Activation of the Cation Channel Long Transient Receptor Potential Channel 2 (LTRPC2) by Hydrogen Peroxide

A SPlice variant reveals a mode of activation independent of ADP-ribose*

Received for publication, December 18, 2001, and in revised form, March 18, 2002

Edith Wehage‡, Jörg Eisfeld‡, Inka Heiner, Eberhard Jüngling, Christof Zittš, and Andreas Lückhoff¶

From the Institute of Physiology, Medical Faculty, Rheinisch-Westfälische Technische Hochschule Aachen, Pauwelstraße 30, D-52057 Aachen, Germany

LTRPC2 is a cation channel recently reported to be activated by adenosine diphosphate-ribose (ADP-ribose) and NAD. Since ADP-ribose can be formed from NAD and NAD is elevated during oxidative stress, we studied whole cell currents and increases in the intracellular free calcium concentration ([Ca$^{2+}$]i) in long transient receptor potential channel 2 (LTRPC2)-transfected HEK 293 cells after stimulation with hydrogen peroxide (H$_2$O$_2$). Cation currents carried by monovalent cations and Ca$^{2+}$ were induced by H$_2$O$_2$ (5 mM in the bath solution) as well as by intracellular ADP-ribose (0.3 mM in the pipette solution) but not by NAD (1 mM). H$_2$O$_2$-induced currents developed slowly after a characteristic delay of 3–6 min and receded after wash-out of H$_2$O$_2$. [Ca$^{2+}$]i was rapidly increased by H$_2$O$_2$ in LTRPC2-transfected cells as well as in control cells; however, in LTRPC2-transfected cells, H$_2$O$_2$ evoked a second delayed rise in [Ca$^{2+}$]i. A splice variant of LTRPC2 with a deletion in the C terminus (amino acids 1292–1325) was identified in neutrophil granulocytes. This variant was stimulated by H$_2$O$_2$ as the wild type. However, it did not respond to ADP-ribose. We conclude that activation of LTRPC2 by H$_2$O$_2$ is independent of ADP-ribose and that LTRPC2 may mediate the influx of Na$^{+}$ and Ca$^{2+}$ during oxidative stress, such as the respiratory burst in granulocytes.

The long transient receptor potential channel 2 (LTRPC2) is a member of the transient receptor potential (TRP) family of cation channels (1). Its function may not be confined to that of a Ca$^{2+}$-permeable ion channel widely expressed in several cell types but may extend to the role of an enzyme, as has been shown for its relative TRP-phospholipase C interacting kinase (2). LTRPC2 contains a Nudix box in its C terminus (3) which is a common motif of enzymes degrading mostly nucleoside diphosphates (4). The protein NUPT9 that is homologous to the C terminus of LTRPC2 is a specific ADP-ribose pyrophosphatase degrading ADP-ribose (3). A similar function may be attributed to LTRPC2. Alternatively, the Nudix box may serve as a regulatory ADP-ribose-binding site because ADP-ribose has been shown to stimulate the channel activity of LTRPC2 (3). Therefore, ADP-ribose can be thought of as a novel second messenger regulating Ca$^{2+}$ influx. However, the stimuli and signaling pathways leading to elevated levels of ADP-ribose have not been elucidated in detail. ADP-ribose can be generated from cyclic ADP-ribose (5–7), an established messenger mobilizing Ca$^{2+}$ from ryanodine-sensitive calcium stores (8–12). Moreover, ADP-ribose can be produced from NAD (13, 14). This links ADP-ribose to the redox state of the cell and may lead to the assumption that ADP-ribose and ADP-ribose-induced Ca$^{2+}$ influx play a role during oxidative stress because a characteristic feature of oxidative stress is an increased ratio of NAD to NADH (15). In this context, it is of interest that NAD has been reported to be a further stimulus of LTRPC2 channels (16, 17).

To study the role of LTRPC2 in oxidative stress, we used an experimental model in which a strong oxidant, H$_2$O$_2$, was applied to LTRPC2-transfected cells. Indeed, H$_2$O$_2$ evoked cation influx and increased [Ca$^{2+}$]i. Furthermore, we studied the effects of H$_2$O$_2$ on splice variants of LTRPC2 identified in HL-60 cells and neutrophil granulocytes. One splice variant was activated by H$_2$O$_2$ as the wild type but did not respond to ADP-ribose, in contrast to the wild type. Thus, oxidative stress leads to the activation of LTRPC2. Channel activation, however, does not need to be directly mediated by ADP-ribose.

EXPERIMENTAL PROCEDURES

Molecular Cloning—For cloning of LTRPC2 (formerly named TRPC7 (18)) with reverse transcriptase-polymerase chain reaction, total RNA was isolated from 1 to 2 × 10$^7$ undifferentiated HL-60 cells using TRIzol (Invitrogen, Groningen, the Netherlands). mRNA was extracted with 15 μl of Oligotex (Qiagen, Hilden, Germany). First strand cDNA synthesis was performed with 500 ng of HL-60 mRNA with Moloney murine leukemia virus reverse transcriptase (Superscript II, Invitrogen) using 500 ng of oligo(dT) primer or 2 pmol of gene-specific primer.

Two adjacent cDNA segments of LTRPC2 that combined to the total open reading frame of LTRPC2 were separately amplified by two PCR reactions. In the first one, primers were chosen to amplify the region of the open reading frame of LTRPC2, 2.5 kb. The abbreviations used are: LTRPC2, long transient receptor potential channel 2; TRP, transient receptor potential; [Ca$^{2+}$]i, intracellular [Ca$^{2+}$]; EGFP, enhanced green fluorescent protein; NMDG, N-methyl-D-glucamine.
μM of the respective primer, 200 μM of each dNTP, 2 mM MgCl₂, 0.5–1 μM of cDNA and 1.25 units of Pfu DNA polymerase (Promega, Mannheim, Germany).

Both cDNA segments of LTRPC2 were prepared for TA-cloning by adding adenine tails with 1 μl of Taq polymerase (Amplitaq Gold, PerkinElmer Life Sciences). All the PCR products were solved in a total volume of 50 μl containing 2 mM MgCl₂, and 10 mM dATP and incubated at 72 °C for 30 min with the polymerase that had been preincubated in buffer at 95 °C for 10 min. Subsequently, the TA cloning (Invitrogen) was carried out.

The 5’-segment was subcloned into pBluescript SK(–) by use of the SpeI and XhoI sites of the multiple cloning site of the vector. The 3’-segment was dissected with the endonucleases SpeI and SphI. Subsequently, ligation of the two segments was performed with T4 ligase (Invitrogen) at the SpeI sites of the segments and the SphI site of the multiple cloning site of pBluescript SK(–).

The resulting 5.2-kb insert was directionally cloned into the eukaryotic expression plasmid construct pcDNA3-EGFP (modified as reported in Ref. 19) by use of the KpnI and XhoI sites of the multiple cloning sites of the pcDNA3-EGFP construct. Thus, the LTRPC2 variant is under control of the cytomegalovirus promoter, and the enhanced green fluorescent protein (EGFP) is under control of the simian virus 40 promoter.

DNA sequencing was performed with a Big-Dye terminator kit (PerkinElmer Life Sciences). A sequence alignment of our clone with LTRPC2I (Genbank accession number AB091535 (18)) indicated that we had obtained a variant with a deletion in the N terminus as well as in the C terminus (see Fig. 1). Additionally, several nucleotides are changed, resulting in the exchange of two amino acids (S1088N and D1292E). The details were submitted to GenBank™ under accession number A411076. Thus, the initial cloning yielded the LTRPC2-ΔNΔC form of LTRPC2 (see below, Fig. 1 and “Results”).

Activation of LTRPC2 by H₂O₂ Independent of ADP-ribose

Isolation of mRNA from Human Neutrophil Granulocytes and Demonstration of LTRPC2 Variants with Reverse Transcriptase-PCR—Vigorous human blood (70 ml) was taken from healthy volunteers of either sex (aged 25–35). Neutrophil granulocytes were isolated by dextran sedimentation, centrifugation through a Ficoll cushion, and separation with magnetically labeled antibodies. The isolation of total RNA (from a total of 102 nucleotides) of the missing part. Pf contains the NofI restriction site, the following sequence up to the missing part, and 60 nucleotides (from a total of 102 nucleotides) of the missing part. Pf contains the stop codon and an Spel restriction site not present in the original LTRPC2 gene. The amplification product with 650 bp encodes the full 3’-segment, from the NoI restriction site to the stop codon, and an additional Spel restriction site. This product was used to create the cDNA of the LTRPC2 variant with the complete 3’-segment coding for LTRPC2-ΔNΔC. This product was used to create the full-length LTRPC2 cDNA by cloning LTRPC2-ΔN and LTRPC2-ΔC (both in pBluescript SK(–)) at the mentioned XbaI and SpelI restriction sites and subsequent ligation of the fragments coding for the long version of the N and C terminus, respectively. Insertion into pcDNA3-EGFP was performed as described above.

All PCR products were sequenced prior to further use, and all constructs were sequenced at the transition sites. All procedures were performed in accordance with the respective manufacturer’s instructions, if not indicated otherwise.

Cell Culture and Transfection—Human embryonic kidney 293 (HEK 293) cells were obtained from ATCC (Manassas, VA). HEK 293 cells were cultured in Dulbecco’s modified Eagle medium (Biochrom, Berlin, Germany) supplemented with 10% (v/v) fetal calf serum (Biochrom), 1 mM sodium pyruvate (Sigma), and 4 mM L-glutamine (Biochrom). Cells were plated onto polystyrene (0.1 μg/μl, Sigma)-coated glass coverslips for 24 h and then transiently transfected with one of the described pcDNA3-EGFP vector channel constructs (5 μg) and Transfectant transfection reagent (7.5 μg; Promega, Mannheim, Germany). As controls, cells were transfected with 2.5 μg of “empty” vector construct and pcDNA3-EGFP as transfection reagent. Electrophysiological and fluorimetric studies were carried out 24–48 h after transfection in cells visibly positive for EGFP.

Electrophysiology—Transfected cells were studied with the patch clamp technique in the whole cell mode, using an EPC 9 equipped with a personal computer with Pulse and X chart software (HEKA, Lambrecht, Germany). The standard extracellular bath solution contained (in mM): 140 NaCl, 1.2 MgCl₂, 6 KCl, 1.8 CaCl₂, 1 NaH₂PO₄, 10 HEPES, pH adjusted with KOH to 7.4. For Na⁺/Ca⁺-free solutions, Na⁺ was replaced by NMDG (150 mM) and the titration was performed with HCl. For the NMDG solutions with Ca⁺², NMDG was partly replaced with CaCl₂ (11.2 mM), preserving an osmolarity of about 300 mosm/l. The pipette solution contained (in mM): 145 Cs-glutamate, 8 NaCl, 2 MgCl₂, 10 CsF, 48 KCl, 10 HEPES, pH 7.2 (CsOH). In some experiments, the pipette solution additionally contained ADP-ribos (0.3 mM) or NAD (final concentration of 1 mM, added to the pipette solution from a stock of 70 mM in ethanol). Cells were held at a potential of ~60 mV, and current voltage (I-V) relations were obtained from voltage ramps from ~90 to +60 mV applied over 400 ms.

Measurements of the Intrasoluble Ca⁺² Transients—Transfected HEK 293 cells grown on poly-L-lysine-coated glass coverslips were loaded with fura-2 by incubation in fura-2 acetoxyethyl ester (2 μM, Calbiochem) for 30 min at 37 °C in a buffer consisting of (in mM): 138 NaCl, 6 KCl, 1 MgSO₄, 1 NaHPO₄, 5 NaHCO₃, 5.5 glucose, 20 HEPES, pH 7.4 (NaOH), supplemented with 0.1% (v/v) bovine serum albumin. Loaded cells were washed twice and then kept in a buffer consisting of (in mM): NaCl, 138 KCl, 1 MgCl₂, 5.5 glucose, 1.6 NaHCO₃, and 20 HEPES, pH 7.4 (NaOH). Cells were illuminated by a 510-nm light-emitting diode (tu.Ll. Photonics, München, Germany). Fluorescence images were recorded with a ×40 oil immersion objective (Zeiss). Emitted fluorescence was recorded after filtering through a 510-nm band pass filter over manually defined regions of interest. Excitation was performed at intervals of 2.5 s with wavelengths of 340 and 380 nm for 4 ms each, yielding the emitted fluorescence signals F₃₄₀ and F₃₈₀ under various conditions.
Bath solutions containing H$_2$O$_2$ (5–30 mM; Merck, Darmstadt, Germany) were freshly prepared daily and stored in the dark until application. Fura-2/AM was kept in a stock solution of dimethyl sulfoxide at a concentration of 1 mM stored at -100°C until use. Unless otherwise indicated, all chemicals were purchased from Sigma. The experiments were carried out at room temperature (21–23°C).

Data are expressed as mean ± S.D. Statistical significance between groups was assessed with the Student’s t test. A p < 0.05 was considered significant.

RESULTS

Molecular Cloning of LTRPC2 and Its Variants—We cloned LTRPC2 (formerly LTRPC7) from undifferentiated HL-60 cells. Initially, we obtained a clone that differs from the original sequence (18) by two deletions, from amino acids 538 to 557 and from 1292 to 1325 (Fig. 1). Additionally, two amino acids are exchanged (S1088N and D1291E). According to the structure model of TRP channels (20), the one deletion is located in the cytosolic N terminus and the other one in the cytosolic C terminus of LTRPC2. We refer to the clone with the two deletions as LTRPC2-N.C (see “Experimental Procedures”). To test whether HL-60 cells and neutrophil granulocytes express not only LTRPC2-N.C but also LTRPC2 variants with the originally reported longer forms of the N terminus and the C terminus, we performed reverse transcriptase-PCR experiments with the three primer pairs indicated in Fig. 1.

Fig. 2 shows that mRNA coding for both forms of the N terminus and for both forms of the C terminus is expressed in HL-60 cells (Fig. 2A) as well as in neutrophil granulocytes (Fig. 2B). Specifically, lanes 1 and 4 of Fig. 2 show two different PCR products from primers P1 and P2 that demonstrate mRNA encoding the short and the long form of the C terminus. Lanes 2 and 5 show a PCR product that can only be formed if mRNA coding for the long form of the N terminus is present. Lanes 3 and 6 indicate the additional presence of the short form because of the size of the PCR product; note that the PCR product indicative of the long form of the N terminus is not visible in lanes 3 and 6 but was demonstrated in a nested PCR (lane 7).
Sequence analysis of all the fragments shown in Fig. 2 confirmed that they represent the expected PCR products. Therefore, mRNA encoding different variants of LTRPC2 exists in HL-60 cells and neutrophil granulocytes. The HL-60 cells from which the mRNA was normally extracted were not differentiated to the granulocytic phenotype. However, mRNA coding for LTRPC2 was also demonstrated (data not shown) in HL-60 cells differentiated with dibutyryl-cAMP, a cell culture model for neutrophil granulocytes (21), but no analysis of the expressed variants was performed.

For functional studies of the LTRPC2 variants, we created four cDNA constructs coding for: (i) the full-length form of LTRPC2 with both the N terminus and the C terminus in its originally described long form; (ii) LTRPC2-ΔN (i.e. with the short form of the N terminus; for details, see Fig. 1 and “Experimental Procedures”); (iii) LTRPC2-ΔC; and (iv) LTRPC2-ΔNAC. Each construct was transiently expressed in HEK 293 cells and electrophysiologically studied with the patch clamp technique in the whole cell mode.

**Activation of LTRPC2 Currents by ADP-ribose**—None of the expressed variants of LTRPC2 produced cation inward currents different from controls (vector-transfected cells identified by EGFP fluorescence) when the standard pipette solution was used (Fig. 3A). However, when ADP-ribose (0.3 mM) was present in the pipette solution in experiments with cells expressing the full-length form of LTRPC2 (Fig. 3B), we consistently observed an inward current that gradually developed within a few minutes after obtaining the whole cell configurations. The time elapsed before the full response to ADP-ribose occurred varied between 0.5 and 8 min. This variability is probably caused by variable diffusion times of ADP-ribose from the pipette into the cytosol and by metabolism of ADP-ribose to products that do not activate LTRPC2 (3); metabolism is expected to delay activation of LTRPC2 particularly when diffusion is already slow.

The ADP-ribose-induced inward current was abolished when extracellular Na⁺ was substituted with the large impermeable cation NMDG (Fig. 3B). When Ca²⁺ (10 mM) was added to NMDG, a small inward current was restituted (Fig. 3, C and D). These data indicate that LTRPC2 enables non-selective cation currents carried mainly by Na⁺, but to a small part also by Ca²⁺, in the inward direction and carried by Cs⁺ in the outward direction under our experimental conditions. Our results with LTRPC2 are essentially identical with those recently reported (3), where also a full-length form of LTRPC2 was used. However, when we extended the analysis to the other three forms of LTRPC2, we did not find ADP-ribose-induced currents (Fig. 4). Slightly enhanced current density levels after extended dialysis with ADP-ribose in some experiments (Fig. 4) cannot be attributed to an effect of ADP-ribose, because small
current increases developed over time also in control cells in the absence of intracellular ADP-ribose (see Fig. 3A). Furthermore, co-transfection of cells with two vectors coding for LTRPC2-ΔC and LTRPC2-ΔN, respectively, failed to result in ADP-ribose-induced currents (n = 4, data not shown). Thus, stimulation with ADP-ribose was exclusively found for the full-length form of LTRPC2.

**Activation of LTRPC2 Currents by Hydrogen Peroxide**—As a model of oxidative stress, we tested the effects of extracellular H$_2$O$_2$ on the four LTRPC2 variants. Fig. 5A shows a representative experiment with a cell expressing LTRPC2 in which H$_2$O$_2$ was added to the bath to a final concentration of 5 mM. After a delay of 4 min, an inward current developed gradually that was blocked by NMDG and was partly carried by Ca$^{2+}$. After wash-out of H$_2$O$_2$, the current initially increased further but then declined almost to baseline levels, although a small NMDG-sensitive current was still present. Re-addition of H$_2$O$_2$ led to a second current rise. When the cells were exposed to H$_2$O$_2$ over more extended times than in Fig. 5, the inward currents reached a plateau after about 15 min.

**Stimulation of currents with H$_2$O$_2$ after a characteristic delay was consistently observed in 10 out of 10 cells expressing LTRPC2. The mean increase in current density was from **

---

**Fig. 5. Stimulation of LTRPC2 and LTRPC2-ΔC with H$_2$O$_2$**—A, original current recording from one cell expressing LTRPC2. The bars on top indicate the composition of the respective bath solutions that contained Na$^+$, NMDG, or NMDG plus Ca$^{2+}$ as the main cation and did or did not contain H$_2$O$_2$ (5 mM). The inset shows a magnification of the current tracing in NMDG solution and in NMDG solution containing additionally 11.2 mM Ca$^{2+}$. B, original current recording from one cell expressing LTRPC2-ΔN. C, original current recording from one cell expressing LTRPC2-ΔC.

---

**Fig. 6. Differential stimulation of LTRPC2 splice variants with H$_2$O$_2$**—See the legend of Fig. 4 for details. The concentration of H$_2$O$_2$ was 5 or 30 mM in the bath.

---

-0.9 ± 0.75 to -20.3 ± 18.8 pA/pF (Fig. 6). H$_2$O$_2$-induced increases in currents were completely abolished when the pipette solution contained the radical scavenger mannitol (10 mM). This result was obtained in 5 cells when H$_2$O$_2$ was applied for 10 min.

Although we normally used a concentration of 5 mM H$_2$O$_2$, currents were induced by H$_2$O$_2$ concentrations of 1 mM (n = 2) as well. Raising the H$_2$O$_2$ concentration to 30 mM resulted in current increases that were nearly irreversible over the time of the experiments, although the currents were still blocked by NMDG.

In control cells (Fig. 6) and in cells expressing either LTRPC2-ΔN (Figs. 5B and 6) or LTRPC2-ΔNAC (Fig. 6), no current increase was induced by H$_2$O$_2$ (5 or 30 mM). However, in cells expressing the variant LTRPC2-ΔC that was insensitive for ADP-ribose, H$_2$O$_2$ was similarly effective (Fig. 5C) as in cells expressing the full-length form of LTRPC2. The currents through either form of LTRPC2 could not be discriminated by current density, time course after addition or removal of H$_2$O$_2$, or by selectivity for Ca$^{2+}$ over Na$^+$ (Figs. 5C and 6).

**Ca$^{2+}$ Transients Induced by H$_2$O$_2$**—Since the extent by which the currents through LTRPC2 were carried by Ca$^{2+}$ was low, we studied with a different approach whether stimulation of LTRPC2 with H$_2$O$_2$ evokes increases in [Ca$^{2+}$]. Ca$^{2+}$ transients in LTRPC2-transfected cells were followed in a digital imaging system after loading of the cells with the fluorescent Ca$^{2+}$ indicator fura-2. After exposure to a bath containing 5 mM H$_2$O$_2$, all cells (LTRPC2-transfected as well as controls) responded with an initial rise in [Ca$^{2+}$]$_i$ (Fig. 7). [Ca$^{2+}$]$_i$ returned quickly to resting levels. In cells transfected with LTRPC2 but not in control cells, a second increase in [Ca$^{2+}$]$_i$ was observed after several minutes (Fig. 7B). Such a second rise in [Ca$^{2+}$]$_i$ after exposure to H$_2$O$_2$ was found in 11 out of 25 LTRPC2-transfected cells in four independent experiments. This increase reached a plateau that remained fairly stable until the end of the experiment when further increases were elicited by ionomycin (10 μM in a buffer with 10 mM Ca$^{2+}$). However, a calibration of the fluorescence signal in terms of Ca$^{2+}$ concentration was not done because many cells detached during the long time required for experiments with H$_2$O$_2$. 

---

**Fig. 6. Differential stimulation of LTRPC2 splice variants with H$_2$O$_2$.** See the legend of Fig. 4 for details. The concentration of H$_2$O$_2$ was 5 or 30 mM in the bath.
No H$_2$O$_2$-induced second rise in [Ca$^{2+}$]$_i$ was seen in any control cells ($n = 23$ in four experiments). Likewise, no such rise was seen in LTRPC2-transfected cells in the absence of H$_2$O$_2$ ($n = 19$ in four experiments).

Similar results as with the full-length LTRPC2 were obtained with LTRPC2-ΔC (Fig. 7C). Here, the second rise in [Ca$^{2+}$]$_i$ occurred in 17 out of 41 LTRPC2-ΔC-transfected cells in five experiments and not in any control cell ($n = 9$).

Lack of LTRPC2 Activation by NAD—As LTRPC2 has been reported to be stimulated by intracellular NAD (16), we also tested this compound which was applied to the cells by dialysis through the patch pipette. However, LTRPC2 did not respond to NAD with an increase in currents (Fig. 8A; $n = 7$). These negative findings were confirmed with the other three forms of LTRPC2 and in controls ($n = 4–5$). To demonstrate that the lack of NAD effects was not a problem of LTRPC2 expression, we applied H$_2$O$_2$ (5 mM) to cells that had been dialyzed with NAD for 5 min without showing any indication of a developing current. After a further delay of 2 min, a gradually increasing current was observed (Fig. 8B). Essentially the same finding as in cells expressing LTRPC2 was obtained in cells expressing LTRPC2-ΔC ($n = 2$). Thus, stimulation with H$_2$O$_2$ was independent of the presence of NAD in the pipette solution, whereas NAD alone did not stimulate currents through LTRPC2.

Differential Sensitivity of LTRPC2-ΔC for H$_2$O$_2$ and ADP-ribose—Furthermore, we tested whether stimulation by H$_2$O$_2$ was independent of ADP-ribose (Fig. 8C). Cells expressing LTRPC2-ΔC were patched with ADP-ribose (0.3 mM) present in the pipette solution. A time of 5 min was allowed during which ADP-ribose failed to induce currents, as in the previous experiments (Fig. 4). Then, H$_2$O$_2$ was applied extracellularly. Again, after a delay of 2 min, the typical H$_2$O$_2$-induced currents were observed ($n = 3$) that were slowly increasing and blocked by the impermeable cation NMDG (Fig. 8C).

**DISCUSSION**

The present study found that the non-selective cation channel LTRPC2 was activated by H$_2$O$_2$. This is an alternative mode of activation for this channel that has previously been demonstrated to be activated by ADP-ribose (3). A splice variant of LTRPC2, LTRPC2-ΔC, identified in HL-60 cells and in neutrophil granulocytes, was differentially sensitive for the two stimuli because it was exclusively activated by H$_2$O$_2$. Thus,
LTRPC2-ΔC reveals some information on the structural requirements for ADP-ribose sensitivity and provides evidence that both stimuli act by distinct mechanisms.

NAD has recently been reported to stimulate LTRPC2 (16, 17). However, we did not see activation of any form of LTRPC2 by NAD, which does not support the idea that this substance activates LTRPC2 directly. Since ADP-ribose is a metabolite of NAD (13, 14), it is conceivable that NAD is converted to ADP-ribose under some experimental conditions to a sufficient extent to stimulate LTRPC2; however, this may depend on very subtle details and obviously did not occur in our experiments.

When we started the functional characterization of LTRPC2, we reasoned that both NAD and ADP-ribose may be mediators of oxidative stress because the intracellular concentrations of NAD and, consequently, of ADP-ribose may be increased when the redox potential of the cell is altered such that the equilibrium of NAD to NADH is shifted in favor of NAD. The same redox state of the cells and mimicked the diabetes-inducing effects of alloxan. Indeed, H$_2$O$_2$ has recently been reported to stimulate LTRPC2 (16, 17). However, we did not see activation of any form of LTRPC2 by NAD, which does not support the idea that this substance represents a different mode of channel activation than ADP-ribose. Thus, LTRPC2 represents a cation channel that links the redox state of the cell to Ca$^{2+}$ homeostasis, as has also been reported by Hara et al. (17) after submission of our manuscript.

Acknowledgment—We thank Ilinca Ionescu for expert technical assistance.

REFERENCES

1. Harteneck, C., Plant, T. D., and Schultz, G. (2000) Trends Neurosci. 23, 159–166
2. Runnels, L. W., Yue, L., and Clapham, D. E. (2001) Science 291, 1043–1047
3. Perraud, A. L., Fleig, A., Dunn, C. A., Bagley, L. A., Launay, P., Schmitz, C., Stokes, A. J., Zhu, Q., Bessman, M. J., Penner, R., Kniet, J. P., and Scharenberg, A. M. (2001) Nature 411, 595–599
4. Bessman, M. J., Frick, D. N., and O’Handley, S. F. (1996) J. Biol. Chem. 271, 25059–25062
5. Howard, M., Grimaldi, J. C., Bazan, J. F., Lund, F. E., Santos-Aragueda, L., Parkhouse, R. M., Walseth, T. F., and Lee, H. C. (1995) Science 262, 1056–1059
6. Lee, H. C., and Aarhu, R. (1993) Biochim. Biophys. Acta 1164, 68–74
7. Takasawa, S., Tohgo, A., Naguchi, N., Koguma, T., Nata, K., Sugimoto, T., Yonekura, H., and Okamoto, H. (1995) J. Biol. Chem. 263, 26652–26654
8. Cancela, J. M., Gerasimenko, O. V., Gerasimenko, J. V., Tepikin, A. V., and Petersen, O. H. (2000) EMBO J. 19, 2549–2557
9. Clementi, E., Riccio, M., Sciacca, J., Nistico, G., and Moldolesi, J. (1996) J. Biol. Chem. 271, 17739–17745
10. Galione, A., Lee, H. C., and Busa, W. B. (1991) Science 253, 1143–1146
11. Meszaros, L. G., Bak, J., and Chan, A. (1993) Nature 364, 76–79
12. Sonnleitner, A., Conti, A., Bertocchini, F., Schindler, H., and Sorrentino, V. (1998) EMBO J. 17, 2790–2798
13. Verheule, V., Tijs, J. M., Muller-Steffner, H., Schuber, F., and Deterre, P. (1998) Biochem. J. 330, 1383–1390
14. Ziegler, M. (2000) Eur. J. Biochem. 267, 1550–1564
15. Wilson, H. L., Dipp, M., Thomas, J. M., Lad, C., Galione, A., and Evans, A. M. (2001) J. Biol. Chem. 276, 11109–11112
16. Sano, Y., Inamura, K., Miyake, A., Mochizuki, S., Yokoi, H., Matsushime, H., and Furutti, R. (2001) Science 293, 1327–1330
17. Hara, Y., Wakamori, M., Ishii, M., Maeno, E., Nishida, T., Yoshida, T., Yamada, H., Shimizu, S., Mori, E., Kudoh, J., Shimizu, N., Kurase, H., Okada, Y., Imoto, K., and Mori, Y. (2002) Mol. Cell 9, 163–173
18. Nagamine, K., Kudoh, J., Minoshima, S., Kawakami, K., Asakawa, S., Ito, F., and Shimizu, N. (1998) Genomics 54, 124–131
19. Halaszovich, C. R., Zitt, C., Jungling, E., and Luckhoff, A. (2000) J. Biol. Chem. 275, 37423–37428
20. Hardie, R. C., and Minke, B. (1992) Neuron 8, 643–651
21. Collins, S. J. (1987) Blood 70, 1233–1244
22. Suzuki, J., Imaizumi, S., Kayama, T., and Yoshimoto, T. (1985) Blood 65, 705–709
23. Xu, X. Z., Moebius, F., Gill, D. L., and Montell, C. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 10692–10697
24. Knebel, A., Rahmsdorf, H. J., Ullrich, A., and Herrlich, P. (1996) EMBO J. 15, 5314–5325
25. Meyer, M., Schreck, R., and Baeuerle, P. A. (1993) EMBO J. 12, 2005–2015
26. Schreck, R., Rieber, P., and Baeuerle, P. A. (1991) EMBO J. 10, 2247–2258
27. Thannickal, V. J., and Fanburg, B. L. (2000) Am. J. Physiol. 278, L1055–L1028
28. Conrad, P. W., Millhorn, D. E., and Beitner-Johnson, D. (2000) Am. J. Physiol. 278, L1036–L1048
29. Cohen, M. S. (1994) Clin. Infect. Dis. 18, Suppl. 2, S170–S179
30. Krause, K. H., Dameraux, N., Jacobi, M., and Lew, D. P. (1993) Blood Cells 19, 163–173
31. Schorr, W., Swandulla, D., and Zeilhofer, H. U. (1999) Eur. J. Immunol. 29, 897–904
32. Walz, A., Meloni, F., Clark-Lewis, I., von Tschcharner, V., and Baggioi, M. (1991) J. Leukocyte Biol. 50, 279–286