Review article

The action of smooth muscle cell potassium channels in the pathology of pulmonary arterial hypertension

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Abstract

Many different types of potassium channels with various functions exist in pulmonary artery smooth muscle cells, contributing to many physiological actions and pathological conditions. The deep involvement of these channels in the onset and exacerbation of pulmonary arterial hypertension (PAH) also continues to be revealed. In 2013, *KCNK3* (TASK1), which encodes a type of two-pore domain potassium channel, was shown to be a predisposing gene for PAH by genetic mutation, and it was added to the PAH classification at the Fifth World Symposium on Pulmonary Hypertension (Nice International Conference). Decreased expression and inhibited activity of voltage-gated potassium channels, particularly *KCNA5* (Kv1.5), are also seen in PAH, regardless of the cause, and facilitation of pulmonary arterial contraction and vascular remodeling has been shown. The calcium-activated potassium channels seen in smooth muscle cells also change from BKca (Kca1.1) to IKca (Kca3.1) predominance in PAH due to transformation, and have effects including the facilitation of smooth muscle cell migration, enhancement of proliferation, and inhibition of apoptosis. Elucidation of these roles for potassium channels in pulmonary vasoconstriction and remodeling may help bring new therapeutic strategies into view.

1. Introduction

Pulmonary hypertension is a refractory disease with the clinical conditions of persistently elevated pulmonary arterial pressure and pulmonary vascular resistance from various causes, and a poor prognosis with progressive exacerbation of right heart failure and respiratory failure. The major pathology in pulmonary arterial hypertension (PAH) is narrowing of the pulmonary artery lumen and develops from three factors: (1) abnormal constriction of peripheral small pulmonary arteries to less than 500 μm in diameter from an imbalance between vasodilators and vasoconstrictors, (2) vascular remodeling from hyperproliferation of vascular endothelial, smooth muscle, and other cells and resistance to apoptosis, and (3) thrombus formation in affected sites. Pulmonary vascular resistance increases as a result of the above, causing elevated pulmonary artery pressure and right heart failure. These conditions are related to the characteristics of pulmonary artery endothelial cells and smooth muscle cells [1-4].

In the early pathological stage, abnormal contraction accounts for much of the condition, after which vascular remodeling become predominant. PAH lesions fall into the general classification of constrictive lesions consisting of gradual stenosis and obstruction of vessel lumens from thickening of the vessel wall, and complex lesions consisting of plexiform lesions, space-occupying lesions, and vasculitis. In the early stage of the disease, isolated medial thickening is seen, but with the continuation of pulmonary hypertension, thickening of the

intima also begins to occur [5-8]. Thickening due to increases in cellular components, such as smooth muscle cells and myofibroblasts, is called cellular intimal thickening, and that due to increases in fiber components, mainly collagen fibers, is called fibrous intimal thickening.

Predisposing genes in this disease include transforming growth factor (TGF)-β signal-related genes such as bone morphogenic protein type II receptor gene (*BMPR2*), activin receptor-like kinase-1 (*ALK-1*) gene (*ACVRL1*), endogolin gene (*ENG*), and SMAD8/9 (*SMAD9*) gene, as well as the caveolin-1 (*CAV1*) gene, an intracellular calcium regulator [9-14]. In 2013, a mutation in the potassium channel gene *KCNK3* (TASK1) was demonstrated in PAH [15], and it was added to the PAH classification at the Fifth World Symposium on Pulmonary Hypertension (Nice) [16]. The mechanism of onset due to BMPR2 mutation is thought to be the initiation of proliferation of smooth muscle cells and other cells and resistance to apoptosis due to an imbalance in bone BMP and TGF-β signal transmission [17-20]. Because the newly discovered potassium channel gene mutation is unrelated to TGF-β signal transmission, there is a possibility that it will lead to new findings related to the mechanism of onset of PAH.

KCNA5 (Kv1.5), a voltage-gated potassium channel, has often been a subject of investigation with regard to potassium channel involvement in the onset and exacerbation of PAH [21, 22]. Decreased Kv1.5 current not only makes the resting membrane potential shallower and causes constriction of the pulmonary vessels, it also affects cell proliferation and

migration [23-26]. Caspase activity is also inhibited by an increased concentration of intracellular potassium ions, and it also acts to induce resistance to apoptosis [27, 28]. Many potassium channels other than *KCNK3* and *KCNA5* are involved in small pulmonary artery contraction/relaxation and remodeling, as well as in pulmonary artery smooth muscle cell proliferation, apoptosis, and migration. In the future, they may occupy a major position in treatment strategies. Today, the use of prostacyclins, endothelin receptor antagonists, and phosphodiesterase 5 inhibitors has become widespread, and data on outcomes with monotherapies and combination therapies are accumulating, including data from randomized controlled clinical trials [29-31]. However, this disease is resistant to treatment, and treatment results remain unsatisfactory. Further breakthroughs are needed [32]. Although pathogenesis of PAH is recognized as a complex and multifactorial process, numerous data has accumulated demonstrating the significant role of potassium channels in the cellular mechanisms underlying abnormal pulmonary arterial smooth muscle cell behavior. In regulating potassium flow across the membrane and subsequent modulation of cytoplasmic free calcium concentration, potassium channels control substantial biological functions. This review summarizes potassium channel actions and control in PAH and discusses the outlook for future treatment strategies.

2. Potassium channels in pulmonary artery smooth muscle cells

Potassium channels are ion channels present in cell membranes that are selectively permeable to potassium ions. They perform important roles in the formation of resting membrane potentials, cell excitability, electrical cellular response, formation and duration of action potentials, synapse transmission, cell division, cell differentiation, periodic activity, tension and various other body regulation processes, and cell function control. The potassium channels that exist in vascular smooth muscle cells are broadly divided into four classes: voltage-gated K^+ channels (Kv), Ca^{2+}-activated K^+ channels (Kca), two-pore domain K^+ channels (K_{2P}), and inwardly rectifying K^+ channels (K_{IR}) (Table 1) [33, 34]. Nearly all potassium channels are tetramers formed of α subunits, with a central pore for the passage of potassium. Depending on differences in electrophysiological characteristics and the α subunit transmembrane region structure, they are broadly divided into six or seven transmembrane-type Kv and Kca; two transmembrane-type K_{IR}; and four transmembrane-type K_{2P}. They are formed from more than 100 types of gene clusters combining the α subunits that make up the ion permeation pathways and β subunits that control current characteristics and membrane expression level. The diversity and versatile functionality of potassium channels are expressed from these abundant molecular species of subunits, α subunit heterotetramer formation, and the formation of further complexes with β subunits.

3. Contraction, dilatation, and remodeling of pulmonary arteries via potassium channels

Pulmonary artery contraction and dilatation are controlled by various vasoactive substances, environments, stresses, and drugs. Figure 1 shows vasoconstriction due to hypoxia, a characteristic response in pulmonary vessels [35-38]. Under normal oxygen partial pressure, the membrane potential of vascular smooth muscle cells is maintained at −50 to −60 mV, and calcium ion influx from voltage-dependent Ca^{2+} channels (VDCC) is inhibited.

Decreased expression and inhibited activity of potassium channels cause decreased potassium current in smooth muscle cells and lead to elevation and depolarization of resting potentials. This results in activation of VDCC and elevation of the intracellular calcium concentration, generating myogenic tension. Contraction of vascular smooth muscle occurs and is established via a signal transduction pathway [39, 40]. This increase in calcium also leads to the action of calcium-induced calcium release, which stimulates the release of Ca^{2+} from the sarcoplasmic reticulum.

In the control system of vascular smooth muscle cell contraction via membrane potentials, BKca is central in the feedback mechanism. The characteristic of activated BKca is suitable for the feedback mechanism with greater elevation in intracellular calcium concentration, or greater depolarization. Hyperpolarization is produced by activation of BKca, facilitating the inhibition of VDCC and other voltage-gated channels. Kv channels are also

activated as a result of depolarization and so contribute significantly to negative feedback.

Depolarization of cell membrane potentials and persistently elevated intracellular calcium concentrations are major factors, both physiologically and pathologically, in the contraction of smooth muscle cells, but they also stimulate cell proliferation. Elevated calcium concentrations in the nucleus and cytoplasm activate calmodulin kinase, mitogen-activated protein kinase, and other Ca^{2+}-dependent kinases as well as transcription factors such as nuclear factor of activated T-cells (NFAT) and cAMP response element binding protein (CREB). This pushes cells in the resting stage to enter the cell cycle and proliferate (Fig. 2) [39]. Kv and other potassium channels are involved in this cell signaling control.

Among Kv channels, Kv1.5 (*KCNA5*) shows greater expression in arterioles than in elastic or muscular arteries. In acute periods, Kv1.5 activity is inhibited by hypoxia, and smooth muscle contracts via decreased potassium current and depolarization of cell membranes. As a result of the hypoxic state, increases in reactive oxygen species, increases in nicotinamide adenine dinucleotide phosphate, and activation of protein kinase C occur from activation of sphingomyelinase. All of these signals inhibit Kv1.5 activity in acute phases. Moreover, alveolar hypoxia not only causes pulmonary vasoconstriction, it also inhibits Kv1.5 expression and promotes remodeling in small pulmonary arteries in the chronic phase [41]. Decreased Kv1.5 expression is seen as a common feature or characteristic regardless of the cause of

pulmonary hypertension, and is thought to be very important in exacerbation of the condition [41-43]. Although the reason for this is not understood, it has been suggested that many factors are involved, and this decreased expression is a potential target for future therapies.

KCNK3 (TASK1) was reported in 2013 as a gene that causes PAH and is one type of two-pore domain potassium channel (K_{2P}). K_{2P} has a subunit structure in which two subunits consisting of two membrane-spanning segments and one P domain are connected serially, and is activated when hypoxia or pH is detected. According to electrophysiological and pharmacological characteristics, K_{2P} channel is classified into six subfamilies (TWIK, TREK, TASK, TALK, THINK, TRAAK). In this report, six heterozygous missense variants were independently identified [15]. *KCNK3* shows activity not only in voltage-gated channels but also near resting membrane potentials, and so abnormalities in this channel are thought to promote pulmonary vasoconstriction and remodeling by hindering the maintenance of membrane potentials.

At the same time, there are few reports on the involvement of the ATP-sensitive K^+ channel (K_{ATP}) that is activated by hypoxia or ischemia in many organs during contraction [44, 45], remodeling, or feedback in pulmonary arteries. In particular, cell membrane K_{ATP} channels are thought to have little importance in contributing to the pathology. Reports suggesting that mitochondrial K_{ATP} channels affect pulmonary artery contraction or remodeling are also seen

occasionally [46], but their role in exacerbation of pathological conditions remains poorly understood [47-49].

4. PAH caused by mutations in the KCNK3 (TASK1) gene

In 2013, Ma et al. announced that gene mutations in *KCNK3* (TASK1), a type of two-pore domain potassium channel, are a cause of PAH [15], and this was added to the pulmonary hypertension classifications at the Fifth World Symposium on Pulmonary Hypertension (Nice International Conference) [16]. Familial cases of PAH have long been recognized and are usually due to mutations in members of the TGF signaling cascade. BMPR2 mutations account for ~70% of familial PAH and 15% of patients with idiopathic PAH. Recent advances in genome sequencing technologies have provided unprecedented opportunities to identify mutations. They conducted whole exome sequencing of three members of one family that included multiple PAH patients and did not show mutations corresponding to known gene abnormalities (BMPR2, ALK1, ENG, SMAD9, CAV1). Screening for the gene mutations identified in whole exome sequencing was done in other familial and idiopathic PAH patients, and channel function was analyzed with the patch clamp method. A heterozygous missense variant c.608 G→A (G203D) in KCNK3 was identified as a candidate gene causing the disease. Five additional heterozygous missense variants in *KCNK3* were independently identified in 92

unrelated familial PAH patients and 230 idiopathic PAH patients (Fig. 3). Electrophysiological examination of the channels showed that function loss had occurred with all six of these missense variants. They considered that the *KCNK3* functional abnormality caused shallower resting membrane potentials and pulmonary artery contraction. The prevalence of KCNK3 mutations was 1.3% in idiopathic PAH and 3.2% in familial PAH [15]. This study provides the first causal relationship between a potassium channels and PAH, and consequently PAH is now considered as a channelopathy.

5. Kv1.5 (*KCNA5*) mutations and functional abnormalities in PAH

Remillard et al. investigated single-nucleotide polymorphisms (SNPs) in *KCNA5* in idiopathic PAH patients, and indicated that they may contribute to the manifestation of clinical symptoms and permeability [50]. *KCNA5* variations may act as a "second-hit" in BMPR2 missense mutations, causing early onset of symptoms and severe symptoms [51]. However, to understand whether or not these variations actually have significant effects on PAH, the extent to which genetic modifications alone induce symptoms will need to be clearly demonstrated, and functional analysis of channels that show variations will need to be conducted. Kv1.5 is controlled by various vasoactive substances. Kv1.5 current is inhibited by endothelin-1 and activated by nitric oxide [50] (Fig. 4). Kv1.5 is preferentially expressed in the small resistance

pulmonary arteries rather than in conduit pulmonary arteries and diminished following hypoxia exposure. [21,22]. Reduced expression of Kv1.5 is a common denominator of human and experimental PAH suggesting an important role of this channel in the pathogenesis of various forms of PH [6,39,41-43]. Although KV1.5 is considered as a potential therapeutic target, the molecular mechanisms leading to its reduced expression in this disease are not clear. PAH is treated with the use of prostacyclin, endothelin receptor antagonists, nitric oxide, phosphodiesterase-V, and other agents, but the fact that Kv1.5 is an intermediary in the intracellular signaling pathway of these principal PAH therapeutic agents suggests that directly controlling Kv1.5 may be a useful therapeutic approach.

6. Plasticity and channel switching in vascular smooth muscle cells

Generally, terminally differentiated cells (skeletal muscle cells, nerve cells, blood cells, etc.) maintain their differentiated phenotype until they die, and do not show dedifferentiation or cell division. Thus, they show what is referred to as “terminal differentiation.” Smooth muscle cells, on the other hand, readily change from the differentiated phenotype to a dedifferentiated phenotype under pathological or special conditions (pulmonary hypertension, arteriosclerosis, diseased blood vessels, culture, etc.). They also show an inherent plasticity, such as cell proliferation while maintaining their differentiated phenotype.

Transformation (dedifferentiation) of vascular smooth muscle cells is the starting point for remodeling, after which thickening of the vessel intima-media wall occurs from proliferation and migration of dedifferentiated vascular smooth muscle cells, exacerbating vessel wall remodeling. In recent years, it has come to be understood that increased or decreased expression of various ion channels is intimately involved in the transformation of vascular smooth muscle cells [52, 53]. In a state in which smooth muscle cells have differentiated and proliferation has ceased, the dominant expression is of VDCC and BKca, which are involved in processes related to excitation contraction. These channels, as mentioned above, act as important regulators of smooth muscle cell calcium influx that is dependent on the cell membrane potential [54]. If proliferation is stimulated in vascular smooth muscle cells in response to this, the expression of VDCC and BKca rapidly decreases [55]. In its place, increased expression of transient receptor potential channels (TRP) and intermediate-conductance Ca^{2+}-activated K^+ channels (IKca; Kca3.1) has been shown (Fig. 5) [53, 55]. VDCC and BKca that are expressed in differentiated smooth muscle cells are activated with strong dependence on membrane potential, whereas TRP and IKca have the characteristic of being activated and open even in the vicinity of resting membrane potential, with almost no effect from the membrane potential. Therefore, in vascular smooth muscle cells subjected to proliferative stimulation that have transformed to a dedifferentiated type (proliferative type), the membrane potential is hyperpolarized as a result of

the hyperpolarizing action from IKca activation, and because a constant calcium inflow is driven via TRP, a pathway that is independent of electric potentials and a large potential difference is maintained [52, 53]. The elevated intracellular calcium concentration via TRP further activates IKca and shows positive feedback. This state is advantageous for the activation of intracellular calcium concentration-dependent transcription factors such as NFAT/CREB/AP-1/NF-κB mentioned above. Actions that cause increased expression of TRP and IKca are also seen in NFAT and NF-κB, and are further reinforced with positive feedback [55].

Figure 6 is a record demonstrating increased expression of IKca in immature proliferative smooth muscle cells. The potassium current in the whole-cell configuration of proliferative smooth muscle cells was only mildly (14%) inhibited by administration of Iberiotoxin, a selective BKca inhibitor. With the subsequent addition of charybdotoxin, a BKca and IKca inhibitor, the potassium current was strongly inhibited. The IKca inhibitor clotrimazole inhibited most (79%) of the charybdotoxin-sensitive current. Almost no IKca is expressed in differentiated smooth muscle cells, and there is thought to be only a very small IKca-mediated current; however, in proliferative smooth muscle cells, IKca current is markedly increased [53]. In smooth muscle cells stimulated with platelet-derived growth factor, marked increases in IKca (KCa3.1) mRNA and protein have been shown [56]. These results confirm

that the major portion of calcium-dependent potassium current in proliferative smooth muscle cells is IKca-mediated current, and that IKca expression is increased.

TRP channels are tetramer channels with six membrane-spanning helices and are non-selective cation channels permeable to sodium, potassium, and calcium. They have a membrane-potential sensor-like structure, but membrane potential sensitivity is either extremely weak or not seen, and these channels are activated by various environmental stimulants from inside and outside the body and intra- and extracellular signals and ligands. More than TRPC1, which attracts attention for its involvement in cardiomegaly or coronary artery remodeling, it is thought that increased activity of the store-operated calcium influx channel from TRPC6 is involved in pulmonary artery smooth muscle cell proliferation and abnormalities. In smooth muscle cells collected from PAH patients, increased activity of store-operated calcium channels and increased proliferative capacity are seen with increased expression of TRPC6. These proliferative changes have been demonstrated *in vitro* to be inhibited as a result of suppressed expression by siRNA [57].

7. Smooth muscle cell migration and potassium channels

In the progression of vascular remodeling, smooth muscle cell migration is a major factor together with proliferation [58]. The basis of cell migration is repeated extension and

protrusion of the cell anteriorly, and retraction and shrinkage of the trailing end. Increases and decreases in cell volume are controlled especially by potassium and other ion channels and transporters, and are produced through cooperative action together with the cytoskeleton, actin filaments, and other structures. First, the Cl^-/HCO_3^- exchanger and Na^+/H^+ exchanger within the anterior part of the cell are activated, and cellular uptake of water accompanying salt movement and changes in osmotic pressure induce expansion of cellular volume. The cell membrane stretches as the cell volume balloons, mechanical receptor (stretch activated) channels are activated, and calcium flows into the cell [59, 60]. This elevation in the intracellular calcium concentration activates IKca in the posterior part of the cell, and as potassium flows out of the cell, the rear part of the cell retracts (Fig. 7) [58]. Migration occurs through repetitions of this process. IKca, which is expressed at high levels in dedifferentiated smooth muscle cells, also plays an important role in this action.

8. Smooth muscle cell apoptosis and potassium channels

Cell proliferation and apoptosis are opposing controls that maintain normal tissue. Pulmonary artery smooth muscle cells in PAH patients are thought to have increased resistance to apoptosis [61]. This resistance to apoptosis is reported to be produced by downregulation of Kv channels [62]. In apoptosis, decreased cell volume from morphological and biochemical

changes is seen initially. This is produced by the flow of K^+, Cl^-, and H_2O out of the cell. Afterward, pyknosis and DNA fragmentation occur. The flow of potassium out of the cell with potassium channel activation is important not only with respect to the decrease in cell volume, but is also thought to serve a major role in inhibition of caspase activity and in DNA fragmentation [63]. It has been suggested that Kv1.5, mitochondrial K_{ATP}, and mitochondrial BKca are involved in the apoptotic resistance of smooth muscle cells in pulmonary hypertension [63].

9. Possibilities and outlook for treatments via potassium channels

PAH treatments to date have focused on signaling pathways related to pulmonary artery contraction and dilatation, such as prostacyclins, endothelin receptor antagonists, and phosphodiesterase 5 inhibitors. These drugs have without question dramatically improved PAH treatment outcomes, but they also have limitations, and research on pulmonary artery wall remodeling has begun to attract attention. Potassium channels in pulmonary artery smooth muscle cells are thought to be a strong candidate for a therapeutic target. For example, in a report on *KCNK3* [15], which was identified as a new predisposing gene for hereditary PAH, an electrophysiological investigation of *KCNK3* showed that all missense variations of this gene produce functional loss. This decrease in *KCNK3* current is reportedly improved with

administration of a phospholipase inhibitor (ONO-RS-082). In this report, the identification of specific therapeutic agents has greater significance than the specification of predisposing genes, and it is important that improvements are seen with pharmacological manipulations. Increased expression and activation of Kv channels was also reportedly seen with administration of the survivin inhibitor YM155 in rats with pulmonary hypertension from hypoxia exposure [64]. Kv channel inhibition is seen regardless of the underlying etiology in PAH patients, and if applied, it is possible that an effect will be obtained in a wide range of cases. In addition, inhibition of vascular remodeling occurs in relation to TRAM-34, an inhibitor of IKca, which plays a central role in migration, proliferation, and transformation [65]. Modulation of TRAM-34 is promising for future clinical application.

Possible therapeutic approaches for the future include not only the pharmacological methods described above, but also methods such as gene transfer of KCNK3 or KCNA5, which show decreased expression in PAH, to pulmonary artery smooth muscle cells. Elucidation of potassium channel inhibition will likely have a large impact on PAH treatment.

Conclusion

This review has shown the many ways in which potassium channels are involved in PAH pathogenesis at multiple levels. Elucidation of the roles of potassium channels in

pulmonary vasoconstriction and remodeling is promising for the establishment of new therapeutic strategies for PAH.

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5

Figure Legends

Figure 1.

Diagram of hypoxia-induced pulmonary arterial contraction and voltage-gated K^+ (Kv) channels.

Vasoconstriction involves hypoxia-induced elevation of intracellular Ca^{2+} and the related signaling pathways. The inhibition of Kv channels, particularly Kv1.5, plays a key role in the mechanism of vasoconstriction.

AMPK, AMP-activated kinase; AP-1, activating protein 1 transcription factors; cADPR, cyclic ADP ribose; DAG, diacylglycerol; Em, membrane potential; ET-1, endothelin-1; GPCR, G protein-coupled receptor; IP3R, Inositol 1,4,5-trisphosphate receptor; K2P, two-pore domain K^+ channels; Kv, voltage-gated K^+ channels; NCX, Na^+–Ca^{2+} exchanger; PDGF, platelet-derived growth factor; PKC, protein kinase C; ROS, reactive oxygen species; RTK, receptor tyrosine kinase; RyR, ryanodine receptor; SOC, store-operated channels; SR, sarcoplasmic reticulum; STIM1, stromal-interacting molecule 1; TRP, transient receptor potential channels; VDCC, voltage-dependent Ca^{2+} channels.

Figure modified from Ward JP and McMurtry IF (ref. 14) with permission.

Figure 2.

Diagram of the pulmonary arterial contraction and vascular remodeling mechanism.

A rise in cytosolic Ca^{2+} can be created by opening voltage-dependent Ca^{2+} channels (VDCC) through decreased voltage-gated K^+ (K_V) channel current and membrane depolarization (Em). Activation of receptors such as G protein-coupled receptors (GPCR) and receptor tyrosine kinases (RTK) induces diacylglycerol (DAG) and inositol 1,4,5,-trisphosphate (IP3) production. In addition, these receptors increase the cytosolic Ca^{2+} concentration by opening receptor-operated Ca^{2+} channels (ROC) and inducing Ca^{2+} mobilization from the sarcoplasmic reticulum (SR). IP3 also directly or indirectly opens store-operated Ca^{2+} channels (SOC) by store depletion to further increase Ca^{2+}. The Ca^{2+}/ Calmodulin (CaM) complex binds to and activates myosin light chain kinase (MLCK), which phosphorylates the myosin light chain (MLC). MLC stimulates the activity of myosin ATPase, which hydrolyzes ATP to generate energy for cycling of myosin cross-bridges with actin filaments. Formation of these cross-bridges underlies pulmonary artery smooth muscle cell (PASMC) contraction, prompting vasoconstriction. Furthermore, an elevation in the intracellular Ca^{2+} concentration induces quiescent cells to undergo mitosis. Increased intracellular Ca^{2+} also activates CaM kinase (CaMK) and mitogen-activated protein kinase (MAPK), as well as transcription factors,

including nuclear factor of activated T cells (NFAT), cAMP response element binding protein (CREB), activator protein-1 (AP-1), and NF-κB, to stimulate proliferation by inducing Ca^{2+}-sensitive steps during cell cycle progression. Chronic and sustained elevation of pulmonary vascular resistance and arterial pressure resulted from vasoconstriction and vascular remodeling. Figure modified from Kuhr HF, et al. (ref. 16) with permission.

Figure 3.

Topologic analysis of the human KCNK3 (hKCHK3) channel and functional consequences of mutations.

Panel A shows a topologic analysis of the hKCNK3 channel, indicating the positions of the mutations. Panel B shows current traces for the nonmutant hKCNK3 channel (NM) and the T8K, G97R, E182K, Y192C, G203D, and V221mutants in whole-cell patch-clamp procedure. Current density is measured as picoamperes per picofarad (pA/pF). For all current traces, the vertical scale is 10 pA/pF and the horizontal scale is 20 mV. The inset shows the ramp protocol (i.e., voltage steps or ramps). The vertical dashed lines represent the current at 60 mV. Figure modified from Ma L, et al. (ref. 4) with permission.

Figure 4.

Kv1.5 current is inhibited by endothelin-1 (ET-1) and activated by nitric oxide (NO).

A: Inhibition of Kv1.5 currents by endothelin-1 (ET-1). Representative Kv1.5 currents elicited by step depolarizations (-60 to +60 mV, holding potential of -80 mV) before (Cont), during (ET-1), and after (Wash) application of 100 nM ET-1 (a). Summarized current amplitude (b, left) and conductance (b, right) at -60 mV from KCNA5-transfected HEK-293 cells before (open bars), during (closed bars), and after (gray bars) treatment with ET-1 are shown. *P<0.001 vs. control

B: Activation of Kv1.5 currents by nitric oxide (NO).

Representative and summarized currents recorded from KCNA5-transfected HEK 293 cells before and after treatment with 0.1 mM S-nitroso-N-acetyl penicillamine (SNAP). Currents were elicited by a step depolarization to potentials ranging between -60 and +80 mV from a holding potential of -70 mV (a). Current amplitudes were significantly greater at all membrane potentials (b), including -60 mV (c).

# P<0.05 vs. control.

Figure modified from Remillard CV, et al. (ref. 22) with permission.

Figure 5.

Diagrams depicting phenotypic switching of vascular smooth muscle cells and ion channel

expression.

Vascular smooth muscle cells (SMCs) can have one of two phenotypes: immature proliferative SMCs and differentiated contractile SMCs. Vascular SMCs change phenotype in response to the surrounding environment. Proliferative immature SMCs proliferate, migrate, and synthesize proteins. In contrast, contractile fully differentiated SMCs adhere to each other and are contractile. Switching to different ion transport systems is also shown.

This phenotypic shift in the $Ca2^{+}$-activated K^{+} channel (Kca) expression pattern produces dramatic alterations in the electrical properties of the cell and has functional consequences, in part due to the effect on Ca^{2+} influx. Activation of IKca enhances Ca^{2+} influx by increasing the transmembrane electrical gradient. This increase in Ca^{2+} influx stimulates distinct cellular processes associated with smooth muscle growth and proliferation.

BKca, large conductance Ca^{2+}-activated K^{+} channel; IKca, intermediate conductance Ca^{2+}-activated K^{+} channel; SMC, smooth muscle cell; TRP, transient receptor potential channels; VDCC, voltage-dependent Ca^{2+} channels.

Figure 6.

Predominant expression of IKca (KCa3.1) in proliferative smooth muscle cells (SMCs).

IKca (KCa3.1) current is observed using the patch-clamp technique. Representative

recording of whole-cell current from an immature SMC held at −60 mV. Establishment of the whole-cell configuration (vertical arrow). The zero current level (dashed line). Iberiotoxin (IbTX) and charybdotoxin (ChTX) were added as indicated (A). IbTX inhibited the ChTX-sensitive currents by 14% in this cell. Clotrimazole (CLT)-sensitive K+ current is shown in Panel B. CLT inhibited the current by 79% in this cell, and ChTX inhibited the remaining current. (C) Percentage inhibition of ChTX-sensitive K^+ current by IbTX or CTL in experiments. The bars shows mean ± SEM. *$p < 0.0001$. KCa3.1 upregulation in activated VSMCs were shown in Panel D. Stimulation with 20 ng/ml PDGF increased KCa3.1 mRNA in human coronary SMCs. mRNA expression of KCa1.1, -2.1, -2.2, and -2.3 was unchanged or decreased. (E) Total KCa3.1 protein expression was increased in VSMCs in a time-dependent fashion. #: $p < 0.05$ versus control.

Figure modified from Hayabuchi Y, et al (ref. 25) and Toyama K, et al. (ref. 28) with permission.

Figure 7.

Schematic of the mechanism underlying changes in the cell volume during cell migration.

As shown in the schematic, cell migration is a continuous cycle of protrusion of the cell front followed by the retraction of the trailing end. This process can be represented as a

cycle of isosmotic volume increases at the cell front and isosmotic volume decreases at the rear end. Extension of the lamellipodium results from salt and osmotically obliged water uptake mediated by the parallel operation of Na^+/H^+ and Cl^-/HCO_3^- exchange as well as Na^+-HCO_3^- cotransport at the front of migrating cells. Increases in volume and membrane tension eventually produce an increase in the intracellular Ca^{2+} concentration via activation of Ca^{2+}-permeable stretch-activated cation channels. The rise in intracellular Ca^{2+} concentration induces retraction of the rear portion of the migrating cell, which is paralleled by massive K^+ efflux and shrinkage of the cell pole.

AE2, Cl^-/HCO_3^- exchanger isoform 2; AQP 1, 4, aquaporin 1, 4; ClC3, ClC3 chloride channel; ENaC, epithelial Na^+ channel; IKca, intermediate conductance Ca^{2+}-activated K^+ channel; MScCa, mechanosensitive cation channel; NHE1, Na^+/H^+ exchanger isoform 1; NKCC1, $Na^+/K^+/2Cl^-$ cotransporter isoform 1; VRAC, volume-regulated anion channels. Figure modified from Schwab A, et al. (ref. 30) with permission.

Figure 1

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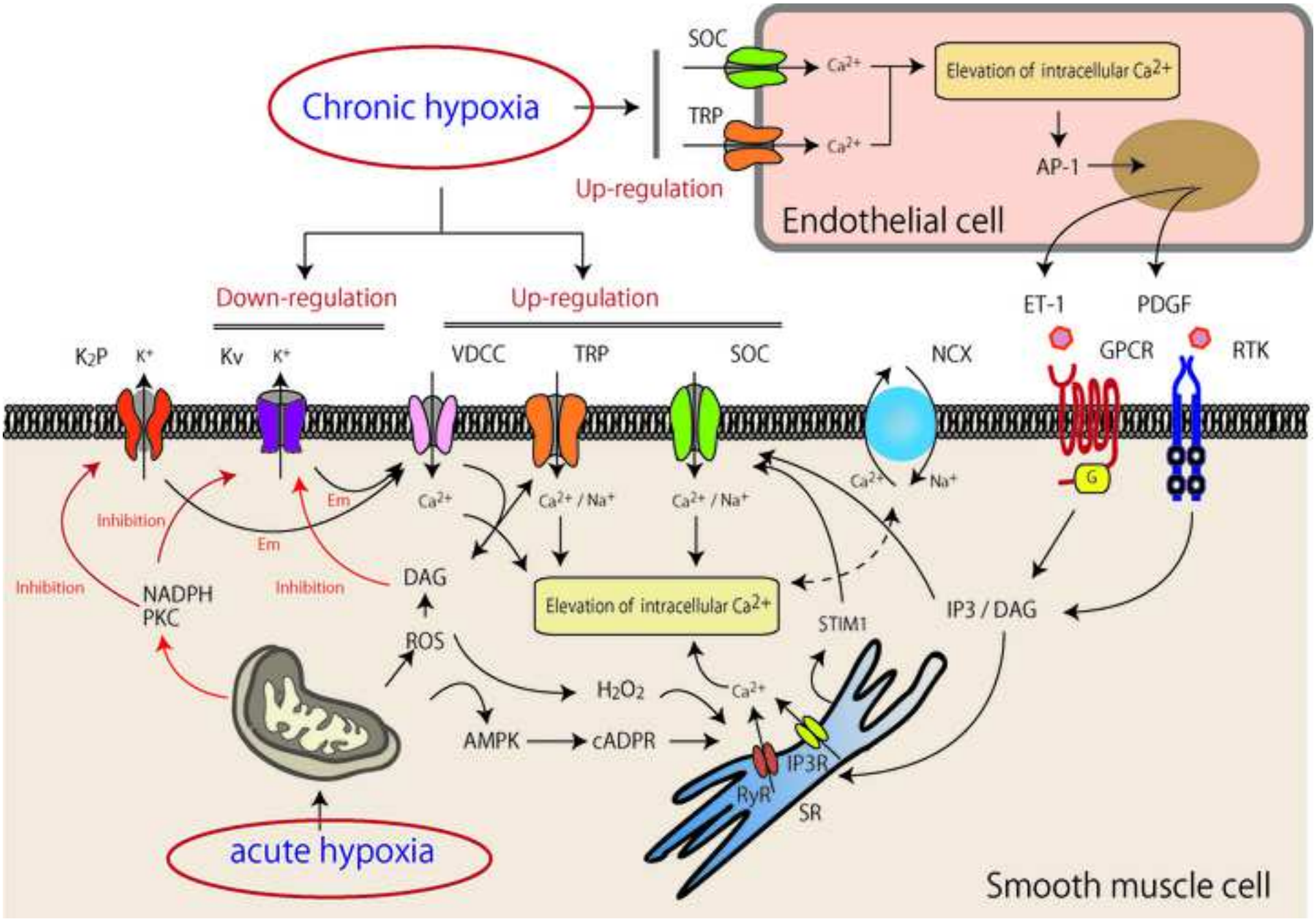

Figure 2

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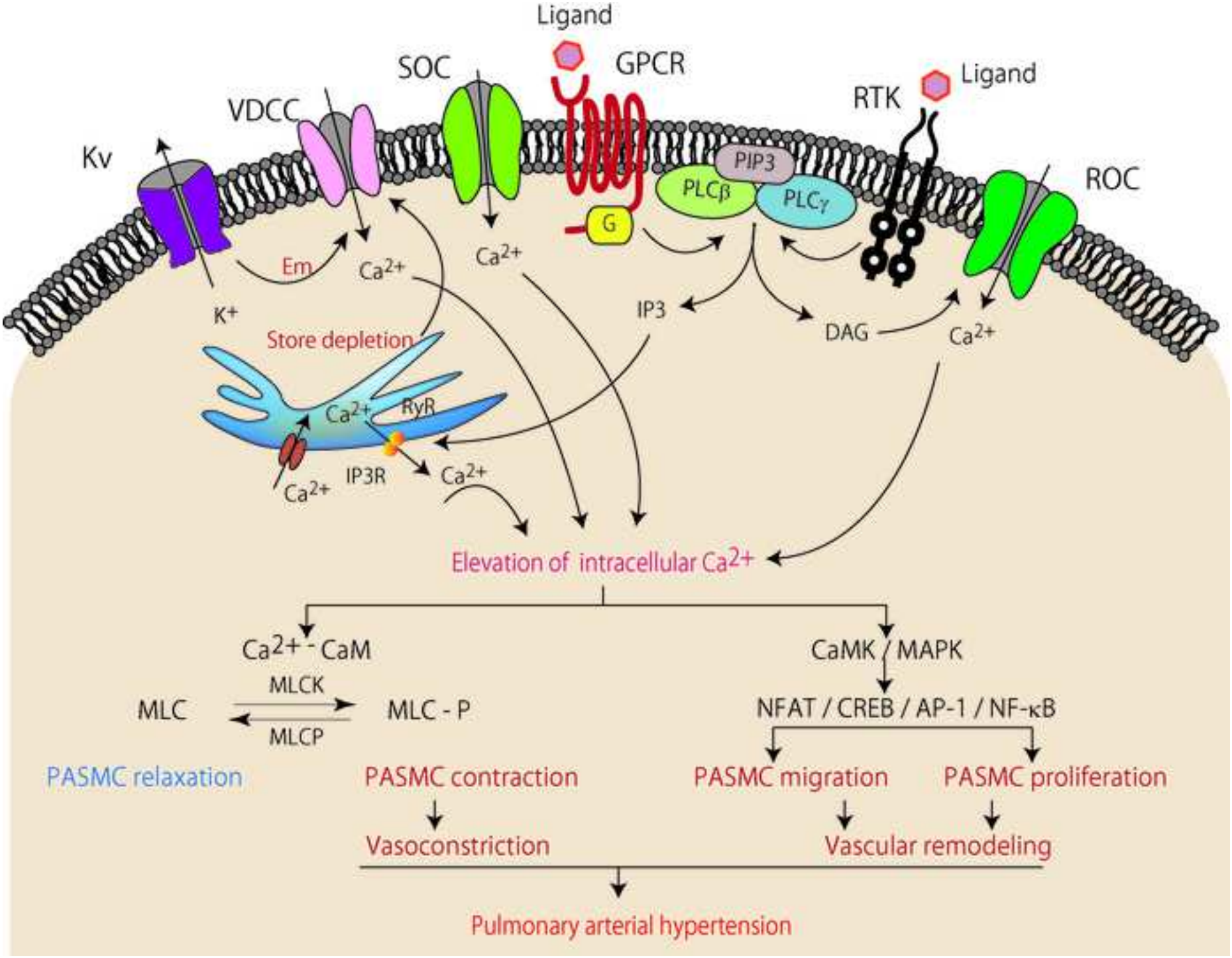

Figure 3

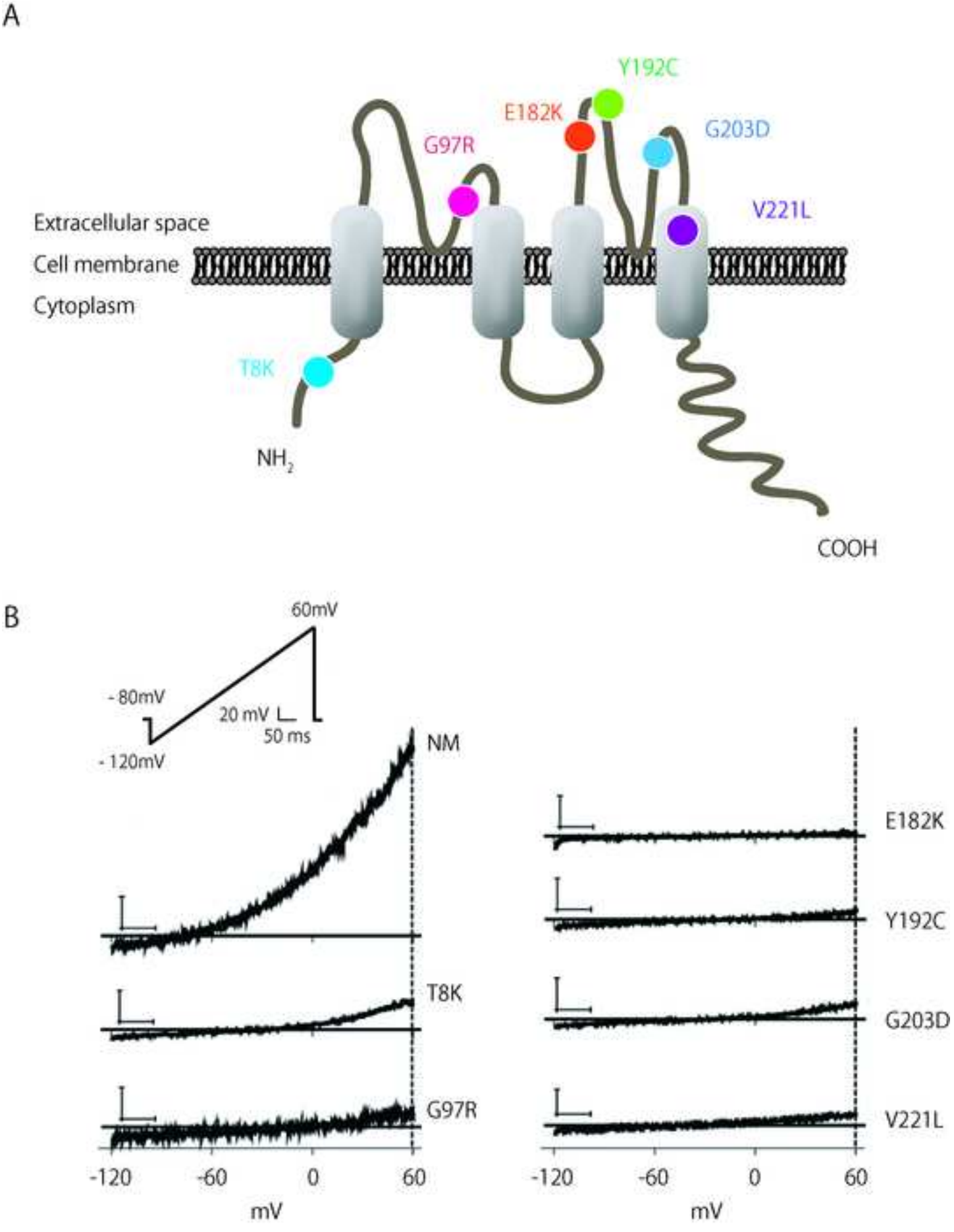

Figure 4

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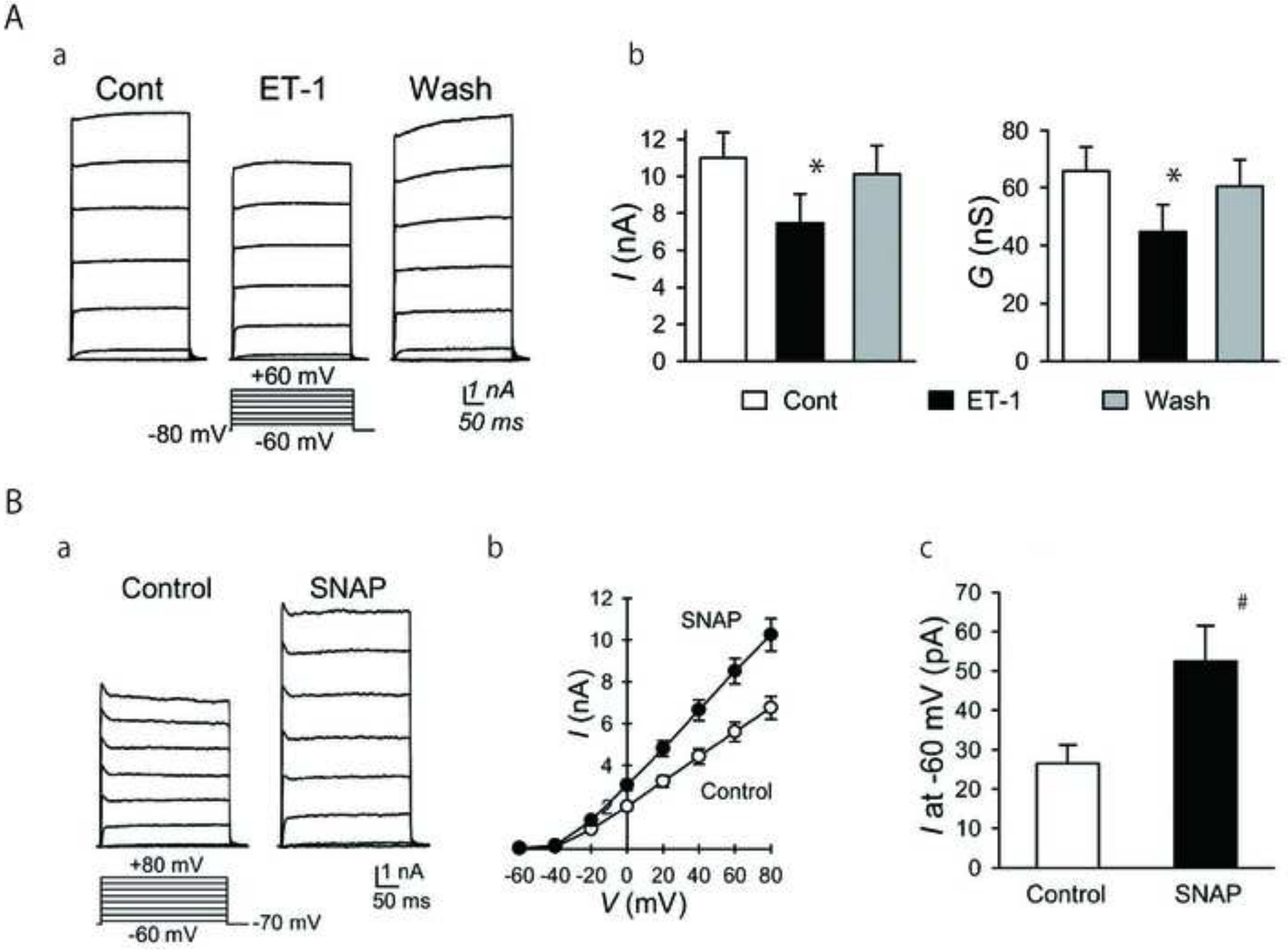
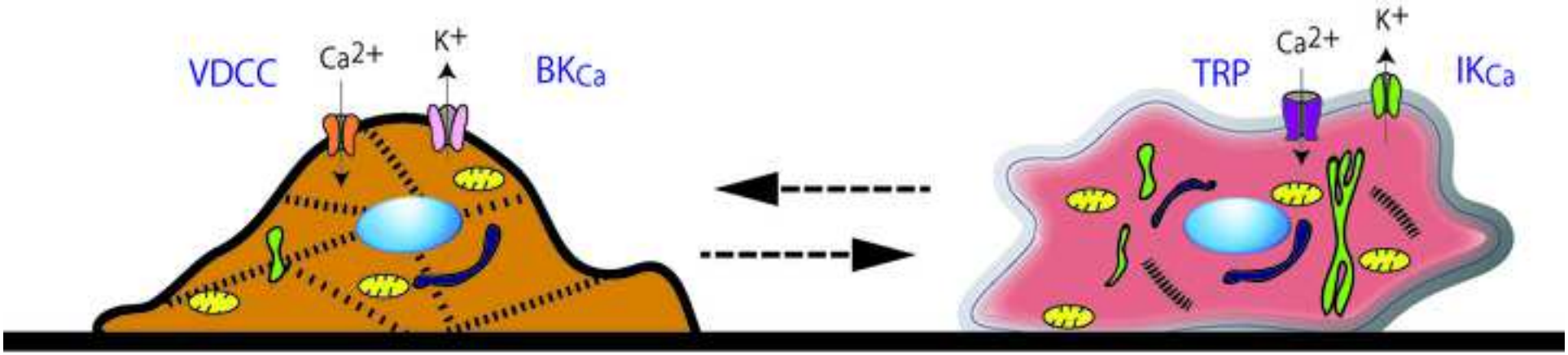


Figure 5



Contractile (fully differentiated) SMC

Proliferative (immature) SMC

Inhibition by the hyperpolarization

VDCC BKca

Activation by the intracellular Ca^{2+} elevation

Negative feedback

Current increasing by the driving force

TRP IKca

Activation by the intracellular Ca^{2+} elevation

Positive feedback

Figure 6

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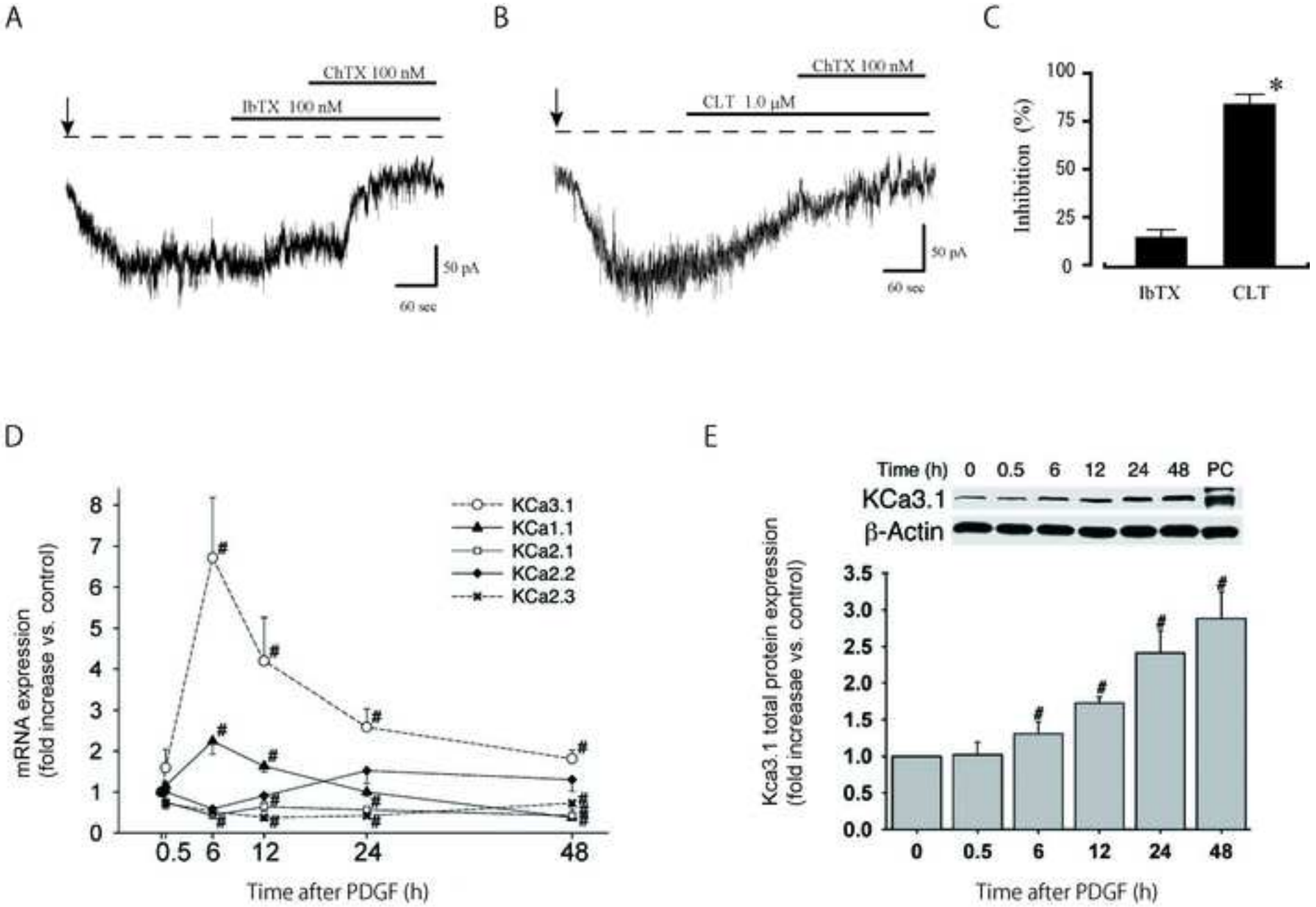

Figure 7

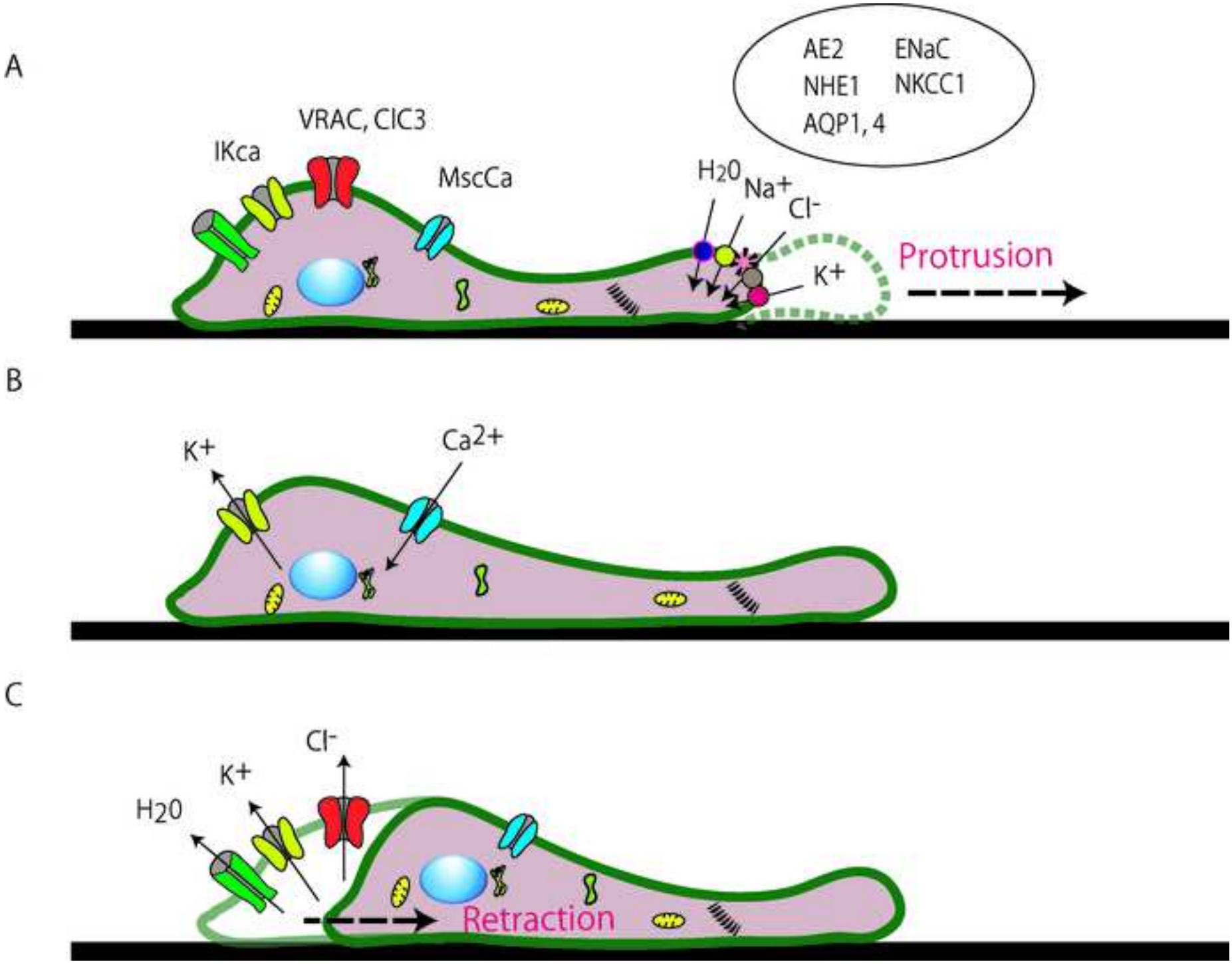

Table 1

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K^+ channel Families	Kv channel (42 isoforms; 12 subfamilies)		K_{Ca} channel (8 isoforms; 5 subfamilies)		K_{2P} channel (15 isoforms; 6 subfamilies)		K_{IR} channel (15 isoforms; 7 subfamilies)	
Nomenclature	HGCN	IUPHAR	HGCN	IUPHAR	HGCN	IUPHAR	HGCN	IUPHAR
Isoforms & Subfamilies	*KCNA1 -A10*	Kv1.1 - Kv1.10	*KCNMA1*	K_{Ca}1.1	*KCNK1*	K_{2P}1.1	*KCNJ1*	K_{IR}1.1
	KCNB1 & B2	Kv2.1 & Kv2.2	*KCNN1-3*	K_{Ca}2.1 – 2.3	*KCNK2*	K_{2P}2.1	*KCNJ2, 12, 4, 14*	K_{IR}2.1 - K_{IR}2.4
	KCNC1 – C4	Kv3.1 – Kv3.4	*KCNN4*	K_{Ca}3.1	*KCNK3*	K_{2P}3.1	*KCNJ3, 6, 9. 5*	K_{IR}3.1 - K_{IR}3.4
	KCND1 – D3	Kv4.1 – Kv4.3	*KCNT1 & T2*	K_{Ca}4.1 & 4.2	*KCNK4*	K_{2P}4.1	*KCNJ10 & 15*	K_{IR}5.1
	KCNF1	Kv5.1	*KCNU1*	K_{Ca}5.1	*KCNK5*	K_{2P}5.1	*KCNJ8 & 11*	K_{IR}6.1 & 6.2
	KCNG1 – G4	Kv6.1 – Kv6.4			*KCNK6*	K_{2P}6.1	*KCNJ13*	K_{IR}7.1
	KCNQ1 – Q5	Kv7.1 – Kv7.5			*KCNK7*	K_{2P}7.1		
	KCNV1 & V2	Kv8.1 & Kv8.2			*KCNK9*	K_{2P}9.1		
	KCNS1 - 3	Kv9.1 – Kv9.3			*KCNK10*	K_{2P}10.1		
	KCNH1 & 5	Kv10.1 & Kv10.2			*KCNK12*	K_{2P}12.1		
	KCNH2,H6, H7	Kv11.1 - Kv11.3			*KCNK13*	K_{2P}13.1		
	KCNH8,H3,H4	Kv12.1 - Kv12.3			*KCNK15*	K_{2P}15.1		
					KCNK16	K_{2P}16.1		
					KCNK17	K_{2P}17.1		
					KCNK18	K_{2P}18.1		

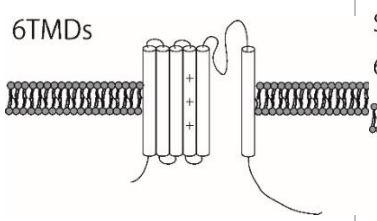
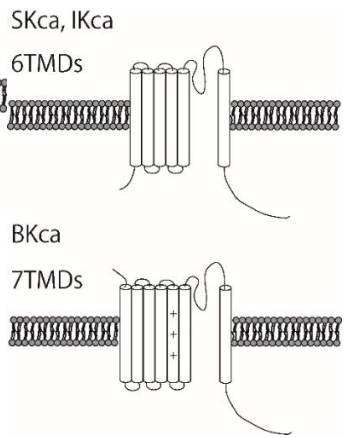
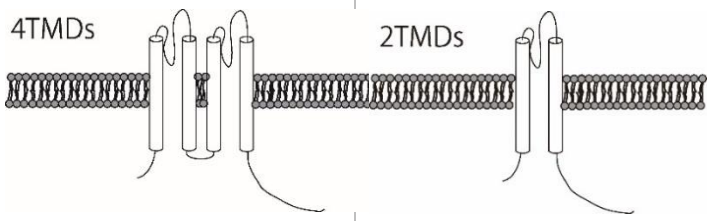
α-subunit membrane topology				
General characteristic of the K^+ channels	Ca^{2+}-insensitive, voltage-sensitive Outward rectification Single-channel conductance: 5 -80 pS	$K_{Ca}1.1$ (BKca, Slo1, or MaxiK) : large-conductance, voltage- and Ca^{2+}-sensitive, outward rectification Single-channel conductance: ≈250 pS $K_{Ca}2.1$-2.3 (SKca), $K_{Ca}3.1$ (IKca) : small- and intermediate-conductance, and voltage independent Single-channel conductance: SKca 10-14 pS, IKca ≈45 pS	Voltage-independent Single-channel conductance: <40pS, except TREK (≈100 pS) TWIK、TREK、TASK、TALK、THINK、TRAAK subfamilies	Single-channel conductance: <30pS and inward rectification K_{IR} subfamily: strong inward rectification K_{ATP} subfamily ($K_{IR}6.1$ & 6.2): intermediate inward rectification, intracellular ATP sensitive

Table 1　Potassium channel families.

Human potassium channels can be broken down into 4 distinct families by their functional characteristics. Kv, voltage-gated; $K_{Ca,}$ calcium activated; K_{2P}, two pore; K_{IR}, inward rectifying; HGCN, HUGO human genome organization nomenclature; IUPHAR, International Union of Pharmacology nomenclature: TMDs, transmebrane domains;　+; voltage sensor