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Stress-Axis Regulated Exon (STREX) in the C terminus of BK_{Ca} channels is responsible for the stretch sensitivity

Keiji Naruse [a,*], Qiong-Yao Tang [b], Masahiro Sokabe [b,c,d]

[a] Department of Cardiovascular Physiology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan
[b] Department of Physiology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan
[c] ICORP Cell-Mechanosensing, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan
[d] Department of Molecular Physiology, National Institute for Physiological Sciences, Myodaiji-cho, Okazaki 444-8585, Japan

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ABSTRACT

We previously reported that SAK_{CA}, a stretch-activated, large-conductance, calcium- and voltage-activated potassium (BK_{Ca}) channel is present in chick embryonic heart. Here, we cloned SAK_{CA} and identified that Stress-Axis Regulated Exon (STREX) is responsible for the stretch sensitivity. Single patch–clamp recordings from CHO cells transfected with the cloned SAK_{CA} showed stretch sensitivity, whereas deletion of the STREX insert diminished the stretch sensitivity of the channel. Sequence analysis revealed that the $ERA_{672–674}$ sequence of the STREX is indispensable for channel stretch sensitivity and single amino acid substitution from Ala674 to Thr674 completely eliminated the stretch sensitivity. Co-expression of chick STREX-EGFP and SAK_{CA} in CHO cells, induced a strong GFP signal in the cell membrane and inhibited the stretch sensitivity significantly. These results suggest that SAK_{CA} senses membrane tension through an interaction between STREX and submembranous components.

Introduction

SA channels have been found in a variety of cell types from bacteria to human and are thought to play a crucial role in mechanosensory system, auditory system, and cell volume regulation [1–4]. Molecular structures of several SA channels, including bacterial MscL and MscS [5,6], eukaryotic TREK/TRAAK channels [7], and Mid1 [8,9] have been reported. However, the molecular and biophysical mechanisms of mechanogating of SA channels remain largely unknown due to a lack of knowledge about the domains or sites responsible for sensing membrane tension. Recently a candidate site in the bacterial SA channel MscL [10] was reported. Considering the physiological significance, it is important to examine the mechanosensing mechanism of eukaryotic SA channels in which the function–structure relationship of channel gating is well studied.

We previously reported that one of five SA channels in chick embryonic cardiomyocytes is a type of the BK_{Ca} channel, thereby we referred to as stretch-activated BK_{Ca} (SAK_{CA}) channel [11]. BK_{Ca} channels play a central role in the regulation of cellular excitability because of its activation by both voltage and intracellular free Ca^{2+}. In addition, the structural basis of their phenotypic variation has been studied extensively [12,13]. KCNMA1, a gene encoding the pore-forming α-subunits of BK channels [12], is shown to have extensive alternative exon splicing. If the SAK_{CA} channel is encoded by one of the alternative exon splicings of the KCNMA1 gene, we thought it could be a very useful model to get insights into the stretch sensitive domain, by comparing molecular structures of BK_{Ca} channels with or without stretch sensitivity.

In the present study, we cloned the channel using degenerate primers against BK_{Ca} channel from the chick embryonic heart cDNA library. We found out that it turned out to be one of BK_{Ca} variants including STREX [13] and that the STREX insertion is responsible for the stretch sensitivity. The electrophysiological characterization of SAK_{CA} channel expressed exogenously in CHO cells has been reported elsewhere [14].

Materials and methods

Cloning and mutagenesis. cDNA library was made from 10 to 12D chick embryonic heart (Superscript Choice system for cDNA synthesis, Gibco BRL) and ligated into λZAP II vector (Stratagene). PCR amplification was carried out with degenerate-sequence primers designed from S7 to S9 of BK channel from human brain (^{529}ELKLGFIA^{536} and ^{773}IGLRNLVM^{780}) [15]. A single band encoded a sequence homologous to the BK_{Ca} channel. Using this PCR product as a probe for plaque hybridization, approximately 10^8 plaques of a chick embryonic heart cDNA library were screened. One phage was posi-

* Corresponding author. Fax: +81 86 235 7430.
E-mail address: knaruse@md.okayama-u.ac.jp (K. Naruse).

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tively identified and the ~4 kbp insert from this clone was excised and was converted into a pBluescript II SK+ vector (Stratagene) by the ExAssist/SOLR system (Stratagene), as described in the manufacturer's protocol. For transient expression experiments, the cDNA of SAK_{CA} was subcloned into a mammalian expression vector (pTarget, Promega). Human BK_{Ca} channel containing STREX ($hSAK_{CA}$) was PCR-cloned from human heart cDNA using sense and antisense primers (5′-gcagcagtcttagaatgagt-3′ and 5′-gtggcagatacagtttcaca-3′) and DNA polymerase (35 cycles, denaturing at 94 °C (30 s), annealing at 55 °C (30 s) and extension at 68 °C (4 min). The PCR product was confirmed by sequencing. The accession numbers for chick and human SAK_{CA} are AB072618 and AB113382, respectively. Mouse BK_{Ca} is a generous gift from Dr. Genetzky, rabbit BK_{Ca} and its STREX variant are generous gifts from Dr. Shao Xiong and Dr. Guggino. The A672T mutant was generated with the Quick Change site-directed mutagenesis kit (Promega) and verified by sequencing.

Antibody and immunohistochemistry. A STREX-specific antibody (anti-STREX) was raised in rabbits against a synthetic oligopeptide corresponding to STREX (^{660}MSGSVHSNMDTLERAFPLSSVSVNDC^{685}), and affinity-purified. The hearts were removed from chick embryos and fixed in 4% paraformaldehyde in phosphate buffered saline (PBS) at 4 °C for overnight. After washing in PBS, the hearts were serially dehydrated in 25%, 50%, and 75% ethanol in PBS for 30 min, respectively, and 100% ethanol for each 1 h at room temperature. Next, the hearts were incubated in xylene for 1 h at room temperature, three times, and in paraffin each for 1 h at 65 °C three times. Finally, the hearts were embedded in paraffin. Immunohistochemistry of the hearts was performed on 4-μm paraffin-embedded sections by the indirect immunoperoxidase method.

Immunoblotting. The proteins were resolved by SDS–PAGE and transferred to polyvinylidene difluoride membranes. The membranes were blocked with 2% bovine serum albumin overnight at 4 °C, washed with phosphate-buffered saline with 0.05% Tween 20 (PBST), incubated with anti-STREX or anti-BK antibody (1:500 dilution, Chemicom Int., Inc.) for 60 min at room temperature, washed with PBST, incubated with peroxidase-conjugated goat anti-rabbit IgG (Sigma) at 1:2000 dilution for 60 min at room temperature, washed with PBST, and developed with ECL-Plus according to the manufacturer's instructions (Amersham Biosciences).

Electrophysiology. Patch–clamp experiments were performed with an Axopatch 200A amplifier (Axon Instruments) in the inside-out configuration. Currents were low-pass filtered at 5 kHz and sampled at 10 kHz. Recordings were made at 23–25 °C. Data were acquired and analyzed with the Digidata and Axoclamp ver. 8. Pipette resistances were 5–8 MΩ. The internal pipette solution for single-channel currents contained 145 mM KCl, 5 mM NaCl, 1 mM EGTA, 10 mM HEPES (pH 7.4) and the external solution contained 145 mM KCl, 5 NaCl, 118 μM $CaCl_2$, 1 mM EGTA, 10 mM

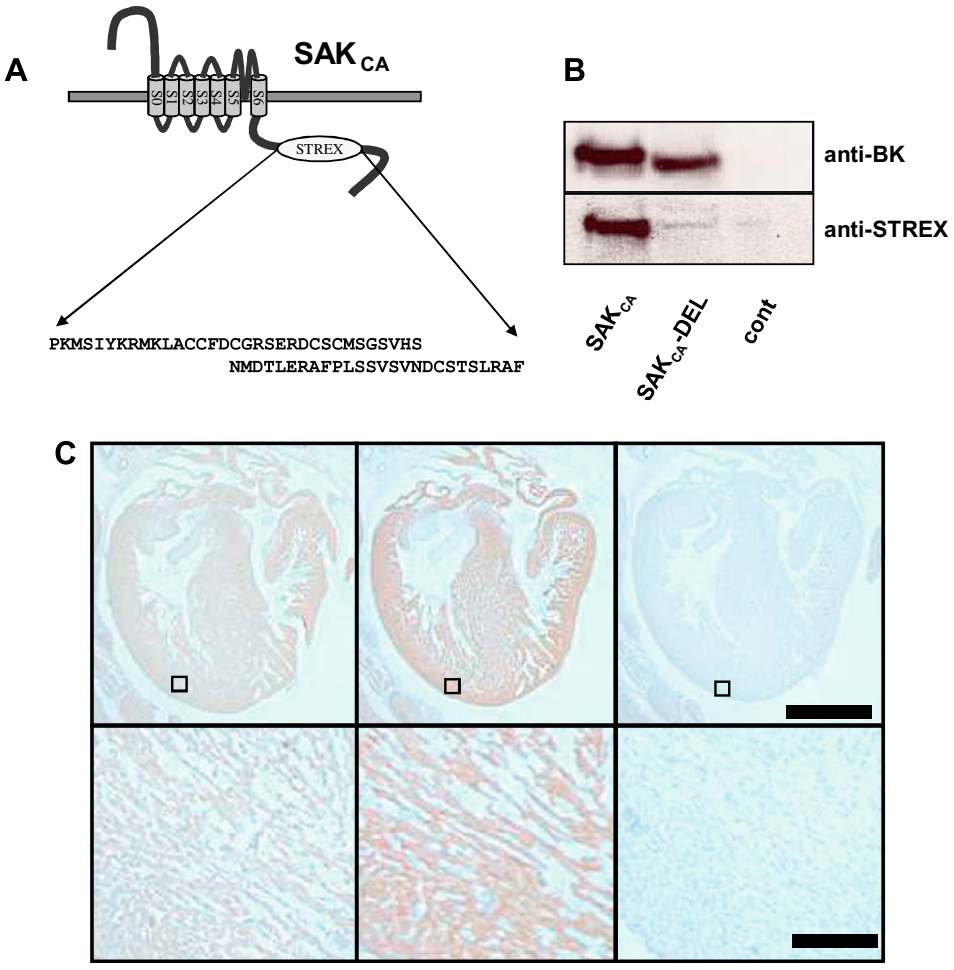

Fig. 1. SAK_{CA} and its expression. (A) Topology of the SAK_{CA} channel. An intracellular sequence contains STREX. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. (B) Expression of SAK_{CA} was assessed by transient transfection of SAK_{CA} (left lane), SAK_{CA}-Del (middle lane), and vector (right lane) into CHO-K1 cells by Western blotting with anti-BK (upper panel) and anti-STREX Ab (lower panel, affinity-purified rabbit antibody to a synthetic oligopeptide corresponding to STREX, respectively. (C) Immunostaining with anti-STREX (left), anti-cardiac actin (middle), and pre-immune serum (right) in chick embryonic heart. Lower panels: Higher magnification of insets in corresponding staining. A bar indicates 1 mm in upper panel, and 100 μm in lower, respectively.