providing no obvious evidence for P_{Ca}/P_{Na} changes over this time scale. We considered that saturation of intracellular Ca^{2+} chelators such as EGTA would likely hinder assessment of time-dependent changes in P_{Ca}/P_{Na} using E_{Rev} measurements, as these assume that the transmembrane Ca^{2+} concentration ratio remains constant throughout. In 10 mM external Ca^{2+}, we performed patch-clamp photometry using pipettes containing 10 mM fura-2. Cells expressing TRPV1 were stimulated with capsaicin for 25 s, with 140 mV ramps applied every 500 ms. We observed a shift in E_{Rev} for the capsaicin-evoked current from 12.2 ± 1.9 to 1.1 ± 1.3 mV (n=4), but this was mostly apparent after the rapid saturation of fura-2 by TRPV1-mediated Ca^{2+} entry. Therefore, caution is strongly encouraged when interpreting time-dependent changes in Ca^{2+} permeability based on E_{Rev} experiments alone.

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Protection of NG108-15 Cells from P2X7 Receptor-Mediated Toxicity by Taurine

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Strong P2X7 receptor (P2X7R) activation could cause Ca^{2+} overload and consequently cell death. We previously showed that Ca^{2+} store depletion and endoplasmic reticulum (ER) stress were involved in P2X7R-mediated cytotoxicity in differentiated NG108-15 neuronal cells. In this work, we studied whether taurine (2-aminoethanesulfonic acid) could reverse P2X7R-mediated death in differentiated neuronal NG108-15 cells. Taurine (10 mM) could prevent cell death and appearance of cleaved caspase-3 after BzATP (a selective P2X7R agonist) treatment. However, taurine did not protect cells from Ca^{2+} overload after P2X7R activation. P2X7R-mediated Ca^{2+} overload by BzATP led to endoplasmic reticulum (ER) Ca^{2+} depletion and ER stress. We found that although taurine did not prevent Ca^{2+} store depletion after BzATP stimulation, it prevented appearance of ER stress markers, namely, phosphorylated eukaryotic translation initiation factor 2α (peIF2α) and C/EBP-homologous protein (CHOP). P2X7R activation did not result in mitochondrial Ca^{2+} ($[Ca^{2+}]_m$) overload, nor did it affect mitochondrial membrane potential. BzATP-induced generation of intracellular reactive oxygen species (ROS) was prevented by taurine. Collectively, neuroprotective effect by taurine may involve suppression of ROS formation and intervention between ER Ca^{2+} depletion and ER stress.

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Co-Expressed Pannexin-1 and the P2X7 Receptor do not Interact Functionally or Physically at the Plasma Membrane of *Xenopus Laevis* Oocytes

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A remarkable feature of a sustained P2X7 receptor activation by ATP is the induction of a large cytolytic pore permeable to cations up to 700 Da. Whether the cytolytic pore is formed by the homotrimeric P2X7 receptor itself or by the downstream activation of secondary channel proteins such as pannexin-1 is not finally settled. Here, we assessed the specificity of the functional and physical interaction between pannexin-1 and the P2X7 receptor. We found that recombinant pannexin-1 and the P2X7 receptor expressed in *Xenopus laevis* oocytes form disulfide-bonded oligomers in the endoplasmic reticulum (ER). However, at the level of the plasma membrane, we could not detect disulfide-bonded oligomers between pannexin-1 and hP2X7 nor any non-covalently linked physically stable complexes between the two proteins. This suggests that the disulfide-bonded oligomers are retained in the ER by the ER quality control system. Also by using two-electrode voltage-clamp electrophysiology, we obtained no evidence for a functional interaction of human or murine P2X7 receptors and pannexin-1 before and during P2X7 receptor activation by ATP. Although our data confirm that certain forms of P2X7 receptors and pannexin-1 interact physically stably with each other, they argue against the view that this interaction takes place at the cell membrane and that pannexin-1 represents the pore-forming principle activated through P2X7 receptors.

We thank the German Research foundation (Deutsche Forschungsgemeinschaft, DFG) for financial support (grants Ma1581/15-1 and Schm536/9-1).

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Structural Elements in the Cytoplasmic Domain of Mammalian Inward Rectifier K^+ Channels Responsible for Gating

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Inward rectifier K+ (Kir) channels consist of the transmembrane and cytoplasmic domains. Various cytoplasmic regulators associate with the cytoplasmic domain to control the gate at the transmembrane domain. Despite increasing knowledge about the structure, it is still unclear how structural elements within the cytoplasmic domain shift their positions to regulate the gate in the functional channels. To address these questions, we focused on two spatially adjacent residues (Glu236 and Met313) of G protein-gated Kir channel subunit Kir3.2, whose side chains face the cytoplasmic pore when it is in a closed state. Mutagenesis for these residues revealed that two unique membrane-facing structural elements (CD element and HI element) in the cytoplasmic domain. The first HI element associated with concerted motion of the subunits and moved concomitantly with the transmembrane domain during gating, implying that the element mediates allosteric coupling between domains. The second CD element appeared to shift its position upon the G protein stimulation. Since the CD element lies adjacent to the HI element by forming a β sheet, the rearrangement of these two structural elements and the constraint between them appear to be crucial for control of gating in Kir channels.

Anion Channels & Transporters

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Kinetic and Energetic Insights into the Gating of a Single Protein Channel

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Gating of a *beta*-barrel membrane protein is a fundamentally important and ubiquitous process in membrane biology. Here, we systematically examined details of the kinetics and energetics of the current fluctuations within the OccK1 protein, a family member of outer membrane carboxylate channels from *Pseudomonsa Aeruginosa*. Temperature-dependent, single-molecule electrophysiology analysis of the native and loop-deletion OccK1 proteins demonstrated the distinctive nature of two energetic barriers: an enthalpic barrier of the large-current amplitude, infrequent O_1 to O_2 transitions and an entropic barrier of the low-current amplitude, highly frequent O_2 to O_3 transitions. Changes in temperature produced asymmetric kinetic and energetic modifications that caused a switch of the most probable open sub-state, O_2 to O_3, at the lowest examined temperature of 4 ^{0}C. Our approach might be used in the future to obtain a semi-quantitative assessment of the discrete fluctuation dynamics in other *beta*-barrel protein channels of the outer membranes of Gram-negative bacteria, mitochondria and chloroplasts.

Acknowledgments: This work is funded in part by grants from the US National Science Foundation (DMR-1006332, L.M.) and the National Institutes of Health (R01 GM088403, L.M. and R01 GM085785, B.v.d.).

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Deciphering the Ion Selectivity of the Phosphate Specific Channel OprP from Pseudomonas Aeruginosa: A Free-Energy Molecular Dynamics Simulation Study

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An enormous variety of biological processes demands the participation of specific ions in the metabolism of living organisms. This selective participation is regulated by various membrane proteins which are able to discriminate between different ion types and thereby allowing the transport of specific ions across membranes [1]. To understand this process, we probed the phosphate selectivity of the porin OprP from Pseudomonas aeruginosa utilizing all-atom free energy molecular dynamics simulations. Energies associated with phosphate, sulfate, chloride, and potassium ion transport through OprP were obtained. On the one hand, further atomic level analysis indicated the role of the overall electrostatic environment of the channel in determining its anion selectivity. On the other hand, a particular balance of interactions between the permeating ions and water as well as channel residues was found to be responsible for the selectivity between different anions. The selectivity of OprP is discussed in the context of well-studied ion channels being highly selective for potassium or chloride.

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Molecular Dynamics Simulations of CFTR Gating and Conductance

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