氏　　名	沼田　朋大
学位（専攻分野）	博士（理学）
学位記番号	総研大甲第968号
学位授与の日付	平成１８年３月２４日
学位授与の要件	生命科学研究科　生理科学専攻 学位規則第６条第１項該当
学位論文題目	TRPM7 is mechanosensitive volume-regulatory cation channel in human epithelial cells
論文審査委員	主　査　教授　岡村　康司 教授　岡田　泰伸 教授　富永　真琴 教授　森　泰生（京都大学）

論文内容の要旨

TRPM7 is mechanosensitive volume-regulatory cation channel in human epithelial cells

Stretch-activated cation (SAC) channels play an essential role in sensing and transducing external mechanical stresses and in cell volume regulation in living cel ls. However, the molecular identity of the mechanosensing channel which is involved in cell volume regulation in mammalian cells has not yet been firmly established. Since members of the transient receptor potential (TRP) channel family are known to be involved in sensory signal transduction for a large variety of stimuli, some member of the TRP family might also be a good candidate for the SAC channel in vertebrates. In the present study, in human embryonic kidney (HEK293T) epithelial cells transfected with the member 7 of human transient receptor potential cation channel subfamily M (TRPM7), constitutively active outward-rectified whole-cell cation currents were first studied by ramp-clamp and step-pulse clamp. The whole -cell currents were augmented, and their outwardly rectifying current-voltage relationship was converted to more ohmic one not only by mechanical stress caused by perfusion of bath solution (flow rate: 15 μl/s or 35 μl/s), but also by osmotic swelling induced by intracellular hypertonicity (by 50 mosmol kg-H_2O^{-1}). The mechano-stress-activated whole-cell current was sensitive to extracellular or intracellular Mg^{2+}, or to extracellular Gd^{3+}, SKF 96365 or ruthenium red (RR). The sequence of relative cation conductance estimated from the slope co nductance at −100 mV was $Cs^+ > Na^+ > Li^+ > Mg^{2+} > Ca^{2+}$. The same sequence of cation permeability was obtained from the reversal potential, when step pulses were applied from a pre-potential of −100 mV; the relative cation permeability coefficients were est imated to be $Cs^+ : Na^+ : Li^+ : Mg^{2+} : Ca^{2+} = 1 : 0.58 : 0.52 : 0.45 : 0.29$. Single-channel recordings revealed expression of the non-selective cation channel activity which is exquisitely sensitive to membrane stretch, requiring a negative pressure of only 4 cm-H_2O for half-maximum activation and also is sensitive to voltage with a half-maximum activation voltage of 14.7 mV. The SAC channel in HEK293T/TRPM7 cells showed a linear current-voltage relationship with a slope conductance of 26 pS.

Next, he studied the SAC channel expressed endogenously in human epithelial HeLa cells. He detected similar single SAC channel activity with a half-maximum activation pressure of 3 cm-H_2O and a half-maximum activation voltage of −2.7 mV. The SAC channel showed a linear current-voltage relationship with a slope conductance of 23 pS. The amplitude of single SAC channel current was suppressed by extracellular or intracellular Mg^{2+} or by extracellular Gd^{3+}, SKF 96365 or RR. Whole-cell recordings revealed cation currents exhibiting an outward-rectified current-voltage relationship in normal conditions. However, osmotic swelling was found to augment and convert the current-voltage relationship to more ohmic one. The swelling-activated ohmic whole-cell current was sensitive to extracellular or intracellular Mg^{2+} or to extracellular Gd^{3+}, SKF 96365 or RR. Both whole-cell cation currents activated by cell swelling and single SAC currents in HeLa cells were

sensitive to extracellular or intracellular Mg^{2+}. From the concentration-inhibition curves for blocking of single-channel current by Mg^{2+}, the half-maximum inhibition concentrations were 3.2 μM at −40 mV and 0.11 mM at +80 mV for extracellular Mg^{2+} and 0.65 mM at −40 mV and 0.66 mM at +80 mV for intracellular Mg^{2+}.

He then conducted reverse transcriptase polymerase chain reaction (RT-PCR) studies to examine expression of TRP family members in HeLa cells. The members tested were TRPC1-7, TRPM1-8, TRPV1-6, TRPP1 and TRPA1. Robust amplification of PCR products of expected size from reverse-transcribed RNA was seen only with two separate sets of TRPM7-specific primers, but no PCR products for other members were detected. To confirm the expression of the TRPM7 protein in HeLa cells, Western blotting of cell membrane proteins prepared fro m HeLa cells was performed using a polyclonal anti-TRPM7 antibody. The major immunoreactive band detected had a molecular mass of around 160 kDa. Since robust expression of TRPM7 in HeLa cells was confirmed by RT-PCR and Western blotting, gene silencing experiments were performed by using small interfering RNA (siRNA) to clarify whether the molecular identity of endogenously expressed SAC channel is TRPM7 or not. Treatment with siRNA targeted against TRPM7 led to abolition of mRNA, TRPM7 protein, single SAC channel events and swelling-induced activation of whole-cell cation currents in HeLa cells.

Since a SAC-channel activated by osmotic swelling is reported to serve as the volume-regulatory Ca^{2+} influx pathway during the regulatory volume decrease (RVD) in epithelial cells, he tested a possible involvement of the TRPM7 channel in RVD by measuring the cell volume in HeLa cells. Removal of extracellular Ca^{2+} or extracellular application of Gd^{3+}, SKF 96365 or RR suppressed the rate of cell volume recovery after osmotic swelling in HeLa cells. Similar suppression of the RVD was also observed, when the silencing of TRPM7 by siRNA was made in HeLa cells. H e thus conclude that TRPM7 is the stretch-activated cation channel endogenously expressed in human epithelial HeLa cells and, by serving as a swelling-induced Ca^{2+} influx pathway, plays an important role in cell volume regulation.

Although endogenously expressed TRPM7 in HeLa cells were activated by mechanical stimuli, he also found that the TRPM7 channel current was sensitive to extracellular osmolarity. The outward, but not inward, TRPM7 single -channel currents were augmented by extracellular, but not intracellular, hypertonicity. Similar sensitivity to extracellular osmolarity was observed in single SAC channel currents in HEK293T/TRPM7 cells, but not in HEK293T cells transfected with TRPV4. Thus, it is concluded that TRPM7 represents not only a membrane stretch-activated cation channel but also an osmosensitive cation channel.

論文の審査結果の要旨

機械受容チャネルは細胞の機械刺激応答や細胞容積の調節に重要であるが、哺乳類細胞の容積調節を担う機械受容チャネルの分子実体はこれまで明確でなかった。細胞容積の調節機構として、低張液処理後に細胞容積が増大した後に減少する、regulatory volume decrease (RVD) と呼ばれる現象が知られており、細胞外からの Ca イオン流入が引き起こされることが重要な経路であるが、その分子実体については未だ不明な点が多かった。本研究では多くの生物種で機械刺激受容能があることが示されてきた TRP チャネルのうち TPRM7 に注目し、まず発現系細胞のパッチクランプ法により機械刺激受容能とその基本的な電気生理学的性質を明らかにした。TRPM7 を発現させた細胞では、容積変化と機械的刺激の両方に対して外向き整流性から線形への電圧依存性の特徴的な変化が起こることを見出した。またこの電流が細胞外 Mg イオンに対して強い感受性をもつことを明らかにするとともに、単一チャネル電流記録を行って約 23pS (ピコシーメンス) のコンダクタンスをもつ機械刺激受容チャネルであることなども明らかにした。次に HeLa 細胞に内在的に発現する TPRM7 チャネルについてもチャネル特性を解析し、HEK 細胞への強制発現系での結果と同様に、浸透圧による容積増大により電流電圧関係の整流性が大きく変化すること、細胞外 Mg イオンに高い感受性を示すことなどを明らかにした。RT-PCR により HeLa 細胞での容積感受性チャネルの候補は TRPM7 に限定されることが示唆され、実際 siRNA による遺伝子ノックダウン実験で TRPM7 の蛋白の発現を減少させると機械刺激受容チャネルの活性と細胞の膨張による活性化が阻害されることを確認した。さらに regulatory volume decrease (RVD) における TRPM7 の役割を解析し、細胞外 Ca を減少させたりガドリニウムイオンの投与などにより TRPM7 チャネル活性を抑制すると RVD が抑制された。更に TRPM7 の siRNA 導入により TRPM7 蛋白を減少させると、RVD は有意に抑制され、TRPM7 を介する経路が RVD の容積調節に重要な働きを担うことを見出した。

本研究結果は、電気生理学的手法と分子生物学的手法を駆使して、これまで明確でなかった容積感受性チャネルを新たに同定するとともに細胞容積の制御という生理機構における役割へ発展させた優れた研究であり、論文は学位取得に十分値すると判断された。