1692-Pos

TRP'Ing on QPatch in Multi-Hole Mode

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Transient receptor potential (TRP) channels are non-selective cationic channels that are widely distributed in mammalian tissues. Their specific physiological functions are largely unknown. Proposed functions include responses to pain, temperature, touch, osmolarity, pheromones, and taste. But due to the lack of specific blockers and the full understanding of their mechanisms of activation studies of TRP channels have been difficult and unexpectedly slow.

The emergence of automated patch clamp (APC) systems has increased the number of new targets available for ion channel drug development and has augmented throughput.

In order to facilitate tests of large compounds libraries on e.g. TRP targets, we have recently developed two multi-hole APC systems: QPatch HTX and QPatch 16X. The multi-hole technology allows the simultaneous recording of 10 cells in parallel per recording site thereby increasing the signal to noise ratio and the success rate.

In this study, we have validated several TRP channels for their activation by their appropriate agonist e.g. Menthol, Capsaicin and temperature. We have found that using the QPatch in multi-hole mode significantly increased the volume of electrophysiology data that can be generated. Our results demonstrate that the QPatch multi-hole systems are capable of generating high quality data from a wide range of the channels belonging to the TRP family of receptors.

We believe that the combination of high throughput and high quality data in a single system has more than a ''transient potential'' to advance the understanding of the complex mechanisms of action exhibited by difficult targets such as the TRP channels.

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TRPV1 Modulates Acetylcholine Release From Motor Nerve Terminals

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Transient receptor potential (TRP) proteins are expressed ubiquitously throughout the body. Previous studies demonstrated expression of TRPV1, the capsaicin receptor, in sensory neurons. Recently, we reported TRPV1 expression in mouse motor nerve endings (MNEs; J. Pharmacol. Exp. Ther. Aug 04. 2009) where we observed that capsaicin protected MNEs from botulinum neurotoxin A (BoNT/A). We hypothesized that capsaicin reduced clathrin coated pit (CCP) dependent endocytosis of BoNT/A and demonstrate the regulatory influence of TRPV1 in exo-endocytic processes of MNEs. Phrenic nerve diaphragm muscle preparations isolated from isoflurane anesthetized adult mice were analyzed for the nerve-evoked twitch and transmitter release (TR). Capsaicin produced a concentration-dependent decline of twitch tension (TT), an effect attributed to suppression of stimulus-evoked acetylcholine release (SEAR) since these capsaisin concentrations reduced the amplitude of endplate currents. These effects of capsaicin were antagonized by capsazepine, the TRPV1 antagonist. To understand the mechanism whereby capsaicin reduced TR, we studied cholinergic Neuro 2a cells. Acute exposure to capsaicin altered the subcellular distribution of clathrin heavy chain (CHc) and AP2, two proteins essential to CCP formation. Wortmannin, (non selective PI3K/PI5K inhibitor), inhibited the TT and SEAR of the isolated nerve-muscle preparations and delocalized CHc and AP2 in Neuro 2a cells. Chlorpromazine, an inhibitor of CCP dependent endocytic pathway [Cell Mol. Biol. Lett. 2004; 9 (3): 475-81], mimicked the effects of capsaicin on AP2 delocalization. These data suggest that endogenous TRPV1 proteins are coupled to the exo-endocytic mechanisms that regulate neuromuscular transmission and that activation of TRPV1 with high capsaicin concentrations reduces exocytosis of acetylcholine by down regulating the compensatory CCP dependent endocytic pathways.

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Direct and Indirect Effectors of the TRPM2 Cation Channel

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TRPM2 is a Ca^{2+} permeable cation channel which plays a role in physiological and pathophysiological processes linked to oxidative stress. TRPM2 channels are co-activated by intracellular Ca^{2+} and ADP ribose (ADPR). In addition, in intact cells, a large number of compounds appear to modulate TRPM2 activity. Superfusion of TRPM2-expressing cells with hydrogen-peroxide (H_2O_2) activates TRPM2 currents, just as intracellular dialysis of cyclic ADPR (cADPR) or nicotinic acid adenine dinucleotide phosphate (NAADP). Importantly, H_2O_2, cADPR, and NAADP enhance ADPR-induced TRPM2 whole-cell currents. Finally, in intact cells AMP acts as a TRPM2 inhibitor. Because in whole-cell recordings the entire cellular machinary involved in nucleotide- and Ca^{2+}-homeostasis is in place, compounds might affect TRPM2 activity either directly, by binding to the TRPM2 protein, or indirectly, by altering the local concentrations of the primary ligands ADPR and Ca^{2+}. To identify direct modulators of TRPM2 activity, we have studied the effects of H_2O_2, AMP, cADPR, NAADP, and nicotinic acid adenine dinucleotide (NAAD) in inside-out patches excised from *Xenopus* oocytes expressing human TRPM2, by directly exposing the cytosolic faces of the patches to these compounds. H_2O_2 (1 mM) and enzymatically purified cADPR (10 μM) failed to activate, while AMP (200 μM) failed to inhibit TRPM2 currents. NAADP acted as a partial agonist (maximal efficacy ~50%) while NAAD was a full agonist, but both with low affinities ($K_{0.5}$=104 and 35 μM). Neither of H_2O_2, cADPR, and NAADP enhanced activation by ADPR. Thus, in a physiological context the above compounds do not directly affect the TRPM2 channel protein. [OTKA grant F68143]

1695-Pos

Trpc3-Mediated Electrical Remodeling of Cardiac Myocytes

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Recent evidence suggests a key role of transient receptor potential (TRP) channels in cardiac pathophysiology with TRPC3 as one potential key player in cardiac remodeling. TRPC3 is typically up-regulated by hypertrophic stimuli and may be involved in distorted Ca2+ signaling that drives pathological remodeling. As TRPC proteins generate non-selective cation conductances, we hypothesized that these channels may not only govern Ca2+-mediated gene expression but exert in addition a severe impact on basic electrical properties and excitability of the myocardium. Utilizing the patch clamp technique we characterized membrane currents and electrical properties of cardiomyocytes in response to enhanced TRPC3 expression in the murine HL-1 model. Stimulation of TRPC3-overexpressing HL-1 cells with endothelin-1 [100 nM] (ET-1) as a Gqi-PLC-activator gave rise to a conductance with features distinctly different from the properties described for TRPC3 conductance in expression systems. In HL-1 cells, the TRPC3 over-expression-induced conductance in physiological solutions reversed at about −50 mV, displayed profound outward rectification and was suppressed by the TRPC3-inhibitor Pyr3 [10 μM] (ethyl-1-(4-(2,3,3-trichloroacrylamide)phenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylate). As a result of this conductance, action potential duration was effectively shortened by ET-1 in HL-1 myocytes over-expressing the TRPC3, while this effect was minute in wild-type myocytes. Moreover, TRPC3 over-expression enabled significant depolarizing effects of ET-1 along with action potential shortening and reduction of refractory period. Our results suggests that increased expression of TRPC3 in cardiomyocytes may significantly contribute to electrical remodeling in hypertrophic hearts, generating changes in action potential morphology that are likely to promote arrhythmias.

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Critical Role of Pertussis Toxin Sensitive G Proteins in the Activation of TRPC4 and TRPC5 Channels

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Canonical transient receptor potential 4 and 5 (TRPC 4 and TRPC5) are non-selective cation channels. Their activation causes membrane depolarization and intracellular Ca^{2+} increases. TRPC4 has been implicated in neurotransmitter release, endothelial-dependent regulation of vascular tone, endothelial permeability, and excitation-contraction coupling of intestinal smooth muscles while TRPC5 has been shown to be important for neurite extension and growth cone morphology and behavior responses to fear conditioning. The activation mechanisms of TRPC4/C5 remain unresolved. Most studies have indicated that stimulation of phospholipase C activates TRPC4/C5 channels. Using whole-cell patch clamp recording and fluorescence membrane potential measurements, we show here that $G_{q/11}$ signaling pathway alone is insufficient for the full activation of TRPC4/C5. Channel activities are greatly enhanced with co-stimulation of $G_{i/o}$-coupled receptors, including μ opioid, $5\text{-}HT_{1A}$ serotonin, M2 muscarinic, and D2 dopamine receptors. Stimulation of the $G_{i/o}$-coupled receptors alone also activates TRPC4/C5 in a pertussis toxin-sensitive manner. We further show that the effect of $G_{i/o}$ proteins cannot be attributed to the stimulation of phospholipase C-β through Gβγ subunits as activation of the $G_{i/o}$-coupled receptors induced no detectable intracellular Ca^{2+} signal unless TRPC4/C5 are co-expressed. In addition, the activated form of $G\alpha_{i/o}$ rather than Gβγ appears to be involved in the TRPC4/C5 activation. A