Biochemical and Biophysical Research Communications 391 (2010) 974–978

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Reduced expression and abnormal localization of the K_{ATP} channel subunit SUR2A in patients with familial hypokalemic periodic paralysis

Sung-Jo Kim [a,1], Yea-Jin Lee [a,1], June-Bum Kim [b,*]

[a] Department of Biotechnology, Hoseo University, 165, Baebang-Myun, Asan, Chungnam, Republic of Korea
[b] Department of Pediatrics, Konyang University College of Medicine, 685 Gasoowon-Dong, Su-Goo, Daejun 302-718, Republic of Korea

ARTICLE INFO

Article history:
Received 22 November 2009
Available online 4 December 2009

Keywords:
Hypokalemic periodic paralysis
Depolarization
Potassium channel
Sulfonylurea receptor

ABSTRACT

Familial hypokalemic periodic paralysis is an autosomal-dominant channelopathy that features episodic attacks of flaccid paralysis with concomitant hypokalemia. Reduced activity of ATP-sensitive K^+ (K_{ATP}) channels is suggested to be responsible for this disorder; however, the molecular mechanisms have not yet been elucidated. In this study, we investigated the molecular mechanism of reduced K_{ATP} channel activity in skeletal muscle cells of patients with familial hypokalemic periodic paralysis. We examined the mRNA and protein levels of SUR2A, a K_{ATP} channel subunit, in cells from patients (patient cells) and normal individuals (normal cells). Our results demonstrated that normal cells exposed to 50 mM potassium buffer, which was used to induce depolarization, did not show significant change in the SUR2A mRNA levels; however, the protein level significantly increased in the cytosolic fraction. When the patient cells were exposed to 50 mM potassium buffer, the SUR2A mRNA level significantly decreased. Further, the protein level of SUR2A significantly increased in the membrane fraction but decreased in the cytosolic fraction in patient cells. These findings suggest that abnormal localization of the SUR2A K^+ channel protein leads to reduced K_{ATP} channel activity in familial hypokalemic periodic paralysis.

Introduction

Familial hypokalemic periodic paralysis (OMIM ID: 170400) is an autosomal dominant disorder that features reversible flaccid paralysis with intermittently developed hypokalemia. Although the initial onset of flaccid paralysis varies from infancy to puberty, it begins around puberty in many patients, following which weakness of the extremities or general paralysis occurs regularly or intermittently at night or early in the morning and lasts several hours to a few days with spontaneous recovery. Additionally, exposure to foods rich in sugar or salt, excessive exercise, cold, or emotional stress can initiate these symptoms [1,2]. Previous molecular genetic studies revealed that mutations in the L-type calcium channel gene *CACNA1S* (OMIM ID: 114208) and the sodium channel gene *SCN4A* (OMIM ID: 603967) of skeletal muscle cells are responsible for this disorder; however, its pathogenesis has not been clearly elucidated [2–4].

The adenosine triphosphate (ATP)-dependent potassium channel (K_{ATP}) is one of the various potassium channels involved in the movement of potassium through the cellular membrane. This channel is found in various organs and/or tissues such as the pancreas, brain, heart, skeletal muscles, and smooth muscles [5–7]. The K_{ATP} channel consists of Kir6.*x* subunits that form the pores and sulfonylurea receptor (SUR) subunits that control channel function [8,9]. Channel activity is controlled by the membrane potential and ligands. ATP and phosphatidylinositol 4,5-bisphosphate directly affect the Kir6.2 subunits, whereas sulfonylurea and Mg-nucleotides affect the SUR subunits. ATP inhibits channel activation through Kir6.2, and Mg-ATP and adenosine diphosphate (ADP) activate the channel through the SUR subunit. Therefore, the intracellular ATP/ADP concentration determines the activity of the K_{ATP} channel [10,11].

SURs are a type of ATP-binding cassette proteins. The SUR subtypes SUR1, SUR2A, and SUR2B are expressed in the pancreatic β cells, heart and skeletal muscles, and smooth muscles, respectively [8,12]. SUR1 increases the ATP in pancreatic β cells to block K_{ATP} channels, which in turn increases the intracellular potassium concentration and induces depolarization; thus, SUR1 plays an important role in the extracellular secretion of insulin [13,14]. Furthermore, it also influences the protection of cells by controlling the membrane potential [15–17].

Previous studies have suggested a relationship between the development of hypokalemic periodic paralysis and the decreased function of K_{ATP} channels located in the membrane of skeletal muscle cells [18,19]. A recent study revealed that normal rats exposed to hypokalemia showed decreased expression of the SUR2A protein, suggesting that familial hypokalemic periodic paralysis may have developed as a result of decreased K_{ATP} channel function

* Corresponding author. Fax: +82 42 600 8835.
E-mail address: hoppdoctor@hanmail.net (J.-B. Kim).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.11.177

[20]. However, as this was the response of genetically normal rats to hypokalemic status, this information cannot be used to suggest a direct relation to patients with genetic defects. In this study, we obtained skeletal muscle cells from patients with familial hypokalemic periodic paralysis (patient cells) and healthy volunteers (normal cells) and investigated the expression patterns of SUR2A mRNA and protein levels therein at normal (4 mM) and high (50 mM, for depolarization) potassium concentrations.

Materials and methods

Subjects. We reviewed 108 patients who were being treated for familial hypokalemic periodic paralysis in the Department of Pediatrics, Konyang University Hospital. Of these patients, we selected three who presented the most severe symptoms. These patients had the Arg1239Gly mutation in the *CACNA1S* gene. Further, three healthy volunteers participated in this study. All participants provided written informed consent, and the study was conducted in compliance with the Institutional Review Board of Konyang University Hospital.

Sampling skeletal muscle specimens. The subjects were asked to rest in the supine position on a bed. Skeletal muscle specimens were collected from the gastrocnemius muscles through surgical incision following local anesthesia with lidocaine. A portion of each collected specimen was fixed on a slide, dried at room temperature, and stained with hematoxylin–eosin.

Preparation of potassium buffer. Potassium buffer (4 mM) in pH 7.2 (4 mM KCl, 145 mM NaCl, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 5 mM glucose, and 10 mM 3-(*N*-morpholino) propanesulfonic acid (MOPS)) was prepared and used for cell exposure to normal physiological concentration of potassium. To induce depolarization of skeletal muscle cells under a high concentration of potassium, 50 mM potassium buffer (50 mM KCl, 145 mM NaCl, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 5 mM glucose, and 10 mM MOPS; pH 7.2) was prepared. The buffers were sterilized before use.

Culture of skeletal muscle cells and treatment with potassium buffer solution. Proliferation and differentiation of skeletal muscle cells were induced according to the method described previously [21]. Briefly, after pretreatment, skeletal muscle specimens collected from patients with familial hypokalemic periodic paralysis and healthy volunteers were cultured using Dulbecco's modified Eagle's medium (DMEM; Thermo Scientific) containing 20% fetal bovine serum (Thermo Scientific) and 1% penicillin–streptomycin (Thermo Scientific) at 37 °C in an incubator containing 95% air and 5% CO_2 (Thermo Scientific). Thereafter, skeletal muscle cells were cultured in DMEM with 2% horse serum (Thermo Scientific) and 1% penicillin–streptomycin and allowed to differentiate for 5 days. Both the normal and patient cells were collected at the 12th passage and used for the analysis after they were treated with the potassium buffers for 1 h.

Quantitative RT-PCR analysis. TriZol reagent (Invitrogen) was used to extract RNA from the skeletal muscle cells, and 100 ng of the total RNA was converted to cDNA using reverse transcriptase. Quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) was performed after the SUR2A primers were mixed with Accupower PCR PreMix (Bioneer). Sets were performed in triplicate. Experimental data were presented as means ± standard deviation, and statistical significance was evaluated by the Student's *t* test. A *P* value of less than 0.05 was considered statistically significant.

Western blot analysis. Skeletal muscle cells obtained from the patients and healthy volunteers were treated with the two different concentrations of potassium buffers for 1 h, and the cytosolic and membranous protein fractions were then separated. A modification of the cell separation method described previously was used for the separation of the cytosolic and membranous proteins [22,23], and a protease-inhibitor cocktail (Sigma) was added at each step to extract the proteins. From each specimen, 10 μg protein was electrophoresed on 12% sodium dodecyl sulfate–polyacrylamide gel (Bio-Rad), and then transferred to polyvinylidene fluoride membrane (Bio-Rad) for Western blot analysis. The protein-containing membrane was blocked using 5% skim milk (Bio-Rad) and then incubated with primary anti-SUR2A antibody (Abcam). Subsequently, anti-goat secondary antibody was incubated with the membrane, and the protein band was then visualized using Super-Signal West Pico Luminal/Enhancer solution (Pierce).

Results

This study was performed to investigate the changes in the expression pattern of SUR2A proteins on depolarization in skeletal muscle cells. These cells were then treated with potassium buffer at normal extracellular concentration (4 mM) and high concentration (50 mM) of potassium in order to induce depolarization, and the SUR2A mRNA expressions were analyzed prior to and 1 h following addition of buffer. The expression levels were compared, and Western blotting was carried out to evaluate the expressions of cytosolic and membranous SUR2A protein.

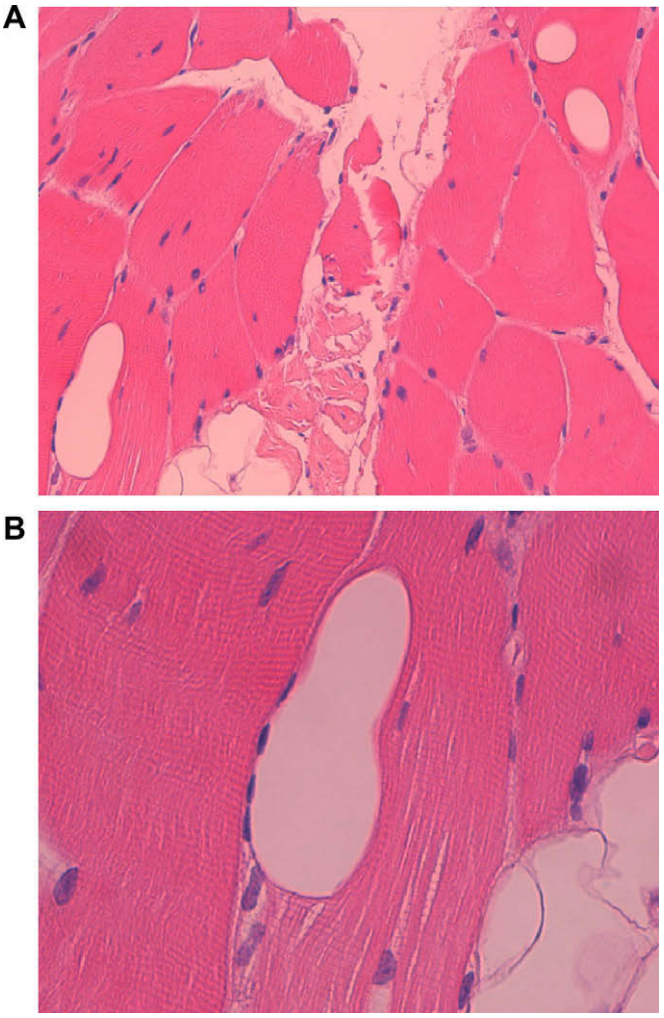

Fig. 1. Histopathological features of muscle biopsy in a patient with hypokalemic periodic paralysis. Intrasarcoplasmic vacuoles were observed on hematoxylin–eosin stained sections of formol fixed and paraffin-embedded skeletal muscle. (A) Magnification: 200×. (B) Magnification: 300×.

Hematoxylin–eosin staining for evaluation of patient muscular tissue

Staining of the gastrocnemius muscles obtained from the patients revealed single or multiple, centrally placed vacuoles, as is typical for hypokalemic periodic paralysis-related vacuolar myopathy (Fig. 1).

Evaluation of SUR2A mRNA expression using quantitative RT-PCR

The expression of the mRNA extracted from each specimen was evaluated by exposing normal cells and patient cells to potassium buffer at normal extracellular concentration (4 mM) and at high concentration (50 mM) to induce depolarization. In both normal and patient cells, the mRNA expression did not differ upon exposure to 4 mM potassium buffer (Fig. 2A and B). Further, normal cells showed no change in mRNA expression after exposure to 50 mM potassium buffer (Fig. 2C). However, decreased SUR2A mRNA expression was detected in the patient cells following exposure to 50 mM potassium buffer (Fig. 2D).

Western blot analysis to evaluate cytosolic and membranous SUR2A protein expression

To investigate the effects of changes in the SUR2A mRNA expression on protein expression, cytosolic and membranous fractions were separated following treatment with potassium buffers of the same condition and protein expression level of SUR2A was evaluated. Results revealed that both patient cells and normal cells that were exposed to 4 mM potassium buffer did not show any significant change in cytosolic and membrane protein, similar to the case of mRNA expression (Fig. 3A and B). However, following treatment with 50 mM potassium buffer to induce depolarization, cytosolic SUR2A protein levels increased (Fig. 3C) whereas the membrane protein levels decreased in normal cells (Fig. 3D). On the contrary, in patient cells, the cytosolic SUR2A protein decreased (Fig. 3C), whereas the membrane SUR2A protein increased (Fig. 3D).

Discussion

Familial hypokalemic periodic paralysis is the most prevalent form of periodic paralysis in Western countries [24], and it has been reported to be more frequent in the Western populations than in Asians. However, recent studies have reported many patients with familial hypokalemic periodic paralysis in Asian countries [1,25–27]. Various ongoing studies are investigating the pathogenesis of this disease, and some have reported that decrease in the membrane K_{ATP} channel function is related to its pathogenesis [19,20]; however, the exact molecular mechanism has not yet been elucidated.

In this study, we aimed to determine the mechanism underlying familial hypokalemic periodic paralysis by observing changes in the SUR2A protein expression in 4 and 50 mM potassium buffers; the former was used to mimic normal extracellular potassium

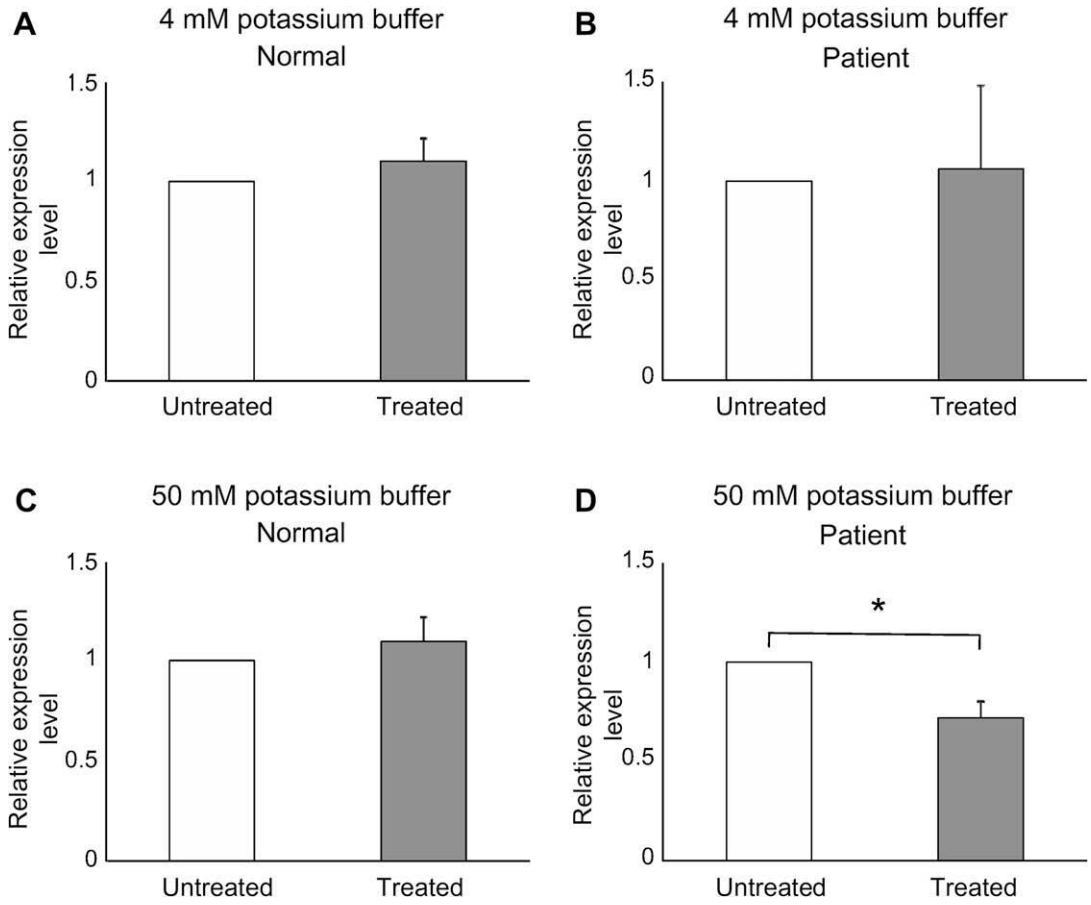

Fig. 2. Quantification of SUR2A mRNA expressions after exposure to 4 and 50 mM potassium buffers, respectively. The mRNA levels of SUR2A did not change in both normal (A) and patient (B) cells in 4 mM potassium buffer. After exposure to 50 mM potassium buffer, the mRNA levels remain unchanged in normal cells (C) but significantly decreased in patient cells (D). Untreated: prior to exposure to potassium buffer. Treated: 1 h after exposure to potassium buffer. In the fold change, untreated samples are marked as value 1.

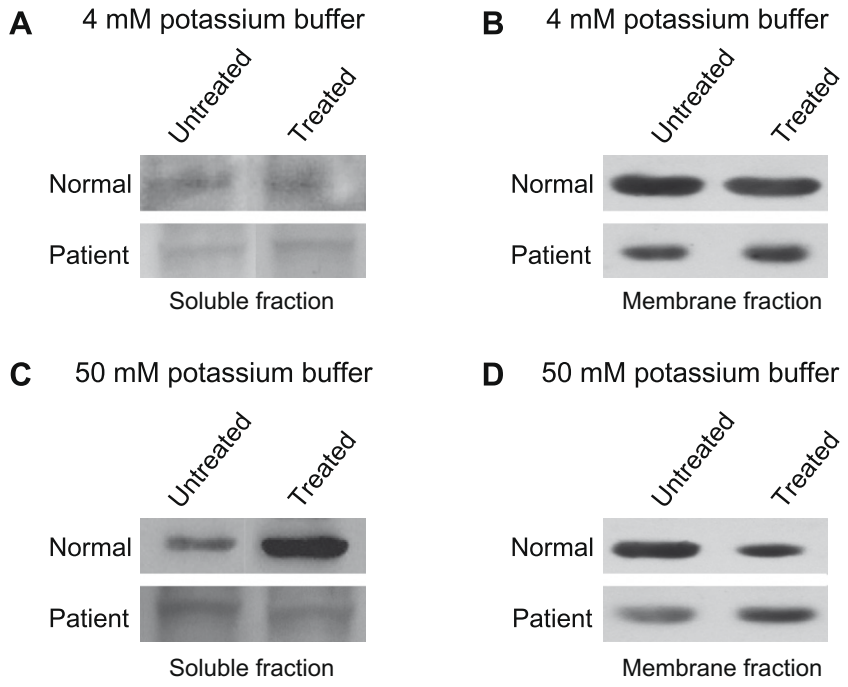

Fig. 3. Western blot analysis of SUR2A protein after exposure to 4 and 50 mM potassium buffers, respectively. The protein levels of SUR2A in the cytosolic (A) and membrane (B) fractions did not change in both normal (upper panel) and patient (lower panel) cells in 4 mM potassium buffer. After exposure to 50 mM potassium buffer, the protein levels of SUR2A in the cytosolic fraction (C) increased in normal cells (upper panel) and decreased in patient cells (lower panel); however, in the membrane fraction (D) those decreased in normal cells and increased in patient cells. Untreated: prior to exposure to potassium buffer. Treated: 1 h after exposure to potassium buffer.

concentrations and the latter, to induce depolarization and to mimic paralytic conditions. As a result, there was no change in the SUR2A mRNA and SUR2A protein expressions in both types of cells after exposure to 4 mM potassium buffer, indicating that under normal physiological concentrations of potassium, the SUR2A protein is used for the normal function of the K_{ATP} channels in the patients. However, when normal cells were exposed to 50 mM potassium buffer, we found that, although the SUR2A mRNA expression did not change, the SUR2A protein level increased in the cytosol and decreased in the cellular membrane. Given that SUR2A binds with the Kir6.*x* membrane channel protein and functions in blocking the potassium channels in normal skeletal muscle cells, this is presumably a mechanism to stabilize cells to a resting membrane potential by resolving the depolarization. To resolve the depolarization, the potassium ions must be released through the potassium channels opened by retrieval of membrane SUR2A to the cytosol following increased intracellular potassium. However, when the patient cells were exposed to 50 mM potassium buffer to induce depolarization, the mRNA expression was found to decrease, and the protein expression in the cytosol decreased but that in the cellular membrane increased. Therefore, we inferred that in patient cells, SUR2A protein is not retrieved to the cytosol following depolarization; rather, it remains overexpressed in the membrane, rendering the K_{ATP} channels unopened, and the resultant continuous intracellular potassium retention leads to a persistent depolarization that induces clinical muscular paralysis. Therefore, decreased intracellular SUR2A mRNA expression in patient cells exposed to 50 mM potassium buffer is probably a physiological compensatory mechanism at the gene level to relieve paralysis due to continuous depolarization.

In conclusion, we analyzed the expression pattern of SUR2A mRNA and the intracellular distribution of the SUR2A protein to investigate the cause of decrease in the functional activity of the K_{ATP} channels in patients with familial hypokalemic periodic paralysis. We observed a decrease in the SUR2A gene expression and abnormal membrane distribution of the protein in these patients. This is a novel finding that explains the pathogenesis of this disease with regard to the K_{ATP} channels in the patient cells. Further research on channel proteins, including Kir6.*x*, which is functionally related to SUR2A, could yield additional information in understanding the pathogenesis of this disorder in detail.

References

[1] J.B. Kim, M.H. Kim, S.J. Lee, D.J. Kim, B.C. Lee, The genotype and clinical phenotype of Korean patients with familial hypokalemic periodic paralysis, J. Korean Med. Sci. 22 (2007) 946–951.
[2] S.L. Venance, S.C. Cannon, D. Fialho, B. Fontaine, M.G. Hanna, L.J. Ptacek, M. Tristani-Firouzi, R. Tawil, R.C. Griggs, The primary periodic paralyses: diagnosis, pathogenesis and treatment, Brain 129 (2006) 8–17.
[3] S.C. Cannon, Pathomechanisms in channelopathies of skeletal muscle and brain, Annu. Rev. Neurosci. 29 (2006) 387–415.
[4] F.J. Gennari, Disorders of potassium homeostasis: hypokalemia and hyperkalemia, Crit. Care Clin. 18 (2002) 273–288.
[5] A. Noma, ATP-regulated K+ channels in cardiac muscle, Nature 305 (1983) 147–148.
[6] K.F. Raab-Graham, C.M. Radeke, C.A. Vandenberg, Molecular cloning and expression of a human heart inward rectifier potassium channel, Neuroreport 5 (1994) 2501–2505.
[7] A.E. Spruce, N.B. Standen, P.R. Stanfield, Voltage-dependent ATP-sensitive potassium channels of skeletal muscle membrane, Nature 316 (1985) 736–738.
[8] N. Inagaki, T. Gonoi, J.P. Clement, N. Namba, J. Inazawa, G. Gonzalez, L. Aguilar-Bryan, S. Seino, J. Bryan, Reconstitution of IKATP: an inward rectifier subunit plus the sulfonylurea receptor, Science 270 (1995) 1166–1170.
[9] S. Seino, ATP-sensitive potassium channels: a model of heteromultimeric potassium channel/receptor assemblies, Annu. Rev. Physiol. 61 (1999) 337–362.
[10] M. Matsuo, K. Tanabe, N. Kioka, T. Amachi, K. Ueda, Different binding properties and affinities for ATP and ADP among sulfonylurea receptor subtypes, SUR1, SUR2A, and SUR2B, J. Biol. Chem. 275 (2000) 28757–28763.
[11] C.G. Vanoye, G.G. MacGregor, K. Dong, L. Tang, A.S. Buschmann, A.E. Hall, M. Lu, G. Giebisch, S.C. Hebert, The carboxyl termini of K(ATP) channels bind nucleotides, J. Biol. Chem. 277 (2002) 23260–23270.
[12] N. Inagaki, T. Gonoi, J.P. Clement, C.Z. Wang, L. Aguilar-Bryan, J. Bryan, S. Seino, A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K^+ channels, Neuron 16 (1996) 1011–1017.
[13] F.M. Ashcroft, P. Rorsman, Electrophysiology of the pancreatic beta-cell, Prog. Biophys. Mol. Biol. 54 (1989) 87–143.

[14] M.L. Ashford, P.R. Boden, J.M. Treherne, Tolbutamide excites rat glucoreceptive ventromedial hypothalamic neurons by indirect inhibition of ATP-K^+ channels, Br. J. Pharmacol. 101 (1990) 531–540.
[15] J. Daut, H.G. Klieber, S. Cyrys, T. Noack, K_{ATP} channels and basal coronary vascular tone, Cardiovasc. Res. 28 (1994) 811–817.
[16] C.G. Nichols, W.J. Lederer, Adenosine triphosphate-sensitive potassium channels in the cardiovascular system, Am. J. Physiol. 261 (1991) H1675–H1686.
[17] J.M. Quayle, M.T. Nelson, N.B. Standen, ATP-sensitive and inwardly rectifying potassium channels in smooth muscle, Physiol. Rev. 77 (1997) 1165–1232.
[18] D. Tricarico, S. Pierno, R. Mallamaci, G.S. Briqiani, R. Capriulo, G. Santoro, The biophysical and pharmacological characteristics of skeletal muscle K_{ATP} channels are modified in K^+ depleted rat, an animal model of hypokalemic periodic paralysis, Mol. Pharmacol. 54 (1998) 197–206.
[19] D. Tricarico, S. Servidei, P. Tonali, K. Jurkat-Rott, D.C. Camerino, Impairment of skeletal muscle adenosine triphosphate-sensitive K^+ channels in patients with hypokalemic periodic paralysis, J. Clin. Invest. 103 (1999) 675–682.
[20] D. Tricarico, A. Mele, B. Liss, F.M. Ashcroft, A.L. Lundquist, R.R. Desai, A.L. George Jr., D. Contecamerino, Reduced expression of Kir6.2/SUR2A subunits explains K_{ATP} deficiency in K+ depleted rats, Neuromuscul. Disord. 18 (2008) 74–80.
[21] D.L. Cottle, M.J. McGrath, B.S. Cowling, I.D. Coghill, S. Brown, C.A. Mitchell, FHL3 binds MyoD and negatively regulates myotube formation, J. Cell Sci. 120 (2007) 1423–1435.
[22] R.J. Poole, D.P. Briskin, Z. Kratky, R.M. Johnstone, Density gradient localization of plasma membrane and tonoplast from storage tissue of growing and dormant red beet: characterization of proton-transport and ATPase in tonoplast vesicles, Plant Physiol. 74 (1984) 549–556.
[23] A. Prinetti, V. Chigorno, G. Tettamanti, S. Sonnino, Sphingolipid-enriched membrane domains from rat cerebellar granule cells differentiated in culture. A compositional study, J. Biol. Chem. 275 (2000) 11658–11665.
[24] P. Lapie, P. Lory, B. Fontaine, Hypokalemic periodic paralysis: an autosomal dominant muscle disorder caused by mutations in a voltage-gated calcium channel, Neuromusc. Disord. 7 (1997) 234–240.
[25] T.H. Kil, J.B. Kim, Severe respiratory phenotype caused by a de novo Arg528Gly mutation in the CACNA1S gene in a patient with hypokalemic periodic paralysis, Eur. J. Paediatr. Neurol. (2009), doi:10.1016/j.ejpn.2009.08.004, PMID: 19822448 (Epub ahead of print).
[26] J.B. Kim, K.Y. Lee, J.K. Hur, A Korean family of hypokalemic periodic paralysis with mutation in a voltage-gated calcium channel (R1239G), J. Korean Med. Sci. 20 (2005) 162–165.
[27] W. Wang, L. Jiang, L. Ye, N. Zhu, T. Su, L. Guan, X. Li, G. Ning, Mutation screening in Chinese hypokalemic periodic paralysis patients, Mol. Genet. Metab. 87 (2006) 359–363.