BA.11 - The VRAC blocker DCPIB opens Ca^{2+}-activated BK channels and increases intracellular Ca^{2+} in melanoma and pancreatic duct carcinoma cell lines

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The Volume Regulated Anion Channel (VRAC) is known to be involved in cancer cell behavior and response to therapies. Since ion channels play an increasingly recognized role in cancer we investigated the effect of DCPIB, a presumably specific VRAC blocker, in pancreatic duct adenocarcinoma (PDAC) and melanoma. We sought to define the mechanisms of DCPIB in two PDAC lines (Panc-1, MiaPaCa-2), as well as on a primary (IGR39) and a metastatic (IGR37) melanoma line. We performed whole-cell patch clamp electrophysiology, gene expression analysis and calcium measurements. DCPIB induced a dramatic increase of currents in Panc1, MiaPaca2 and IGR39, but not in IGR37 cells. Currents were sensitive to tetraethylammonium and thus not mediated by K2P channels, known to be activated by DCPIB. Rather, currents were mostly mediated by the Ca^{2+}-dependent BK channel. DCPIB activation of BK as verified in transfected HEK293 cells. Further experiments showed that in IGR39, and to a smaller degree also in Panc-1 cells, DCPIB induces a rapid Ca^{2+} influx. This, in turn, indirectly potentiates not only BK but, in IGR39 cells, additionally activates other Ca^{2+}-dependent channels. However, Ca^{2+} influx is not required for BK activation by DCPIB. The direct activation of BK by DCPIB involves the extracellular part of the protein, as no effect was detectable when DCPIB was delivered inside the cell via the patch pipette. We conclude that the BK channel is a new target of DCPIB, and that the compound can acutely increase intracellular Ca^{2+}, elongating the list of DCPIB side-effects that need to be taken into consideration for future development of DCPIB-based activators/inhibitors of ion channels and other membrane proteins.
Keywords: BK channel, PDAC cancer, Melanoma
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BA.12 - Steady state kinetic analysis of Legionella pneumophila Cu+ transport ATPase. The activation by Cu+ and ATP

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P-type ATPases are a family of membrane proteins which couple ATP hydrolysis to the transport of substrates across biological membranes. Within them, Cu+-ATPases are the most widespread and conserved heavy metal ion transporting ATPases (PIB-ATPases). Its reaction cycle is assumed to be described by the so-called Albers-Post model postulated for the most studied P-ATPases such as the Na+,K+-ATPase or the Ca2+-ATPases. However, as some structural and functional particularities arise for Cu+-ATPases, several authors posit some doubts about their reaction cycle mechanism. The aim of our work is to perform a functional characterization of Legionella pneumophila Cu^{+}-ATPase (LpCopA) by measuring steady state ATPase activity. Cu+-ATPase activity of the enzyme presents a maximum at ∼37ºC and pH 6.6-6.8. Phospholipids enhance LpCopA Cu+-ATPase activity in a non-essential mode where optimal activity is achieved at an asolectin mole fraction of 0.15 and an amphiphile-protein ratio of ~30000. As described for other P-ATPases, Mg2+ acts as an essential activator. When evaluating the role of ATP and Cu+ in the reaction cycle of LpCopA we observed that ATPase activity increases as Cu+ concentration increases with a functional dependence that can be described by a sum of two hyperboles. On the other hand, the increment on ATP concentration in the reaction media produces an increment of ATPase activity that can be described by a hyperbola plus a constant value. Based on that, and the [Cu+] and [ATP] dependencies of the best fitting parameters of the functions pointed above, we propose a minimal reaction scheme for LpCopA catalytic mechanism that contemplates two enzyme conformations with different affinities for ATP, enzyme phosphorylation and binding of at least two Cu+ ions with different affinities. This model is compatible with the structural information available and the main characteristics of the reaction cycle models for the most characterized P-Type ATPases.
Keywords: Cu(I) transport ATPase, Kinetic mechanism, reaction cycle
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