Communicative & Integrative Biology 3:6, 530-532; November/December 2010; ©2010 Landes Bioscience

Unraveling the role of polycystin-2/inositol 1,4,5-trisphosphate receptor interaction in Ca^{2+} signaling

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Key words: Ca^{2+} channels, intracellular Ca^{2+} release, endoplasmic reticulum, kidney, signal transduction, autosomal dominant polycystic kidney disease, polycystin-2, renal pathophysiology, inositol 1,4,5-trisphosphate, the inositol 1,4,5-trisphosphate receptor

Abbreviations: AC-VI, adenylyl cyclase type 6; ADPKD, autosomal dominant polycystic kidney disease; $[Ca^{2+}]_{cyt}$, free cytosolic Ca^{2+} concentration; CICR, Ca^{2+}-induced Ca^{2+} release; ER, endoplasmic reticulum; IICR, inositol 1,4,5-trisphosphate-induced Ca^{2+} release; IP_3, inositol 1,4,5-trisphosphate; IP_3R, inositol 1,4,5-trisphosphate receptor; LBD, ligand-binding domain; PDE, phosphodiesterase; TRPP2, polycystin-2

Submitted: 06/18/10

Accepted: 06/18/10

Previously published online: www.landesbioscience.com/journals/cib/article/12751

DOI: 10.4161/cib.3.6.12751

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Addendum to: Sammels E, Devogelaere B, Mekahli D, Bultynck G, Missiaen L, Parys JB, et al. Polycystin-2 activation by inositol 1,4,5-trisphosphate-induced Ca^{2+} release requires its direct association with the inositol 1,4,5-trisphosphate receptor in a signaling microdomain. J Biol Chem 2010; 285:18794–805; PMID: 20375013; DOI: 10.1074/jbc.M109.090.

Autosomal dominant polycystic kidney disease (ADPKD) arises as a consequence of mutations of the genes *PKD1* and *PKD2*, encoding respectively the integral membrane proteins polycystin-1 and polycystin-2 (TRPP2), resulting in a disturbance in intracellular Ca^{2+} signaling. Previously we investigated the interaction between TRPP2 and the inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R), an intracellular Ca^{2+} channel in the endoplasmic reticulum (ER). We identified the molecular determinants of this interaction and observed an enhanced IP_3-induced Ca^{2+} release (IICR). Since we found that TRPP2 strongly bound to a cluster of positively charged amino acids in the N-terminal ligand-binding domain (LBD) of the IP_3R, we now investigated whether TRPP2 would interfere with the binding of IP_3 to the IP_3R. In in vitro experiments we observed that TRPP2 partially inhibited the binding of IP_3 to the LBD of the IP_3R with an IC_{50} of ~350 nM. The suppressor domain, i.e., the N-terminal 225 amino acids of the LBD of the IP_3R, mediated this inhibitory effect of TRPP2 on IP_3 binding. The observation that the interaction between the IP_3R and TRPP2 decreased IP_3 binding is in apparent contrast to the increased IICR. The data can be explained however by a subsequent activation of Ca^{2+}-induced Ca^{2+} release (CICR) via TRPP2. Implications of this mechanism for cellular Ca^{2+} signaling are discussed in this addendum.

The inherited human disorder ADPKD affects more than 1 in 1,000 live births and is the most common monogenic cause of kidney failure in man.[1] It is characterized by the progressive formation and enlargement of renal cysts, typically leading to chronic renal failure by late middle age.[2] In most cases, the disease arises as a consequence of mutations in the *PKD1* or *PKD2* genes, which respectively encode the proteins polycystin-1 and TRPP2.[1,3] TRPP2 is a 968-amino-acid (aa) protein with six predicted transmembrane domains, highly conserved among multicellular organisms and widely expressed in various tissues.[4] TRPP2 has been implicated in diverse functions depending on its subcellular localization. TRPP2 has been detected (1) in the plasma membrane, where it is supposed to form a receptor-operated, non-selective cation channel,[5] (2) in the primary cilium, where it could act as a mechanosensitive channel, possibly in association with other TRP-family members,[6] (3) in the ER, where it is proposed to function as an intracellular Ca^{2+}-release channel,[7] and (4) also in centrosomes and in mitotic spindles of dividing cells (reviewed in refs. 8–10). However, the predominant subcellular localization of TRPP2 is in the ER, as shown by its sensitivity to endoglycosidase H, immunofluorescence experiments and its co-localization and co-distribution with ER-resident proteins.[7,11] Previously we investigated the interaction between TRPP2 and the IP_3R, an ubiquitous intracellular Ca^{2+}-release channel.[12] We observed a strong interaction between TRPP2 and the IP_3R and identified a conserved positively charged cluster in the N-terminal LBD of the IP_3R and an acidic cluster located at the end of the ER-retention signal in the C-terminal

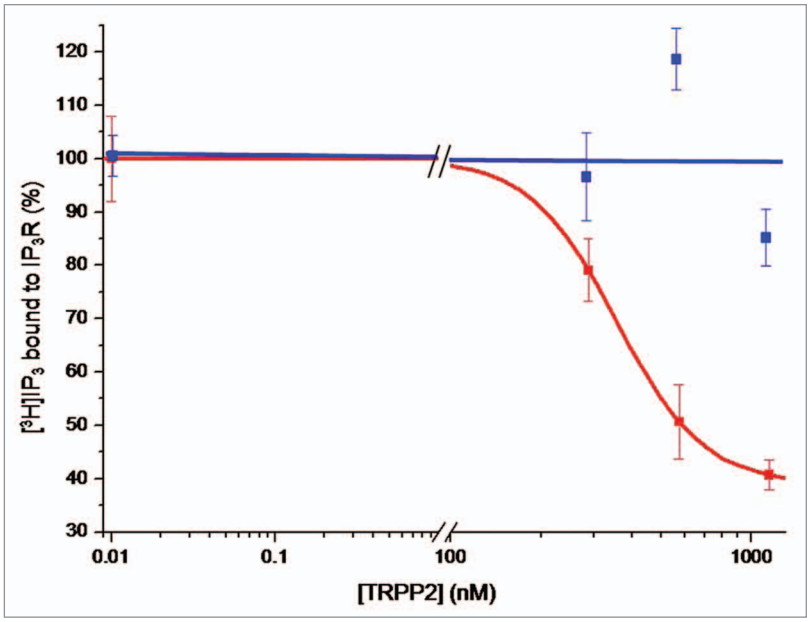

Figure 1. The effect of TRPP2-CT on IP_3 binding to the IP_3R LBD. Specific binding of 1.5 nM [^{3}H]IP_3 to recombinant HIS-fusion proteins of the LBD of the IP_3R, consisting of the suppressor domain and the IP_3-binding core (red) and of the IP_3-binding core, which lacks the suppressor domain, (blue) in the presence of increasing concentrations of recombinant GST-fusion protein of the C-terminal tail of TRPP2. The mean ± S.E.M. of three independent experiments is shown.

tail of TRPP2 as being crucial for their interaction. When full-length TRPP2 was re-introduced in TRPP2$^{-/-}$ mouse renal epithelial cells, there was a clear potentiation of agonist-induced intracellular Ca^{2+} release in intact cells and of IICR in permeabilized cells. Further analysis using pathological mutants of TRPP2 and competing peptides revealed that this effect on IICR was dependent on the TRPP2-channel function but in addition required interaction with the IP_3R.[12]

Since we found that TRPP2 interacted with the LBD of the IP_3R, we investigated whether TRPP2 was able to affect the IP_3-binding properties of a HIS-fusion protein of the LBD of IP_3R1 (LBD-HIS). The LBD consists of an IP_3-binding core (aa 226–581) and a suppressor domain (aa 1–225).[13] A [^{3}H]IP_3-binding assay[14] was performed with purified LBD-HIS (aa 1–581) and with LBD-HIS Δ1-225 (aa 226–581) (described in ref. 15). In the presence of increasing concentrations of a GST-fusion protein of the C-terminal tail of murine TRPP2 (GST-TRPP2-CT, aa 679–966) we observed inhibition of the binding of IP_3 to the complete LBD, with an IC_{50} of 350 nM (**Fig. 1**). This inhibition was partial and amounted to maximally 60% of the total binding. Deletion of the N-terminal 225 amino acids of the LBD, including the positive cluster that interacts with TRPP2,[12] completely abolished the effect of GST-TRPP2-CT on IP_3 binding (**Fig. 1**). This confirms the role of the suppressor domain of the IP_3R as the major determinant for binding to the C-terminal tail of TRPP2.

The attenuation of IP_3 binding did however not inhibit Ca^{2+} release in functional assays of agonist-induced Ca^{2+} release or IICR with the full-length proteins. In contrast, we observed an enhanced Ca^{2+} release in the presence of TRPP2, which could be ascribed to the activation of TRPP2 via CICR. Moreover, in the functional assays we observed no difference in Ca^{2+} release between cells treated with a control adenovirus or cells expressing the pathological dead-channel mutant of TRPP2, D509V, which can still bind to the IP_3R.[12] The inhibitory effect of TRPP2 on IP_3 binding in in vitro experiments was thus not observed in an intact cell context. Several arguments can explain this observation.

First, the inhibition of IP_3 binding was only partial. Apparently a conformational change induced by an allosteric interaction with the suppressor domain attenuates IP_3 binding to the IP_3-binding core but does not preclude subsequent IICR. This behavior is reminiscent to the interaction of the suppressor domain with calmodulin,[16] which modulates but not by itself inhibits IICR. Secondly, it is possible that our functional assays, measuring global Ca^{2+} signals in a whole population of cells, did not have sufficient resolution to measure probably relatively small effects caused by TRPP2-mediated attenuation of IP_3 binding to the IP_3R. Thirdly, it is possible that interaction with TRPP2 resulted in modulation of the IP_3 response from a more graded into an all-or-none Ca^{2+} response. At low doses of IP_3 TRPP2 would inhibit the binding of IP_3 to the IP_3R and thus reduce IICR, thereby affecting the elementary events produced by IP_3Rs (called Ca^{2+} puffs).[17,18] At higher doses of IP_3 this inhibition will be overcome and will induce IICR, which further activates CICR via the TRPP2 channel itself, resulting in an increased global Ca^{2+} signal. Discrimination of graded versus all-or-none Ca^{2+} response is difficult to achieve by measuring global Ca^{2+} changes at relatively high [IP_3] as was done by Sammels et al.[12] Measuring elementary Ca^{2+} events in single cells could possibly elucidate this issue.

We can only speculate on the cellular significance of such a mechanism. All-or-none Ca^{2+} signals would be expected to be more restricted in time and space and to be localized in the immediate environment of the IP_3R/TRPP2 complexes. In this way the ER localization of TRPP2 would not result in Ca^{2+} increase at low or basal [IP_3] but would only result in local rises in free cytosolic [Ca^{2+}] ([Ca^{2+}]$_{cyt}$) evoked upon appropriate cell stimulation. It is conceivable that in the direct vicinity of the IP_3R/TRPP2 protein complex other signaling proteins are localized as adenylyl cyclases or phosphodiesterases (**Fig. 2**). In that respect, it is important to note that the Ca^{2+}-dependent adenylyl cyclase VI was already found to be associated to IP_3Rs.[19] As a result, our model (**Fig. 2**) proposes that the specificity of downstream Ca^{2+}-dependent effects is further increased, e.g., by modulation of the [cAMP]. This can be relevant for the pathology of ADPKD, since increased

levels of cAMP are a common finding in the kidneys of ADPKD animal models.[20]

We conclude that a signaling complex involving TRPP2 and the IP_3R is important for modulating intracellular Ca^{2+} signaling. Disturbance of this interaction, which occurs in pathologically relevant mutants of TRPP2, will lead to altered intracellular Ca^{2+} homeostasis and might contribute to the development of ADPKD caused by loss-of-function mutations in TRPP2. We found that TRPP2 activation via CICR required an initial rise in $[Ca^{2+}]_{cyt}$ via IICR and an interaction with the IP_3R. We observed that TRPP2 could inhibit the binding of IP_3 to its receptor in vitro. Taken together, these properties could favour the specificity of intracellular Ca^{2+} signaling evoked by ER-localized TRPP2.

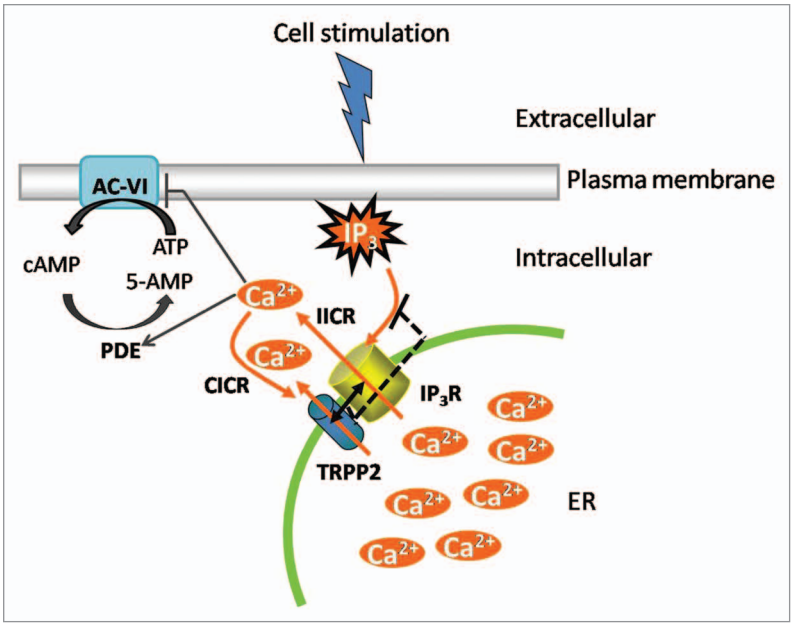

Figure 2. Proposed model. The binding of TRPP2 to the suppressor domain of the IP_3R partially inhibits IP_3 binding in vitro (dashed line), thus possibly suppressing Ca^{2+} release events at very low $[IP_3]$. Upon appropriate cell stimulation, when a sufficiently high $[IP_3]$ is produced, the IP_3R is activated leading to a local $[Ca^{2+}]_{cyt}$ rise that subsequently activates the TRPP2 channel as a CICR channel. We suggest that a signaling microdomain, where TRPP2 interacts with the IP_3R through the charged residues, is required to facilitate CICR by TRPP2. In this microdomain Ca^{2+} could play a role in the inhibition of adenylyl cyclase VI (AC-VI) or stimulation of Ca^{2+}-dependent phosphodiesterase (PDE), thereby lowering [cAMP].

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