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COMMENTARY

Inhibition of TRPM2 function by PARP inhibitors protects cells from oxidative stress-induced death

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TRPM2 is a member of the transient receptor potential (TRP) protein superfamily of calcium-permeable, voltage-independent ion channels expressed in nonexcitable cells. Activation of TRPM2 by oxidative stress results in calcium influx and susceptibility to cell death, whereas inhibition of TRPM2 function enhances cell survival. In the present edition of this journal, Fonfria *et al*. demonstrate a role for poly(ADP ribose) polymerase (PARP) as a mediator between oxidative stress and TRPM2 activation. They present evidence that inhibition of either PARP or TRPM2 protects cells from plasma membrane damage and cell death. The therapeutic implications of this important observation are discussed.
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Abbreviations: ADPR, adenine 5′-diphosphoribose; H_2O_2, hydrogen peroxide; NAD, nicotinamide adenine dinucleotide; PARP, poly(ADP ribose) polymerase; TNFα, tumor necrosis factor α; TRPM, transient receptor potential protein, melastatin subfamily

Calcium is an important and universal intracellular second messenger, which modulates many cell functions including susceptibility to cell death (Berridge *et al*., 2000). Although the majority of ion channel studies have been conducted in excitable cells, a transient receptor potential (TRP) protein superfamily has been described recently, which is a diverse group of calcium-permeable channels involved in cation influx in nonexcitable cells (Harteneck *et al*., 2000; Montell, 2001; Montell *et al*., 2002; Clapham, 2003; Alexander *et al*., 2004). Structurally related to the archetypal *Drosophila* TRP, this superfamily has been divided into seven subfamilies designated C, V, M, A, N, P, and ML based on amino-acid sequence. Vertebrate members for all subfamilies have been identified except for TRPN. All members have six transmembrane domains, a putative pore loop between the fifth and sixth transmembrane domains, and are proposed to function as tetramers. Members are involved in many important physiological processes including vasoactivation, sensation, fertility, cell proliferation, and neurodegeneration. The TRPM (transient receptor potential protein, melastatin subfamily) of TRP channels was named after its first described member, melastatin, a putative tumor suppressor protein (Duncan *et al*., 1998). TRPM2 and TRPM8 are other members of the TRPM family, which have been shown to have roles in cell proliferation (Clapham, 2003).

TRPM2 was the second member of the TRPM family to be described (Nagamine *et al*., 1998; Sano *et al*., 2001; Hara *et al*., 2002). Although brain and the hematopoietic system are the best studied, TRPM2 is expressed in many cell types, suggesting that observations made regarding its function may be applicable to a wide range of tissues. Intracellular adenine 5′-diphosphoribose (ADPR) activates TRPM2, inducing Ca^{2+} influx, by binding to the TRPM2 C-terminal NUDT9-H domain, which has significant homology with NUDT9 ADPR hydrolase (Wehage *et al*., 2002; Heiner *et al*., 2003; Perraud *et al*., 2003). Nicotinamide adenine dinucleotide (NAD) has also been reported by some investigators to directly activate TRPM2, although other evidence suggests that NAD activates TRPM2 through conversion to ADPR. Intracellular Ca^{2+} sensitizes cells to gating by ADPR and provides positive feedback for channel activation (McHugh *et al*., 2003). Calcium influx through TRPM2 is induced by oxidant stress, hydrogen peroxide (H_2O_2), or tumor necrosis factor α (TNFα). Three physiological splice variants of TRPM2 have been identified, which have different activation properties: TRPM2-S (Zhang *et al*., 2003), TRPM2-ΔN (Wehage *et al*., 2002), and TRPM2-ΔC (Wehage *et al*., 2002). TRPM2-S is a short variant, which consists only of the N-terminus and directly interacts with full-length TRPM2 to inhibit calcium influx. TRPM2-ΔN has a deletion of 20 amino acids in the N-terminus, and does not respond to either H_2O_2 or ADPR, suggesting that the TRPM2-ΔN mutation dominantly disrupts channel gating or interferes with surface trafficking or channel assembling (Wehage *et al*., 2002). In contrast, TRPM2-ΔC has a deletion of 34 amino acids in the CAP domain in NUDT9 in the C-terminus; the CAP domain enhances affinity of the CORE domain for ADPR. This mutant displays loss of affinity for ADPR and loss of ADPR gating, although the ability to induce calcium influx in response to oxidative stress is unaltered. These data raise the possibility that oxidant stress activates TRPM2 through a different, undefined mechanism not involving ADPR or the NUDT9-H domain, a possibility that still requires unambiguous resolution. Activation of TRPM2 by either H_2O_2 or TNFα results in calcium influx and susceptibility to cell death

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(Hara *et al*., 2002; Zhang *et al*., 2003), and TRPM2 is likely to be an important modulator of cell fate in many tissues. Inhibition of TRPM2 activity, either by downregulation with antisense constructs or by inhibition with dominant negative splice variants, results in enhanced cell survival *in vitro*. Inhibition of TRPM2 function has been recognized as a potential *in vivo* approach to protecting cells from death following oxidant stress and possibly other adverse stimuli, but no selective inhibitors have yet been identified.

Poly(ADP ribose) polymerase (PARP) enzymes catalyze the attachment of ADPR subunits from NAD to nuclear proteins following DNA damage by toxic stimuli. A role for PARP in cell death has previously been demonstrated. PARP knockout mice are resistant to the development of diabetes induced by the beta-cell toxin streptozocin; PARP−/− mice maintained intracellular NAD levels and resisted streptozocin-induced lysis (Burkart *et al*., 1999). PARP gene disruption also rendered mice resistant to neuronal damage following cerebral ischemia (Eliasson *et al*., 1997). In this edition of the journal, Fonfria *et al*. demonstrate for the first time a role for PARP as a mediator between oxidative damage and downstream TRPM2 activation. PARP inhibitors blocked calcium influx through TRPM2, protecting cells from plasma membrane damage and from cell death. These current data demonstrate that PARP mediates its toxic effects on cells through TRPM2 activation. It provides support for the conclusion that inhibition of either PARP or TRPM2 function will protect cells from oxidant-induced death. Unfortunately, PARP inhibitors are not likely to be viable drugs to inhibit ischemic injury in patients, based on their toxicity and blockade of DNA repair enzymes. This manuscript by Fonfria *et al*. focuses attention on the importance and broad applicability that inhibitors targeted to TRPM2 may have, protecting cells in a wide range of tissues from ischemic injury and potentially from other toxic stimuli including those that result in islet cell injury and diabetes. It also raises again the possibility that downregulation of TRPM2 by other means, for example antisense strategies, will also protect cells from ischemic or toxic death.

The mechanisms through which PARP inhibitors block TRPM2 activation were not explored in the manuscript by Fonfria *et al*. One explanation is that H_2O_2 treatment may result in PARP activation, possibly through peroxide damage of DNA. PARP activation results in increased production of polyADP-ribose, from which ADPR is generated, activating TRPM2, inducing Ca^{2+} influx, and providing positive feedback for channel activation, resulting in cell death. PARP inhibitors may directly or indirectly inhibit PARP, reducing ADPR formation and inhibiting TRPM2 activation and Ca^{2+} entry. This mechanism remains to be confirmed. However, as noted above, previous work with the TRPM2-ΔC mutant suggests that H_2O_2 can gate TRPM2 through an ADPR-independent pathway. Since Fonfria *et al*. demonstrate that PARP inhibitors do not directly block TRPM2, their data raise the possibility that PARP inhibitors may block TRPM2 function through an alternative pathway. Elucidation of this pathway is of key importance, both in understanding the mechanisms of induction of cell death by PARP and TRPM2, and also in identifying potential drug targets to inhibit TRPM2 function with minimal toxicity.

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