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Sustained Depolarization and ADP-Ribose Activate a Common Ionic Current in Rat Peritoneal Macrophages

Brice Campo, Annmarie Surprenant, and R. Alan North

Phagocytosis is associated with large changes in the membrane potential of macrophages, but the functional significance of this is unknown. Whole cell recordings were made from rat peritoneal macrophages. Sustained (>30 s) depolarization of the cells progressively activated a conductance that remained high (several nS) for several tens of seconds. This current: 1) was linearly dependent on potential between −100 and +50 mV; 2) reversed close to 0 mV in a physiological external solution; 3) could also be carried in part by N-methyl-D-glucamine (P_{NMDG}/P_{Na} 0.7), chloride (P_{Cl}/P_{Na} 0.5), or calcium (P_{Ca}/P_{Na} 1.3); and 4) was blocked by intracellular ATP (5 mM) or ADP (10 mM) and by extracellular lanthanum (half-maximal concentration 1 mM). A current with all the same properties was recorded in cells when the intracellular solution contained ADP-ribose (10–300 μM) or β-NAD (1 mM) (but not any other nucleotide analogs tested). The results suggest that prolonged depolarization leads to an increased intracellular level of ADP-ribose, which in turn activates this nonselective conductance(s). The Journal of Immunology, 2003, 170: 1167–1173.

The membrane potential of macrophages undergoes a prolonged depolarization following phagocytosis of an immune complex (1). This depolarization follows an increase in superoxide by a variable period, but typically tens of seconds, and it is subsequently maintained for several minutes. The underlying current shows little rectification and reverses close to 0 mV. The current is blocked by an inhibitor of NADPH-oxidase, and mimicked by xanthine and xanthine oxidase, leading to the conclusion that the depolarization results from free radical release within the cell following particle engulfment. This finding is in keeping with a body of work in a range of cells, which indicates that oxidative stressors, such as H₂O₂, can activate inward currents (2, 3). The capacity of such currents to deliver calcium to the cell has been considered to contribute to subsequent oxidative damage.

Although intracellular calcium plays a key role in several aspects of macrophage function, including activation, migration, and proliferation as well as generation of superoxide anion and stimulation of NO production, calcium influx is not immediately required for phagocytosis (4–6). It is unlikely that the well-studied store-operated or capacitative calcium entry current, which is present in all nonexcitable cells, could underlie the current associated with phagocytosis in macrophage because it shows a characteristic strong rectification, the currents are very small (only a few pA at −60 mV), and it is relatively impermeable to sodium under normal conditions (6–11). Recently, a current has been described in the human macrophage U937 cell line that is activated by extracellular H₂O₂, or intracellular β-NAD free acid (βNAD⁺; 1 mM) (12, 13) and might therefore underlie the current following phagocytosis described by Holevinsky and Nelson (1). This current is also activated by intracellular ADP-ribose at concentrations (10–300 μM) lower than those required for βNAD⁺. It corresponds in some of its properties to the currents observed with heterogeneous expression of TRPM2 subunits (formerly called LTRPC2; for nomenclature, see Ref. 14) (12, 13, 15).

These results are consistent with the interpretation that the prolonged depolarization that follows phagocytosis described by Holevinsky and Nelson (1) results from an inward current, such as that activated by βNAD⁺ in U937 cells (12, 13). However, during the course of voltage-clamp recordings from rat peritoneal macrophages, we noticed that a sustained inward current could actually be activated by prolonged depolarization. In this work, we describe some properties of this current, and compare these with the current activated by βNAD⁺ and ADP-ribose in the same cells. Both currents showed the same permeability to sodium, calcium, N-methyl-D-glucamine (NMDG), chloride, and gluconate ions, and sensitivity to block by lanthanum. The current activated by intracellular ADP-ribose occluded the depolarization-activated current. Thus, we conclude it is likely that these two stimuli activate the same conductance.

Materials and Methods

Culture of rat peritoneal macrophages

Rat peritoneal cells were obtained from male Wistar rats (10 wk old) killed by cervical dislocation. The peritoneal cavity was lavaged with PBS (Life Technologies Cell Culture, Invitrogen, Paisley, U.K.) After centrifugation, the macrophage fraction was separated from the mast cells by fluorescence-activated cell sorting. Two populations of cells were separated by forward and side scatter. Cells were then collected by centrifugation and resuspended in DMEM (Life Technologies Cell Culture) containing 10% (v/v) FCS (Sigma-Aldrich, Poole, U.K.), 1% glutamine (Calbiochem, La Jolla, CA), and 1% antibiotics (Life Technologies Cell Culture, Invitrogen). Cells were plated onto glass coverslips and kept at 37°C in a humidified atmosphere containing 5% CO₂. The cultured macrophages were used in patch-clamp experiments 1–3 days after plating. Cells were identified as macrophages using immunohistochemistry staining with the macrophage specific Abs ED1–10 and ED-2 (Serotec, Oxford, U.K.).

HEK293 cells expressing P2X₂/₃ receptors

HEK293 cells were transfected, as previously described (16). Briefly, cells were electroporated (Gene Pulser; Bio-Rad, Hercules, CA) in the presence of 30 mg of plasmid containing both P2X₂ and P2X₃ receptor cDNAs. Following electroporation, cells were plated and selected for neomycin resistance, subcloned by FACS sorting, and resuspended in standard conditions.
growth medium (Dulbecco's) supplemented with 10% heat-inactivated FCS and 2 mM t-glutamine. Cells were grown to confluence and plated on coverslips, and recordings were made 2–48 h later.

Whole cell recording

Ion currents were studied using the whole cell configuration of the patch-clamp technique, as described by Hamill et al. (17). Glass electrodes (Harvard Apparatus, Holliston, MA) ranged from 6 to 8 MΩ in resistance, and were fire polished. The indifferent electrode was an Ag-AgCl electrode connected to the bath through a short 3 M KCl agar bridge. All the experiments were performed at room temperature using an EPC9 patch-clamp system (HEKA, Lambrecht, Germany). The cells were kept in an extra-

Liquid junction potentials were calculated directly from ionic mobilities. The value of $P_{\text{Cl}} / P_{\text{Na}}$ in subsequent calculations in which three permeant ions constant, $F \frac{P_{\text{Cl}}}{P_{\text{Na}}}$ cose, $12$ (pH 7.3). Series resistance was compensated by $x$ $P_{\text{Cl}} / P_{\text{Na}}$ $\text{Cl}^-$, $0.3$ mM; glucose, 236 (pH brought to 7.3 with 4.2 mM NaOH). The isotonic solution contained (mM): NaCl, 147; HEPES, 10; CaCl2, 2; glucose, 13. The pH was adjusted to 7.3 by adding NaOH (4 mM). The standard intracellular solution contained (mM): NaCl, 148; HEPES, 10; EGTA, 10. We used intracellular sodium rather than potassium in most experiments so that voltage-dependent potassium currents did not complicate our estimates of reversal potential. We used a high EGTA concentration so as to reduce any involvement of store-operated conduc-
tances in those cases in which we had calcium-free extracellular solutions.

The pH was adjusted to 7.3 by adding NaOH (24 mM). Drug application and solution exchanges were conducted using a fast flow RSC 200 system (Biologic Science Instruments, Claix, France). Cells were held at $-80$ mV, except where stated, and current-voltage plots were obtained from ramp commands (−130 to 500 mV in 500 ms). Permeability experiments were conducted using standard intracellular solution and extracellular solution of different composition. The low NaCl solution contained (mM): NaCl, 40; HEPES, 10; CaCl2, 0.3 mM; glucose, 236 (pH brought to 7.3 with 4.2 mM NaOH). The NMDG solution contained (mM): NMDG, base 154; HEPES, 10; NaCl, 23; glucose, 23 (pH brought to 7.3 with HCl; final Cl concentration 146 mM). The gluconate solution contained (mM): Na-glucurate, 147; HEPES, 10; glucose, 33 (pH brought to 7.3 with 4 mM NaOH). The isotonic calcium solution contained (mM): CaCl2, 110; NaOH, 2; HEPES, 10; glu-
cose, 12 (pH 7.3). Series resistance was compensated by $\sim 75\%$.

Liquid junction potentials were calculated directly from ionic mobilities (18). Measured potentials were corrected for the junction potential existing at the recording pipette before seal formation (pipette solution $-1.9$ mV with respect to bath solution) and for any change in junction potential at the indifferent electrode when the bath solution was changed ($<4$ mV in all cases).

Intracellular sodium measurement

Cells were loaded with 1.3-benzenedicarboxylic acid, 4,4'-[1,4,10-tioxo-

Measurements of permeability

We first measured the permeability of chloride relative to that of sodium ($P_{\text{Cl}}/P_{\text{Na}}$) in bi-ionic conditions. $P_{\text{Cl}}/P_{\text{Na}}$ was then calculated from $I_{\text{Cl}} / I_{\text{Na}}$ in bi-ionic conditions. $P_{\text{Cl}}/P_{\text{Na}}$ and $P_{\text{Cl}}/P_{\text{Na}}$ were measured using two concentrations of lanthanum chloride, $0.8$; NMDG, xanthine, and xanthine oxidase were purchased from Sigma-Aldrich. $\gamma$-NAD, $\beta$-NAD di-
sodium salt (\(\gamma\)NADH), $\beta$-NAD phosphate disodium salt (\(\gamma\)NADP$^+$), $\beta$-NADPH disodium salt (\(\gamma\)NADPH), and 1-[b-(3-(4-methoxyphenyl)-

Results

Macrophages had a capacitance of $11.4 \pm 0.4$ pF ($n = 110$). The cell diameter was $9.1 \pm 0.2$ $\mu$m ($n = 30$). Step depolarizations from a holding potential of $-80$ mV (10 mV increments to $+40$ mV, 500 ms duration) revealed no time-dependent currents when a sodium chloride-based internal solution was used. When a po-
tassium chloride-based solution was used (150 mM KCl, 10 mM HEPES, 10 mM EGTA), the resting potential of the cells was $-30.9 \pm 0.7$ mV ($n = 11$).

Current activated by depolarization

Cells held at $-80$ mV showed no change in holding current or conduction during 5–10 min of whole cell recording. However, if the cell was held at $+10$ mV for several tens of seconds and then returned to $-80$ mV, a large inward current was observed (Fig. 1A). This current quickly (5 mV) reached its peak amplitude (sev-
eral hundred pA) and then declined during the next few minutes (Fig. 1A; in 10 cells the amplitude after 2 min was $19 \pm 4\%$ of the peak amplitude). The duration of the depolarization required to evoke the subsequent inward current was not studied in detail; however, a depolarization to $+10$ mV for only 10 s was not ef-
fec
tive ($n = 5$). After an interval of several minutes, a second depolarization again elicited a large inward current with similar properties ($n = 10$).

The inward current was associated with a large increase in cell conductance. The current-voltage relation of the cell remained essentially linear, and reversed close to 0 mV ($-3.1 \pm 0.24$ mV, $n = 18$) in normal external solution (Fig. 1, B and C) (Table I). The time course of the current increase was followed by holding the cell at $+10$ mV, but repeatedly applying brief ramp voltage commands ($-130$ to $+50$ mV in 500 ms; 5 s intervals). Fig. 1C shows the time course of the resulting conductance increase. The current (at $-80$ mV) grew more slowly and was much smaller with a sustained holding potential of $-40$ mV (Fig. 1D), and it was larger with depolarization to $+40$ mV.

In low chloride extracellular solution (40 mM NaCl), the current at $-80$ mV was reduced to $51 \pm 16\%$ ($n = 3$) of its control value; the residual current reversed polarity at $-13.1 \pm 1.6$ mV ($n = 4$) (Fig. 2A); from equation 1 this corresponds to $P_{\text{Cl}}/P_{\text{Na}}$ of $0.5$ (Table I). When extracellular sodium was substituted by NMDG, the current evoked by depolarization was reduced to $79 \pm 2\%$ ($n = 6$) of its control value (measured at $-80$ mV). The residual current reversed polarity at $-5.4 \pm 0.45$ mV ($n = 7$) (Table I; Fig. 2A), which indicates that NMDG is significantly permeable ($P_{\text{Cl}}/P_{\text{Na}} 0.74$; from equation 2). When extracellular gluconate replaced chloride, the current at $-80$ mV was reduced to $91 \pm 4\%$ ($n = 4$), and when extracellular calcium replaced sodium, the inhibition was to $48 \pm 6\%$ ($n = 6$) of control (Fig. 2B). By measuring the reversal potential of the residual current (Table I), it was evident
that the conductance activated by prolonged depolarization is significantly permeable to chloride and gluconate, NMDG, and calcium ions. In each of these ion substitutions, the current-voltage relation remained essentially linear (Fig. 2).

We conducted control measurements of the permeability of P2X receptors expressed in HEK293 cells, using the same intracellular and extracellular solutions. We used the agonist αβmeATP (30 μM) to activate the inward current in cells expressing P2X2/3 subunits, which was typically ~1 nA in amplitude (20). We observed that the agonist-induced current reversed polarity at 0.8 ± 0.5 mV (n = 23) in control extracellular solution, −33 ± 1.4 mV (n = 4) in 40 mM NaCl, and −86.5 ± 0.4 mV (n = 4) in NMDG solution. These reversal potentials correspond (from equations 1 and 2) to relative permeabilities of \( P_{Cl}/P_{Na} = 0.02 \) and \( P_{NMDG}/P_{Na} = 0.03 \) (see Ref. 21).

The current induced by depolarization was substantially blocked by lanthanum (10 μM) (Fig. 1); the IC₅₀ for this action was ~1 μM (Fig. 3A). The current was also significantly inhibited (76 ± 10%, n = 4) by SKF96365 (100 μM); at 10 μM SKF96365 inhibited the current in some (75 ± 8%, n = 4), but not other (n = 3) cells. SKF96365 has been widely used to block nonselective cation channels and store-operated calcium entry in nonexcitable cells since its introduction by Merritt et al. (22). The current evoked by depolarization was also greatly reduced by including in the pipette either ATP (5 mM, inhibited by 89 ± 5%, n = 4) or ADP (10 mM, inhibited by 97 ± 4%, n = 5) (Fig. 3B).

**Current activated by ADP-ribose**

An inward current progressively developed over tens of seconds in cells held at −80 mV, when the recording electrode contained

### Table 1. Reversal potentials and relative permeabilities for two macrophage currents

<table>
<thead>
<tr>
<th>Depolarization</th>
<th>Remaining Current (%)</th>
<th>( E_{rev} ) (mV)</th>
<th>( P_{Na}/P_{Cl} )</th>
<th>ADP-Ribose</th>
<th>Remaining Current (%)</th>
<th>( E_{rev} ) (mV)</th>
<th>( P_{Na}/P_{Cl} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>51 ± 16 (3)</td>
<td>−3.1 ± 0.24 (18)</td>
<td>0.47</td>
<td>Control</td>
<td>69 ± 2 (4)</td>
<td>−2.7 ± 0.65 (11)</td>
<td>0.45</td>
</tr>
<tr>
<td>Low chloride</td>
<td>70 ± 2 (6)</td>
<td>−13.1 ± 1.6 (4)</td>
<td>0.74</td>
<td>Low chloride</td>
<td>75 ± 2 (4)</td>
<td>−13.5 ± 1.2 (4)</td>
<td>0.84</td>
</tr>
<tr>
<td>NMDG</td>
<td>79 ± 4 (4)</td>
<td>−5.4 ± 0.45 (7)</td>
<td>0.96</td>
<td>NMDG</td>
<td>84 ± 4 (4)</td>
<td>−4.8 ± 0.35 (4)</td>
<td>0.90</td>
</tr>
<tr>
<td>Gluconate</td>
<td>91 ± 4 (4)</td>
<td>−8.8 ± 0.6 (6)</td>
<td>1.33</td>
<td>Gluconate</td>
<td>42 ± 6 (3)</td>
<td>−8.3 ± 0.34 (4)</td>
<td>1.15</td>
</tr>
<tr>
<td>Isotonic calcium</td>
<td>48 ± 6 (6)</td>
<td>0.20 ± 0.50 (6)</td>
<td></td>
<td>Isotonic calcium</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± SEM for number of cells in parentheses.*

**FIGURE 1.** Current activated by depolarization of cell membrane. A, Example in one cell of current activated by sustained depolarization (+10 mV). When the holding potential is returned to −80 mV after 30 s depolarization, a large inward current develops and then progressively declines during the next minutes. The current remaining after 90 s is quickly and completely blocked by lanthanum (10 μM). B, Current-voltage plots at the times indicated in A, before, during, and after activation of the current. C, Current-voltage plots recorded at different times after moving to a holding potential of +10 mV. A voltage ramp (−130 to 50 mV in 500 ms) was applied at 5 s intervals (time indicated beside selected traces). D, Time course of development of current at −80 mV, taken from experiments such as those in C. A large current was evoked by holding at +10 mV (●); this was smaller when the potential was held at −40 mV (▲), and did not occur with a holding potential of −80 mV (●). Points are mean amplitude (±SEM) for eight (+10 mV), three (−40 mV), and five (−80 mV) cells.
ADP-ribose (Fig. 4A). The current-voltage relation remained linear throughout this period, and reversed close to 0 mV (−2.7 ± 0.6 mV, n = 11) (Fig. 4B). With 1 μM ADP-ribose, the development of the current was fitted by an exponential with a time constant of ~1 min (Fig. 4C). The lowest concentration of ADP-ribose that was effective was 100 nM, and maximum value of the current was reached with ~100 μM; the EC_{50} was ~10 μM (Fig. 4D). Changing the level of intracellular calcium buffering did not obviously alter the effectiveness of ADP-ribose to activate this current. In our control, strongly buffered solution (10 mM EGTA, 0 mM calcium, ~1 nM [Ca\text{\textsubscript{i}}]) ADP-ribose (300 μM) elicited a current of 703 ± 119 pA (n = 3); with a higher level of intracellular calcium (11 mM EGTA, 2 mM calcium, ~100 nM [Ca\text{\textsubscript{i}}]), this was 811 ± 149 pA (n = 5). No similar current developed when the recording electrode contained ADP (100 μM), ADP-glucose (100 μM), cyclic ADP-ribose (100 μM), AMP (100 μM), ATP magnesium salt (100 μM), β-ribose (100 μM), β-ribose 5-phosphate (100 μM), βNADH (300 μM or 1 mM), βNADPH (300 μM) (n = 3 cells in all cases). However, βNAD^+ at a concentration of 1 mM (n = 4) evoked a current similar to that observed with ADP-ribose, although 100 and 300 μM were ineffective. The peak current elicited by βNAD^+ (1 mM) was 758 ± 428 pA (n = 4), which is not different from that seen with ADP-ribose (Fig. 4).

The relative permeability of the current induced by ADP-ribose (300 μM) was examined by measuring the reversal potential in different extracellular solutions. From the values given in Table I, it can be seen that the relative permeability to chloride, NMDG, gluconate, and calcium ions did not differ markedly from those determined for the currents elicited by depolarization.

The current evoked by ADP-ribose was also very sensitive to block by extracellular lanthanum (Fig. 3A), with an IC_{50} of ~10 μM. It was also blocked by SKF96365 (100 μM, 77 ± 4% inhibition, 5 of 5 cells tested; 10 μM, 70 ± 7%, 7 of 12 cells tested) and by intracellular either ATP (5 mM, inhibited by 66 ± 11%, n = 5) or ADP (10 mM, inhibited by 76 ± 3%, n = 4) (Fig. 3B). These relative inhibitions were not different from those observed for the current activated by depolarization (ANOVA, p > 0.05).

A membrane current with similar properties was also elicited by applying xanthine (0.1 mM) and xanthine oxidase (0.05 U/ml), to generate superoxide anions; this current was completely blocked by 100 μM lantanum (Fig. 5A). Application of xanthine alone had no effect. In parallel experiments, we used intracellular sodium imaging as an index of depolarization of undisturbed macrophages. In these cells, H\text{2}O\text{2} (10 mM) evoked a progressive increase in intracellular sodium concentration ([Na\text{\textsubscript{i}}]) (Fig. 5B). The time course of the [Na\text{\textsubscript{i}}] increase was similar to that of the inward current observed with xanthine/xanthine oxidase, or depolarization, and the increase in [Na\text{\textsubscript{i}}] was also strongly reduced by lanthanum (1 mM) (Fig. 5B).

**Occlusion of the two currents**

The similar properties of the two currents led us to hypothesize that sustained depolarization and intracellular ADP-ribose were
activating the same conductance. We recorded from 10 cells with electrodes containing ADP-ribose (300 μM); after 4 min, when the inward current reached its steady value, we applied a depolarization to +10 mV for an additional 4 min. This depolarization evoked no additional current. In time-matched controls, the depolarization elicited large inward currents recorded at −80 mV (Fig. 6).

Discussion
We found that sustained depolarization of rat peritoneal macrophages activates a large inward current. The current is carried by a conductance that is nonselective for cations, and that also has quite significant permeability to chloride and glucuronate ions. The permeability to chloride ions was surprising, and for this reason we compared the results directly with those for the permeability of another cation-selective membrane channel. In the low chloride solutions that we have used ([Na]i = 172 mM, [Cl]i = 148 mM, [Na]o = 44 mM, [Cl]o = 41 mM), the observed reversal potential for the P2X2/3 receptor corresponds to a very low chloride permeability (PCl/PNa of ~0.02). The estimates of permeability to NMDG, calcium, and glucuronate should be considered as less reliable than those for PCl/PNa. This is because in those cases there are three permeant ions present, and the estimate involves using the value previously determined for PCl/PNa.

Likewise, the reversal potential observed with extracellular NMDG indicates that the P2X2/3 receptor is essentially impermeable (P[NMDG]/PNa < 0.03), whereas both the currents presently studied in macrophages have significant permeability to NMDG. From experiments of the type described, it is difficult to exclude the possibility that the sustained depolarization is activating more than one set of ionic channels; for example, one permeable to anions and another permeable to cations. All that can be said in this case is that each has a very similar, and linear, current-voltage relation and each has a similar sensitivity to block by lanthanum. We shall return later to discuss the question of how a sustained depolarization might lead to the activation of such an ionic conductance(s).

There are many similarities between these permeation properties and those reported by Holevinsky and Nelson (1) for the current following phagocytosis of fluorescein-labeled immune complexes recorded from macrophages derived from human blood monocytes. That was a large current (typically ~1 nA), similar in amplitude to the currents that we presently describe; this is in marked contrast to the very small (~50 pA) current elicited by thapsigargin in the same cells (10). The underlying conductance had P[NMDG]/PNa of 0.69 in the work of Holevinsky and Nelson (1), which is close to our present estimate. Furthermore, they also reported that the underlying channels had significant anion permeability, and that glutamate appeared to be more permeable than chloride. That current was also completely blocked by 1 mM lanthanum, although the effects of lower concentrations were not examined. Holevinsky and Nelson (1) found that a current with similar properties could be activated by xanthine plus xanthine oxidase, although not by xanthine alone, and they suggested that the release of free radicals following phagocytosis activated the underlying conductance. Holevinsky and Nelson (1) did not investigate whether a sustained depolarization could elicit such a current, as we have reported; they did report that, under current clamp, the current led to a sustained depolarization of the cells.

It has recently become clear that oxidants such as H2O2 can indeed activate a cation conductance in certain immune cells (12). In the monocytic cell line U937, as well as Jurkat T lymphocytes and the eosinophil line EOL1, this can be mimicked by intracellular βNAD+, but not by βNADH (12, 13). This implies that βNAD+, formed by the action of free radicals on intracellular...
βNADH mediates the effect. The molecular identity of the underlying channel has been approached in two ways. First, heterologous expression of TRPM2 cDNA results in the appearance of channels permeable to cations that can be activated by intracellular NADH (1 mM) (12, 13). Second, treatment of U937 monocytes with antisense oligonucleotides that reduced the expression of TRPM2 (measured by Western blotting and immunocytochemistry) also reduced the calcium entry and cell death elicited by H2O2 (12). These experiments strongly suggest that one or more TRPM2 subunits are a key component of the channel expressed by these immune cell lines that is activated by oxidative damage (12, 13).

In contrast, it has recently been found that H2O2 can activate heterologously expressed TRPM2 channels by a mechanism that appears not to involve changes in ADP-ribose or NAD levels (23). The homomeric channels formed by expression of TRPM2 subunits are directly activated also by ADP-ribose (13, 15), although not by several closely related analogs (cyclic ADP-ribose, βNADH, βNADP+, βNADPH, ATP, and ADP) (12, 13, 15). The activation results from binding of the ADP-ribose to a Nudix domain in the C terminus of the protein, because it is prevented by point mutations of key residues in this motif (12, 15). The concentration of ADP-ribose causing 50% activation of the whole cell current is ~30–100 μM (15). When activated by intracellular diffusion of ADP-ribose during the course of whole cell recordings, these TRPM2 currents show close to linear I/V relations and other properties that resemble the current first described by Wilding et al. (24) in ascidian oocytes.

The current that we now report in rat peritoneal macrophages, which is activated by ADP-ribose, has some, but not all, of the same features. These include the effectiveness of ADP-ribose and βNAD+ (although not a range of other structurally related compounds), the effective concentrations of ADP-ribose (EC50 ~30 μM; Fig. 4D) and βNAD+ (1 mM), and the block by ATP (5 mM) (13). We considered that an excess of ADP might inhibit the binding of ADP-ribose to its Nudix domain (25), and consistent with this we observed that intracellular ADP also blocked the currents activated by both ADP-ribose and depolarization. However, there also appear to be important differences between the properties of heterologously expressed TRPM2 channels and the macrophage currents. The most notable is the significant permeability to NMDG, chloride, and gluconate that we observed, whereas substitution of external cations by NMDG almost completely blocks the current through homomeric TRPM2 channels (13, 15). Furthermore, we observed a relatively high sensitivity to lanthanum (IC50 ~
1–10 μM) on the depolarization-evoked and ADP-ribose-evoked current in peritoneal macrophages, but the sensitivity of TRPM2 currents to lanthanum has not yet been studied systematically.

In contrast, other currents have been described with properties that more closely resemble the macropage currents observed in the present study. For example, heterologous expression of PKD1 and PKD2, genes named for the polycystic kidney disease that arises when they are mutated in humans (26), gives rise to a similar conductance (27). Those channels have $P_{\text{Na}_c}/P_{\text{Na}_c}$ of 0.51. The $P_{\text{Cl}_c}/P_{\text{Na}_c}$ cannot be calculated directly from the data of Hanaoka et al. (27) (they had three permeant ions present: cesium, sodium, and chloride); however, the 45 mV shift in reversal potential would indicate $P_{\text{Cl}_c}/P_{\text{Na}_c}$ of $\sim$0.3. The lanthannum sensitivity of the PKD1/2 current (50% inhibition by $\sim$50 μM) is also not very different from that which we observed in peritoneal macrophages. It is not clear how polycystin channels could be activated by βNAD$^+$ and ADP-ribose, because their amino acid sequence does not contain a Nudix domain. Further work, such as recordings of single channel activity and antisense oligonucleotide approaches, will be needed to establish the molecular composition of the macrophage channel.

The occlusion experiment illustrated in Fig. 5 strongly suggests that the two currents that we describe pass through the same channels (although it is also formally possible that intracellular ADP-ribose could in some way inhibit the effect of depolarization to activate a separate population of channels). This is consistent with the overall similarity in the properties of the currents with respect to permeability (Table 1) and lanthanum blockade (Fig. 3). This raises the question deferred earlier: what might be the mechanism by which sustained depolarization activates this cell conductance? Speculations might include that depolarization evokes the release by which sustained depolarization activates this cell conductance? This is consistent with that the two currents that we describe pass through the same channel. Further work, such as recordings of single channel activity and antisense oligonucleotide approaches, will be needed to establish the molecular composition of the macrophage channel.

In conclusion, an imposed depolarization of macrophages activates an inward current that is significantly permeable to large cations as well as anions, and that is blocked by low μM concentrations of lanthanum. Under more physiological circumstances (i.e., without voltage-clamp), this current could amplify the depolarization by opening further channels. In this way, the effect of an initial stimulus that leads to the release of free radicals within the cell (such as phagocytosis) would be further enhanced. Such a positive feedback process, in which activation of the current leads to depolarization of the macrophage and depolarization leads to further activation, might contribute to the eventual cell death that readily follows exposure to exogenous oxidants.

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References