TRP Channels II

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Role of Polyphosphate in Cancer Cell Proliferation
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Inorganic polyphosphates (polyPs) are linear polymers of orthophosphate residues linked by phosphoanhydride bonds similar to ATP. PolyPs are ubiquitously distributed in nature from bacteria to mammalian cells where they play multiple roles. Here we investigated intracellular localization of polyP in cancer cell lines and tested the idea about the involvement of polyP in cell growth.
To study PolyP localization and cellular levels we used DAPI-based method of polyP staining. Experiments were performed using the following cell lines: H1299 (lung cancer), U251 (glioma) and HEK293 as a control cell line. PolyP was also visualized by immunocytochemistry with X-press tagged PolyP-binding domain of exopolyphosphatase (PPX) and subsequent use of specific antibodies. The role of PolyP in cancer cell proliferation was studied using MTS proliferation assay. In these experiments endogenous PolyP was either blocked by spermine, a polyamine with high PolyP-binding affinity, or depleted by transient expression of PPX, an enzyme that specifically hydrolyzes PolyP into orthophosphate.
PolyP levels were increased in glioma and lung cancer cells compare to HEK 293 cells. Proliferation of lung carcinoma (H1299) cells was significantly inhibited when PolyP was blocked with spermine compare to control cells. However, proliferation of glioma cells (U251) was not affected by spermine, even at high concentrations of this polyamine (60 μM), suggesting a cancer type dependent effect or, involvement of divers mechanisms of actions of PolyP in different cell types. These results suggest that PolyP plays an important role in the regulation of proliferation of cancer cells. Further investigation of PolyP contribution to cancer development and progression may provide tools to detect, prevent and/or treat cancer.

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Upregulation of TRPM7-Like Current in Ischemia Damaged Human Atrial Cardiomyocytes
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Transient receptor potential of melastatin subfamily (TRPM7) channels has been detected in the heart using molecular approaches. Recently we have identified electrophysiologically the TRPM7-like current in native human cardiomyocytes, and discovered that in ~1/3 of patients the current was already activated at patch rupture. One of possible versions to explain this phenomenon might be related with heart pathology such as ischemic heart disease (IHD). To test this assumption we used cardiomyocytes from right atrial appendages obtained from 77 adult patients with and without IHD. With voltage-dependent and other ion channels inhibited, TRPM7-like current was recorded using the whole-cell patch-clamp technique. The TRPM7-like current studied in 136 myocytes showed a marked variation of outward (at +80 mV) and inward (at −120 mV) current densities ranging from 1.21 to 11.63 pA/pF and from −0.14 to −0.7 pA/pF, respectively. The densities of TRPM7-like current did not correlate with membrane capacitance or changes of biophysical properties. The TRPM7-like current density was homogeneous for a given sample. We established that higher TRPM7-like current values, at rupture and steady-state, were recorded in cardiomyocytes obtained from patients with IHD (in pA/pF): from 2.14 ± 0.12 to 5.39 ± 0.36 and from -0.39 ± 0.02 to -0.55 ± 0.03 for outward and inward current, respectively, versus without IHD (from 1.68 ± 0.22 to 4.03 ± 0.4, and from -0.29 ± 0.04 to -0.39 ± 0.04, respectively, at +80 mV and −120 mV). In addition, we tested influence of pH that is major component of acute myocardial ischemia. Change of extracellular pH from 7.4 to 4 markedly increased inward and outward magnitude of TRPM7-like current, which again was more pronounced in cardiomyocytes from patients with IHD. Our experiments revealed the enlarged TRPM7-like current in atrial cardiomyocytes from patients with IHD, and those cells were associated with the greatest response to the extracellular acidification.

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Development of TRPC Assays on Automated Electrophysiology Platforms
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TRPC3, TRPC6 and TRPC7 are Ca^{2+} permeable non-selective cation channels that have been implicated in cancer, cardiovascular, respiratory and kidney diseases. In this study, CHO-K1 cells stably expressing human M3 muscarinic acetylcholine receptors were infected with human TRPC3, TRPC6 or TRPC7 BacMam viruses and measured on QPatch and IonWorks Quattro automated electrophysiology platforms. In the QPatch HT mode (single cell recording), TRPC currents were rapidly activated by the muscarinic agonist carbachol and then decayed irreversibly with a rank order of TRPC7>TRPC3>TRPC6. In both HT and HTX (10 cells per well) mode there was large variation in current levels between recordings. The lack of a good well-to-well comparison means that QPatch has low suitability for TRPC screening assays. The Quattro in PPC mode (64 cells per well), sufficiently normalised well-to-well variation in carbachol activated TRPC currents to enable assays to be developed for TRPC3 and TRPC6. The time delay on the Quattro between carbachol addition and the first current measurement resulted in TRPC7 currents having almost completely decayed so remaining currents were too small. The TRPC3 and TRPC6 assays were validated with two recently described TRPC inhibitors. Compound 8 [1] inhibited TRPC3 and TRPC6 currents with respective IC_{50} values of 1.1 ± 0.2 μM and 24 ± 7 nM (Mean ± SD, n=4-5). 2-(amino)-thiazole-4-carboxamide [2] inhibited TRPC3 and TRPC6 currents with respective IC_{50} values of 1.4 ± 1.1 and 0.9 ± 0.2 μM (Mean ± SD, n=3). These values are in good agreement with the published data.
This study demonstrates that TRPC3, TRPC6 and TRPC7 currents can be measured on automated electrophysiology platforms. For TRPC channels, the QPatch is suitable to profile channel biophysics whereas the Quattro is more applicable for compound profiling.
[1] Patent WO2011/107474 A1.
[2] Patent WO2012/037349 A2.

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A New Class of Analgesics Exerts a Dual Modulation on TRPA1 and TRPV1 Channels
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The Transient Receptor Potential Vanilloid 1 (TRPV1) and Ankyrin 1 (TRPA1) ion channels belong to the TRP superfamily and are both integrators of a variety of noxious stimuli.
Both channels are non selective cation channels which exert pleiotropic functions in a variety of cells; more specifically they colocalize in primary sensory neurons of the trigeminal, vagal and dorsal root ganglia (DRG).
TRPA1 is activated by a number of pungent and irritant reactive chemical compounds including allyl isothiocyanate (mustard oil), cinnamaldehyde (cinnamon oil), allicin (onions), carvacrol (oregano), polygodial (Tasmanian pepper) and formaldehyde (formalin); all of these molecules elicit a painful burning and a prickling sensation.
TRPV1 is activated by noxious stimuli including high temperature, pH, and vanilloid compounds such as capsaicin. Moreover increased expression of TRPV1 was detected in prostate, colon, and pancreatic cancers, revealing a relevant role of TRPV1 as tumor suppressor.
In this context, we describe a new class of water soluble derivatives of lipoic acid [1], which proved to block TRPA1 channels.
We screened a small molecule compounds library, by patch- clamp recordings on culture cells expressing both the human and the mouse isoforms of TRPA1 channel, to compare the potency of each molecule towards well-known TRPA1 agonists (AITC, Menthol, cinnamaldehyde, oxaliplatin); selectivity studies showed no appreciable block by these molecules of TRPM8, hERG and Na_V channels.
Finally we showed that a subgroup of these compounds is able to activate TRPV1 channels, suggesting interesting applications of our molecules for the treatment of many diseases.
[1] Nativi C., Gualdani R. et al. (2013) 'A TRPA1 antagonist reverts oxaliplatin-induced hyperalgesia'. Scientific Reports, 3, 1-10.
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