}-activated, voltage-dependent K^+ channel	No	Yes (23)
Ca^{2+}-activated, voltage-independent K^+ channel	Yes*	Yes (19, 24)
Ca^{2+}-activated Cl^- channel	Yes*	No

Properties are given as found in this study (*) or as described in the respective references. Studies on membrane potential in neutrophils were mainly done with charged lipophilic dyes or radiotracers, studies in macrophages used mainly microelectrode impalement techniques. Although some studies reported very high (6) or very low (11) resting plasma membrane potential in neutrophils, most studies suggest values of approximately −60 mV (7–10, 12). Our data (Fig. 1 and footnote 1) is consistent with a membrane voltage between −30 and −60 mV.

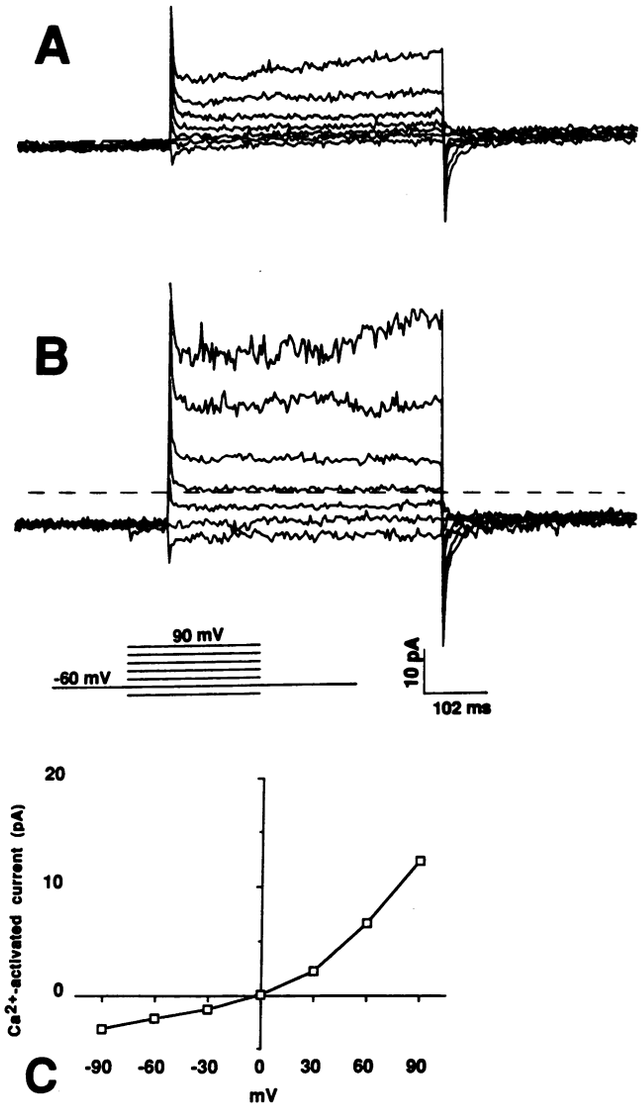

Figure 7. Ca^{2+}-activated current in the absence of Cl^-. (*A*) An unstimulated, neutrophil exhibiting a voltage-dependent K^+ current (*B*) was exposed to 1 μM ionomycin. Solutions: bath K-aspartate, pipette K-aspartate (B4, P3). *Insets* show the voltage protocol and current and time scale. (*C*) Whole-cell current–voltage relationship of the Ca^{2+}-activated current obtained by subtraction of currents in *A* from those in *B*.

sensitivity to 4-amino-pyridine (16, 17). The third is distinguished from the neutrophil channel by its sensitivity to Ca^{2+} (28).

To assess the potential role of Ca^{2+}-activated channels in the regulation of membrane voltage, we compared the effect of Ca^{2+} on neutrophils and macrophages (Table II). Macrophages, like neutrophils, possess cell surface-receptors for the Ca^{2+}-mobilizing agonist FMLP. However, while Ca^{2+} ionophores and FMLP depolarize neutrophils, they hyperpolarize macrophages (21). The Ca^{2+}-induced hyperpolarization in macrophages is thought to result from Ca^{2+}-activated K^+ channels. As shown in this study, neutrophils also have Ca^{2+}-activated K^+ channels; but in addition they have Ca^{2+}-activated Cl^- channels, which were not found in macrophages (Table II). Ca^{2+} activation of both the Cl^- and K^+ channels might result from either a direct ligand-type interaction of Ca^{2+} with the channel or some indirect effect of Ca^{2+}.

Could Ca^{2+} activation of Cl^- channels be responsible for Ca^{2+}-induced depolarization in neutrophils? The intracellular Cl^--concentration in neutrophils is thought to be around 80 mM (12). Thus, for an extracellular Cl^- concentration of 140 mM, the Cl^- reversal potential would be −14 mV, which is less negative than the resting potential. Activation of the Cl^- channel by Ca^{2+} could therefore depolarize the cell, depending on the relative contribution of Ca^{2+}-activated Cl^- channels versus Ca^{2+}-activated K^+ channels. However, depolarization of neutrophils in response to FMLP is only partially Ca^{2+}-dependent and Ca^{2+}-independent depolarization is observed in response to phorbol esters (13). Thus, while Ca^{2+} activation of Cl^- channels is likely to contribute to depolarization in neutrophils, it is unlikely to be the only mechanism.

The effect of a concomitant Ca^{2+}-induced opening of Cl^- and K^+ channels on net salt fluxes would be a loss of KCl to the extracellular space. Such a loss of intracellular KCl has been shown in other cell types to be accompanied by a loss of intracellular water and a decrease in cell volume (25, 26). What could be the physiological significance of a Ca^{2+}-induced decrease of cell volume?

In other cell types a Ca^{2+}-conductive pathway sensitive to cell volume has been described (27, 28). Exposure of such cells to hypotonic solutions and consequent increase in cell volume, activates Ca^{2+} influx, which in turn activates Ca^{2+}-gated K^+ and Cl^- channels. This leads to net loss of KCl and water and therefore decreases cell volume towards normal. Neutrophils might have such a mechanism, inasmuch as they are resistant to hypotonic solutions: brief exposure to distilled water, a routine step in neutrophil purification which lyses red blood cells, does not damage neutrophils. However, no volume-sensitive Ca^{2+}-influx has yet been described in neutrophils.

Alternatively, changes in cell volume might be part of the response to the increase in intracellular [Ca^{2+}] that occurs during cellular activation. Many neutrophil functions that are mediated or accompanied by rises in intracellular Ca^{2+}, such as chemotaxis, adherence to surfaces and spreading, pseudopod formation, and phagocytosis, might necessitate changes in cell volume. The possible involvement of Ca^{2+}-activated Cl^- and K^+ channels in these neutrophil functions will be an important subject of further studies.

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