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

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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$_R$, inositol 1,4,5-trisphosphate receptor; LBD, ligand-binding domain; PDE, phosphodiesterase; TRPP2, polycystin-2

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$_R$), 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$_R$, we now investigated whether TRPP2 would interfere with the binding of IP$_3$ to the IP$_R$. In in vitro experiments we observed that TRPP2 partially inhibited the binding of IP$_3$ to the LBD of the IP$_R$ with an IC$_{50}$ of ~350 nM. The suppressor domain, i.e., the N-terminal 225 amino acids of the LBD of the IP$_R$, mediated this inhibitory effect of TRPP2 on IP$_3$ binding. The observation that the interaction between the IP$_R$ 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. It is characterized by the progressive formation and enlargement of renal cysts, typically leading to chronic renal failure by late middle age. 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. TRPP2 is a 968-amino-acid (aa) protein with six predicted transmembrane domains, highly conserved among multicellular organisms and widely expressed in various tissues. 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, (2) in the primary cilium, where it could act as a mechanosensitive channel, possibly in association with other TRP-family members, (3) in the ER, where it is proposed to function as an intracellular Ca$^{2+}$-release channel, 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. Previously we investigated the interaction between TRPP2 and the IP$_R$, an ubiquitous intracellular Ca$^{2+}$-release channel. We observed a strong interaction between TRPP2 and the IP$_R$ and identified a conserved positively charged cluster in the N-terminal LBD of the IP$_R$ and an acidic cluster located at the end of the ER-retention signal in the C-terminal domain of kidney failure. The results of this study provide further understanding of the role of TRPP2 in Ca$^{2+}$ signaling and its implications for ADPKD.
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\(_3\), R.\(^{12}\)

Since we found that TRPP2 interacted with the LBD of the IP\(_3\), R, we investigated whether TRPP2 was able to affect the IP\(_3\), binding properties of a HIS-fusion protein of the LBD of IP\(_3\), R (LBD-HIS). The LBD consists of an IP\(_3\),-binding core (aa 226–581) and a suppressor domain (aa 1–225).\(^{13}\) A \([\text{H}]\)IP\(_3\),-binding assay was performed with purified LBD-HIS (aa 1–581) and with LBD-HIS D1-225 (aa 226–581) (described in ref. 15). In the presence of increasing concentrations of a GST-fusion protein of the C-terminal tail of TRPP2, the mean ± S.E.M. of three independent experiments is shown.

Figure 1. The effect of TRPP2-CT on IP\(_3\), binding to the IP\(_3\), R LBD. Specific binding of 1.5 nM \([\text{H}]\)IP\(_3\), to recombinant HIS-fusion proteins of the LBD of the IP\(_3\), R, 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.

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\(_3\), R.\(^{12}\) The inhibitory effect of TRPP2 on IP\(_3\), binding 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\(_3\), R. 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\(_3\), R and thus reduce IICR, whereby affecting the elementary events produced by IP\(_3\),Rs (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 Sammel 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\(_3\),/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\(^{2+}\)] (\([\text{Ca}^{2+}]_{\text{cyt}}\)) evoked upon appropriate cell stimulation. It is conceivable that in the direct vicinity of the IP\(_3\),/TRPP2 protein complex other signaling proteins are localized as adenyl cyclases or phosphodiesterases (Fig. 2). In that respect, it is important to note that the Ca\(^{2+}\)-dependent adenyl cyclase VI was already found to be associated to IP\(_3\),Rs.\(^{19}\) 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.\(^\text{20}\)

We conclude that a signaling complex involving TRPP2 and the IP\(_R\) 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\(^{2+}\)]\(_{\text{cyt}}\) via IICR and an interaction with the IP\(_R\). 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.

References
1. Gabow PA, Grantham JJ. Polycystic kidney disease. In: Seifert RW, Gortzschalk CW, (eds). Diseases of the kidney, 6th edn. Little Brown and Company: Boston USA; 1997:821-60.
2. Majumdar A, Redz-Otoo E, Kellet J, Bhatia R, Schnier RW. Developments in the management of autosomal dominant polycystic kidney disease. Ther Clin Risk Manag 2008; 4:494-407.
3. Harris PC, Torres VE. Polycystic kidney disease. Annu Rev Med 2009; 60:321-37.
4. Mochizuki T, Wu G, Hayashi T, Xenophonotos SL, Veldhuisen B, Sarris J, et al. PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. Science 1996; 272:1339-42.
5. Tsiokas L, Veldhuisen B, Saris JJ, et al. PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. Science 1996; 272:1339-42.
6. Zhou J. Polycystins and primary cilia: primers for cell cycle progression. Annu Rev Physiol 2009; 71:83-113.
7. Keuleten P, Cai Y, Geng L, Maida Y, Nishimura S, Honore E, et al. Co-assembly of polycystin-1 and -2 produces unique cation-permeable currents. J Biol Chem 2007; 282:17722-34.
8. Tsiokas L, Kim S, Ong EC. Cell biology of polycystins-1, 2. Cell Signal 2007; 19:444-53.
9. Giamarchi A, Padilla F, Coste B, Raoux M, Crest M, Honore E, et al. The versatile nature of the calcium-permeable cation channel TRPP2. EMBO Rep 2006; 7:787-93.
10. Kortgen M. TRPP2 and autosomal dominant polycystic kidney disease. Biochim Biophys Acta 2007; 1772:836-50.
11. Cai Y, Maida Y, Cederich A, Torres VE, Wu G, Hayashi T, et al. Identification and characterization of polycystin-2, the PKD2 gene product. J Biol Chem 1999; 274:28557-65.