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Comparison of functional properties of the Ca^{2+}-activated cation channels TRPM4 and TRPM5 from mice

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Summary

Non-selective cation (NSC) channels activated by intracellular Ca^{2+} ($[Ca^{2+}]_i$) play an important role in Ca^{2+} signaling and membrane excitability in many cell types. TRPM4 and TRPM5, two Ca^{2+}-activated cation channels of the TRP superfamily, are potential molecular correlates of NSC channels. We compared the functional properties of mouse TRPM4 and TRPM5 heterologously expressed in HEK 293 cells. Dialyzing cells with different Ca^{2+} concentrations revealed a difference in Ca^{2+} sensitivity between TRPM4 and TRPM5, with EC_{50} values of 20.2 ± 4.0 μM and 0.70 ± 0.1 μM, respectively. Similarly, TRPM5 activated at lower Ca^{2+} concentration than TRPM4 when $[Ca^{2+}]_i$ was raised by UV uncaging of the Ca^{2+}-cage DMNP-EDTA. Current amplitudes of TRPM4 and TRPM5 were not correlated to the rate of changes in $[Ca^{2+}]_i$. The Ca^{2+} sensitivity of both channels was strongly reduced in inside-out patches, resulting in approximately 10–30 times higher EC_{50} values than under whole-cell conditions. Currents through TRPM4 and TRPM5 deactivated at negative and activated at positive potentials with similar kinetics. Both channels were equally sensitive to block by intracellular spermine. TRPM4 displayed a 10-fold higher affinity for block by flufenamic acid. Importantly, ATP^{4-} blocked TRPM4 with high affinity (IC_{50} of 0.8 ± 0.1 μM), whereas TRPM5 is insensitive to ATP^{4-} at concentrations up to 1 mM.

Keywords: Non-selective cation channels; TRPM; Mouse; Activation by Ca^{2+}; ATP block; Polyamines; Flufenamic acid

1. Introduction

TRPM4 and TRPM5 are two closely related members of the melastatin-related subfamily of TRP ion channels [1–4]. The homology between TRPM4 and TRPM5 within the same species is approximately 50%, whereas the human and mouse orthologues for both channels show a homology of only approximately 80%. TRPM4 is expressed in a wide range of tissues, with notably high expression levels in heart, pancreas and placenta [5,6]. TRPM5 has been identified in taste receptor cells, where it participates in the signal transduction of the sweet, bitter and umami taste [3,7,8]. It has further been detected in various other tissues of the digestive system (small intestine, pancreas, liver) as well as in lungs, testis and brain [3,7,9].

TRPM4 and TRPM5 exhibit two salient features that are unique within the TRP superfamily. First, they represent the only known TRP channels that are directly gated by increases in intracellular Ca^{2+} ($[Ca^{2+}]_i$). Second, both TRPM4 and TRPM5 are impermeable to Ca^{2+}. This contrasts to all other functionally expressed TRPs, which form either Ca^{2+}-permeable NSC channels or even highly Ca^{2+}-selective channels. Both channels are permeable for monovalent cations with a conductance of approximately 20–25 pS [5,6,10].

Although it is generally agreed that TRPM4 is activated by Ca^{2+}, the exact concentration range as well as the kinetic properties are still a matter of debate [5,6,11]. The mechanism of TRPM5 activation is more controversial. It has been reported that TRPM5 is activated in a store-operated man-

Abbreviations: TRPM, transient receptor potential channels, melastatin subfamily

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ner [7], via a G protein-dependent mechanism independently of an elevation of $[Ca^{2+}]_i$ [8], by elevation of $[Ca^{2+}]_i$ [10] and in response to rapid changes in $[Ca^{2+}]_i$ [9]. Interestingly, both channels show remarkable voltage sensitivity. They activate at positive and deactivate at negative potentials, and are characterized by a Boltzmann type voltage dependence of the open probability [5,10]. Very little is known about the pharmacological modulation of these channels. TRPM4 has been shown to be blocked by intracellular nucleotides and polyamines [11], whereas TRPM5 seems to be regulated by PIP_2 [12].

In several tissues Ca^{2+}-activated, Ca^{2+}-impermeable NSC currents have been characterized that share functional properties with expressed TRPM4 or TRPM5 channels. Notably, in cardiomyocytes Ca^{2+}-activated NSC channels have been found whose single channel conductance and ATP-sensitivity are comparable to that of TRPM4 [13–19]. Furthermore, NSC channels with properties similar to TRPM4/5 have been described in excitable cells including neurons from entorhinal cortex [20,21], dorsal root and vomeronasal neurons [22], neuroblastoma cells and astrocytes [23,24], vascular and myometrial smooth muscle [25–27] and endocrine pancreas cells [28], but also in non-excitable cells such as red blood cells [29,30], exocrine cells from the pancreas [31–33], brown adipocytes [34,35], kidney epithelial cells [36], cochlear hair cells [37] and vascular endothelial cells [38,39]. However, the identity of these NSC channels with TRPM4 or TRPM5 has not yet been proven.

As for most if not for all of these native channels, TRPM4 or TRPM5 are potential molecular candidates, and both channels may have a broad physiological impact on various cell functions. However, both channels have not yet been sufficiently described with respect to their biophysical properties, modes of regulation and pharmacology. In addition, some of the published properties appear contradictory, especially concerning the mode of activation for TRPM5, the concentration range of activating Ca^{2+}, the de-sensitization properties and modulation [3,7–10,12]. The aim of this study was therefore to directly compare functional properties of mouse TRPM4 and TRPM5 heterologously expressed in HEK 293 cells. TRPM4 has been characterized so far only from the human but not from mouse clone. We will report on differences in Ca^{2+} sensitivity, gating behavior and pharmacology between mTRPM4 and mTRPM5. Our results form a basis for further functional investigations of both channels, and may be instrumental for the molecular identification of NSC in native tissues.

2. Material and methods

2.1. Cell culture

HEK293 cells and 3T3 mouse embryonic fibroblasts were grown in DMEM containing 10% (v/v) fetal calf serum, 4 mM L-alanyl-L-glutamine, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin at 37 °C in a humidity controlled incubator with 10% CO_2.

2.2. Transient expression of mTRPM4 and mTRPM5

We used the recombinant bicistronic expression plasmid pdiTRPM4/5, which carried the entire protein-coding region for the mouse TRPM4 (accession number AJ575814) or mouse TRPM5 (accession number AY280364) and for the green fluorescent protein (GFP) coupled by an internal ribosomal entry site (IRES) sequence. HEK293 and 3T3 cells were transiently transfected with the pdiTRPM4/5 vector using previously described methods [40], and successfully transfected cells were visually identified by their green fluorescence in the patch-clamp set-up.

2.3. Solutions

The extracellular solution for whole-cell measurements contained: 156 mM NaCl, 6 mM CsCl, 1.5 mM $CaCl_2$, 1 mM MgCl, 10 mM glucose, 10 mM HEPES, buffered at pH 7.4 with NaOH. The pipette solution for whole-cell patch clamp experiments contained: 20 mM CsCl, 100 mM CsAsp, 1 mM $MgCl_2$, 4 mM Na_2ATP, 10 mM HEPES, pH 7.2 with CsOH. The external solution in inside-out patch clamp experiments consisted of: 156 mM NaCl, 1.5 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, pH 7.4 with NaOH. The bath solution in inside-out experiments contained: 150 mM NaCl, 1 mM $MgCl_2$, 10 mM HEPES, pH 7.3 with NaOH. In both whole-cell and inside-out studies, the Ca^{2+} concentration at the inner side of the membrane was adjusted between 100 nM and 100 μM by adding appropriate amounts of $CaCl_2$ to 10 mM EGTA or EDTA calculated by the CaBuf program ftp://ftp.cc.kuleuven.ac.be/pub/droogmans/cabuf.zip. For Ca^{2+} concentrations between 100 μM and 1 mM, $CaCl_2$ was added to an EGTA-free solution. In inside-out studies, all internal solutions were ATP-free. All experiments were performed at room temperature (22–25 °C).

2.4. Electrophysiology

Whole-cell membrane currents were monitored with an EPC-7 or EPC-9 patch-clamp amplifier (HEKA Elektronik, Lambrecht, Germany) using ruptured patches. Patch electrodes had a DC resistance between 2 and 4 MΩ. An Ag–AgCl wire was used as a reference electrode. Capacitance and access resistance were monitored continuously. Between 50 and 70% of the series resistance was compensated electronically to minimize voltage errors. Data were sampled at 2–5 kHz and filtered at 1–2 kHz.

2.5. Photolytic release of Ca^{2+} and measurement of $[Ca^{2+}]_i$

For photolytic release of Ca^{2+} in whole-cell experiments, caged Ca^{2+}, 1-(4,5-dimethoxy-2-nitrophenyl)-EDTA