Editorial

New insights into the interaction between ADP-ribose and human TRPM2 channel

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Comment on: Fliegert R, Watt JM, Schöbel A, *et al.* Ligand-induced activation of human TRPM2 requires the terminal ribose of ADPR and involves Arg1433 and Tyr1349. Biochem J 2017;474:2159-75.

Received: 09 October 2017; Accepted: 30 October 2017; Published: 08 November 2017.

doi: 10.21037/biotarget.2017.10.01

View this article at: http://dx.doi.org/10.21037/biotarget.2017.10.01

Since adenosine 5'-diphosphoribose (ADPR) has been discovered (1) as stimulus of the human apoptosis-related ion channel transient receptor potential melastatin 2 (TRPM2), the compound has undergone a rapid ascent from being considered a mere spin-off of the cellular energy metabolism to a generally acknowledged important intracellular second messenger. In clear distinction to post-translational modifications of proteins in the process of ADP-ribosylation [e.g., reviewed in (2)], ADPR activates the Ca^{2+}-permeable cation channel TRPM2 in the manner of a receptor agonist. This activation does not involve a covalent modification but constitutes a classical interaction between ADPR as a ligand to a specific binding site.

The ADPR binding pocket of TRPM2 is special in so far in that it represents an intrinsic protein domain which is homologous to the human Nudix hydrolase NUDT9, a mitochondrial/cytosolic enzyme that specifically binds and hydrolyzes ADPR (1). Therefore, TRPM2 may be considered a member of the exclusive club of chanzymes, i.e., ion channels containing a functionally relevant enzymatic domain (1,3-5). However, already the original study of Perraud *et al.* [2001] provided experimental evidence suggesting that a catalytic function of the NUDT9 homology (NUDT9H) domain of TRPM2 is not essential for ADPR-directed channel activation. In the meantime, it has been clearly demonstrated that the rudimentary ADPRase function of NUDT9H does not contribute to channel gating of TRPM2 (6-8).

On the basis of these findings, further questions go into two directions. One is how binding of ADPR to the NUDT9 domain results in gating and which parts of the channel protein participate in which manner. The other is about the detailed mechanisms of binding. What are the structural requirements on the ligand and on the protein for binding and what decides whether binding has agonistic or antagonistic effects? It is for the latter question that Fliegert *et al.* provide remarkable progress with their strategy combining chemical modifications of the ligand and site-directed mutagenesis of the putative ADPR binding pocket.

As an indispensable starting point for further detailed structure-function analysis of the NUDT9H domain of TRPM2, the crystal structure of the human NUDT9 enzyme has been provided at 1.8 Å resolution (9). Based on that, predictions on structural requirements of ADPR binding to the NUDT9H domain of TRPM2 have been proposed and experimentally validated (10,11). This approach is not free from possible pitfalls, however, because NUDT9H domain and NUDT9 enzyme share only 39% of their sequences and there exist some important differences between NUDT9 and TRPM2 in terms of substrate recognition and ligand binding, most notable for O-acetyl-ADPR (9,12). As even more critical point, reservation must be kept in mind, that principally apply to comparisons of an independent soluble protein with a domain structurally and functionally integrated within an ion channel, as well as to comparisons between an enzyme and a binding domain without catalytic activity. Clearly, data from cryo-electron microscopy or crystallography of TRPM2, rather than only of NUDT9, are highly desirable, but for the time being approaches like that of Fliegert *et al.* highly valuable and promising for the further understanding and exploiting of

how ligand-binding to TRPM2 takes place. The particular strength of Fliegert *et al.*'s work is that it tests structural predictions with novel ADPR analogues and thereby not only creates insight into interactions of ligands with their binding sites but also provides new pharmacological tools—and ideas how further of those tools may be designed by expert chemists.

Already previous studies on modified ADPR analogues (13) have revealed that ADP alone had no significant effect at TRPM2 and that especially the terminal ribose moiety of ADPR is crucial for substrate recognition. Accordingly, Fliegert *et al.* demonstrate in whole-cell patch-clamp experiments that four different ADPR analogues lack the ability to activate human TRPM2 because of modifications of the terminal ribose. In an important extension of previous work, ADPR modified by hydrophobic substitutions at the beta-phosphate of ADP, either by a beta-methyl group or by a beta-tetrahydrofuran-2-yl-methyl (THF) group, proved to be significantly antagonistic to ADPR-dependent channel activation. The antagonistic efficacy was clearly enhanced by increasing shape similarity to the terminal ribose. Specifically it increased from beta-methyl-ADP (low) to THF-ADP (moderate) and even more to alpha-1''-O-methyl-ADPR (high). Interestingly, the anomeric form beta-1''-O-methyl-ADPR showed the weakest antagonistic effect, strongly suggesting that the C1''-hydroxyl group of the terminal ribose and its proper configuration play a pivotal role in ADPR-dependent activation of TRPM2.

In a next step Fliegert *et al.* identified, in the NUDT9H domain of TRPM2, some potential molecular interaction partners of the terminal ribose by site-directed mutagenesis guided by homology modelling/docking. As correctly anticipated by the authors, the high flexibility of ADPR within the putative binding pocket of the channel with respect to side chain orientation hampers reliable predictions about specific interaction partners within the TRPM2 structure. This may explain why another study (10) has come to different conclusions on the potential interaction partners, although an apparently similar homology modelling approach was used. Fliegert *et al.* focus on five amino acid residues (T1347, Y1349, L1381, R1433, Y1485) as potential interaction partners of the terminal ribose. When two of them were individually subjected to relatively conservative amino acid substitutions (T1347V, Y1349F), functional consequences (although moderate) were confirmed in calcium imaging experiments.

A third point mutation (R1433M) induced the most pronounced functional effect (59% reduction) on TRPM2 activation. Here, however, the interpretation may be ambiguous because exactly this channel variant seems to show a reduced expression level in comparison to all other variants tested. In the simultaneous study by Yu *et al.* [2017], the same critical residue R1433 was either replaced by alanine, glycine, leucine, glutamine or lysine. The ADPR-stimulated whole-cell currents of these TRPM2 variants (R1433A, R1433G, R1433L, R1433Q and R1433K) were abolished (with the exception of R1433G), while the surface expression of, e.g., R1433A was indistinguishable from that of wild-type TRPM2.

As a personal comment from a lab that uses both approaches for functional studies on TRPM2, patch-clamp and calcium imaging, it may be added that the ADPR sensitivity of TRPM2 is best evaluated with whole-cell current recordings in the presence of a rigorously controlled intracellular Ca^{2+}-concentration. This method keeps the activity-boosting effects of Ca^{2+} under control, although Ca^{2+} is a co-agonist to ADPR and enters the cell through open TRPM2 channels, thereby creating a positive feed-back on the channel activation (14,15).

In summary, while searching for the critical interactions between ADPR and the NUDT9H domain of the human TRPM2 channel Fliegert *et al.* provide convincing evidence that the terminal ribose moiety and especially the conformation of its C1''-hydroxyl group play pivotal roles for the proper binding of ADPR. Together with Yu *et al.* [2017], they have identified the most probable interaction partners of ADPR within the NUDT9H domain. For further studies on ADPR degradation by the NUDT9 enzyme the next logical step would be the elucidation of the catalytic mechanism that hydrolyzes ADPR upon binding. Such studies benefit from the fact that there are many Nudix-pyrophosphohydrolases widely distributed in all kingdoms of life showing similar substrate specificities (16-18). In the case of ADPR as channel activator, however, TRPM2 is the unique target. In this situation the comparative analysis of species variants might be a promising tool to elucidate the complex structural-function relationship of TRPM2. The recent discovery of a TRPM2-like channel from the sea anemone *Nematostella vectensis* could be a first step in this direction (19). Although ADPR seems to interact with an additional site outside of the NUDT9H domain of the sea anemone channel (7), the basic mechanism of the subsequent gating process might well be similar.

Last but not least, the present findings by Fliegert *et al.*

may have impact not only on the understanding of basic mechanisms of TRPM2 channel activation but moreover provide promising pharmacological and even therapeutic prospects. TRPM2 is involved in cell death (which may be apoptosis or necrosis) as well as in other conditions of medical interest, notably granulocytic inflammation. Potential inhibitors that use the principle of pore blocking can hardly be specific for TRPM2, or any other individual member of the TRP family. On the other hand, the unique activation mechanism of TRPM2 involving binding of ADPR to the NUDT9H domain offers the opportunity for highly specific pharmacological interactions. The exact information on binding requirements given by Fliegert *et al.* in their present paper may be of invaluable help for a rationale design of drugs targeting TRPM2 and aiming at modulation of the beneficial as well as the detrimental biological functions of this channel.

Acknowledgments

Thanks to Andreas Lückhoff for helpful discussions on the manuscript.
Funding: FJ Kühn is supported by the grant KU2271/4-1 by the German Research Foundation (DFG).

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by Editor-in-Chief Maorong Jiang (Laboratory Animal Center of Nantong University, Nantong, China).

Conflicts of Interest: The author has completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/biotarget.2017.10.01). The author has no conflicts of interest to declare.

Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Perraud AL, Fleig A, Dunn CA, et al. ADP-ribose gating of the calcium-permeable LTRPC2 channel revealed by Nudix motif homology. Nature 2001;411:595-9.
2. Palazzo L, Mikoč A, Ahel I. ADP-ribosylation: new facets of an ancient modification. FEBS J 2017;284:2932-46.
3. Ramjeesingh M, Li C, Garami E, et al. Walker mutations reveal loose relationship between catalytic and channel-gating activities of purified CFTR (cystic fibrosis transmembrane conductance regulator). Biochemistry 1999;38:1463-8.
4. Runnels LW, Yue L, Clapham DE. TRP-PLIK, a bifunctional protein with kinase and ion channel activities. Science 2001;291:1043-7.
5. Schlingmann KP, Weber S, Peters M, et al. Hypomagnesemia with secondary hypocalcemia is caused by mutations in TRPM6, a new member of the TRPM gene family. Nat Genet 2002;31:166-70.
6. Tóth B, Iordanov I, Csanády L. Putative chanzyme activity of TRPM2 cation channel is unrelated to pore gating. Proc Natl Acad Sci U S A 2014;111:16949-54.
7. Kühn FJ, Kühn C, Winking M, et al. ADP-Ribose Activates the TRPM2 Channel from the Sea Anemone Nematostella vectensis Independently of the NUDT9H Domain. PLoS One 2016;11:e0158060.
8. Iordanov I, Mihályi C, Tóth B, et al. The proposed channel-enzyme transient receptor potential melastatin 2 does not possess ADP ribose hydrolase activity. eLife 2016;5:e17600.
9. Shen BW, Perraud AL, Scharenberg A, et al. The crystal structure and mutational analysis of human NUDT9. J Mol Biol 2003;332:385-98.
10. Yu P, Xue X, Zhang J, et al. Identification of the ADPR binding pocket in the NUDT9 homology domain of TRPM2. J Gen Physiol 2017;149:219-35.
11. Fliegert R, Watt JM, Schöbel A, et al. Ligand-induced activation of human TRPM2 requires the terminal ribose of ADPR and involves Arg1433 and Tyr1349. Biochem J 2017;474:2159-75.
12. Grubisha O, Rafty LA, Takanishi CL, et al. Metabolite of SIR2 reaction modulates TRPM2 ion channel. J Biol Chem 2006;281:14057-65.
13. Moreau C, Kirchberger T, Swarbrick JM, et al. Structure-activity relationship of adenosine 5'-diphosphoribose at

the transient receptor potential melastatin 2 (TRPM2) channel: rational design of antagonists. J Med Chem 2013;56:10079-102.
14. Heiner I, Eisfeld J, Warnstedt M, et al. Endogenous ADP-ribose enables calcium-regulated cation currents through TRPM2 channels in neutrophil granulocytes. Biochem J 2006;398:225-32.
15. Starkus J, Beck A, Fleig A, et al. Regulation of TRPM2 by extra- and intracellular calcium. J Gen Physiol 2007;130:427-40.
16. Bessman MJ, Frick DN, O'Handley SF. The MutT proteins or "Nudix" hydrolases, a family of versatile, widely distributed, "housecleaning" enzymes. J Biol Chem 1996;271:25059-62.
17. Gabelli SB, Bianchet MA, Bessman MJ, et al. The structure of ADP-ribose pyrophosphatase reveals the structural basis for the versatility of the Nudix family. Nat Struct Biol 2001;8:467-72.
18. Srouji JR, Xu A, Park A, et al. The evolution of function within the Nudix homology clan. Proteins 2017;85:775-811.
19. Kühn FJ, Kühn C, Lückhoff A. Functional characterisation of a TRPM2 orthologue from the sea anemone Nematostella vectensis in human cells. Sci Rep 2015;5:8032.

doi: 10.21037/biotarget.2017.10.01
Cite this article as: Kühn FJ. New insights into the interaction between ADP-ribose and human TRPM2 channel. Biotarget 2017;1:14.