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Canonical and noncanonical transducers for calcium signaling: role of Na,K-ATPase and angiotensin receptor

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ABSTRACT

Calcium (Ca^{2+}) is the most universal and versatile signal in the cell. This versatility is based on the speed, amplitude and spatio-temporal patterning of the Ca^{2+} events. The inositol 1,4,5-trisphosphate receptor (IP_3R) is one of the main Ca^{2+} channels responsible for the Ca^{2+} release from the intracellular stores. In a response to various stimuli, IP_3R is able to generate variety of Ca^{2+} signals, ranging from Ca^{2+} transients to Ca^{2+} oscillations of different frequencies.

In this thesis we studied regulation of canonical and non-canonical signaling pathways that activate IP_3R-mediated Ca^{2+} signaling. The canonical pathway is represented in this thesis by angiotensin II type 1 receptor (AT1R) $G\alpha q_{/11}$ protein-coupled signaling, which triggers IP_3R-mediated Ca^{2+} signals by stimulation of IP_3 production. The non-canonical pathway is represented by Na,K-ATPase, which activates IP_3R independently on the presence of IP_3 through allosteric effect.

AT1R, together with dopamine D1-like receptor (D1R), represent a counter-regulatory system that controls sodium uptake in renal proximal tubules. We have shown that AT1R and D1R form a heterodimer. We have demonstrated that the stimulation of either of the receptors induce heterologous desensitization of the other receptor. Activation of D1R resulted in rapid and reversible uncoupling of AT1R from its G protein-coupled signaling pathway followed by internalization of the receptor and *vice versa;* stimulation of AT1R abolished D1R mediated cAMP production and triggered D1R internalization.

Na,K-ATPase, in addition to its function as an ion pump, serves also as a signal transducer. Ouabain, a highly specific Na,K-ATPase ligand, was shown to trigger slow Ca^{2+} oscillations through the Na,K-ATPase/IP_3R signaling complex. We have described that the cytoskeleton associated protein, ankyrin B, stabilizes the Na,K-ATPase and IP_3R interaction. Down-regulation of ankyrin B in COS7 cells using siRNA, resulted in reduction and dysregulation of ouabain-triggered Ca^{2+} oscillations, and abolishment of NF-kB activation.

In 2006, Hilgenberg et al. (Cell 2006 Apr 125:359) reported that 20-kD C-terminal fragment of agrin (agrin C20) is a new ligand of Na,K-ATPase α3 that inhibits its pumping activity. The original purpose of our study was to examine whether agrin C20 has also capacity to induce signaling function of Na,K-ATPase α3. We have shown that the solubilized agrin C20, which we received from the authors of the Cell paper, is not selective for Na,K-ATPase α3, but has a capacity to trigger slow Ca^{2+} oscillations in COS7 cells *via* Na,K-ATPase α1 and also to inhibit the pumping activity of the Na,K-ATPase α1. These effects were dependent on the intact ouabain binding site. Solubilized agrin C20 was also found to trigger slow Ca^{2+} oscillations superimposed on the spontaneous fast frequency Ca^{2+} oscillations in rat primary hippocampal neurons. The naturally occurring 22-kD C-terminal fragment of agrin did not trigger Ca^{2+} signal in COS7 cells. Mass-spectrometry analysis revealed presence of 5-7 mM ouabain in the solubilized agrin C20 sample. Ouabain-free agrin C20 was not found to have any effect on the Na,K-ATPase activity in the mouse brain lysate.

In conclusion we have described new mechanisms regulating canonical and non-canonical activators of IP_3R Ca^{2+} signaling through protein-protein interaction and allosteric modulation.

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