

*Current Perspective***Endocytic Regulation of Voltage-Dependent Potassium Channels in the Heart**Kuniaki Ishii^{1,*}, Ikuo Norota¹, and Yutaro Obara¹¹Department of Pharmacology, Yamagata University School of Medicine, 2-2-2 Iida-nishi, Yamagata 990-9585, Japan

Received September 19, 2012; Accepted October 15, 2012

Abstract. Understanding the regulation of cardiac ion channels is critical for the prevention of arrhythmia caused by abnormal excitability. Ion channels can be regulated by a change in function (qualitative) and a change in number (quantitative). Functional changes have been extensively investigated for many ion channels including cardiac voltage-dependent potassium channels. By contrast, the regulation of ion channel numbers has not been widely examined, particularly with respect to acute modulation of ion channels. This article briefly summarizes stimulus-induced endocytic regulation of major voltage-dependent potassium channels in the heart. The stimuli known to cause their endocytosis include receptor activation, drugs, and low extracellular $[K^+]_o$, following which the potassium channels undergo either clathrin-mediated or caveolin-mediated endocytosis. Receptor-mediated endocytic regulation has been demonstrated for Kv1.2, Kv1.5, KCNQ1 (Kv7.1), and Kv4.3, while drug-induced endocytosis has been demonstrated for Kv1.5 and hERG. Low $[K^+]_o$ -induced endocytosis might be unique for hERG channels, whose electrophysiological characteristics are known to be under strong influence of $[K^+]_o$. Although the precise mechanisms have not been elucidated, it is obvious that major cardiac voltage-dependent potassium channels are modulated by endocytosis, which leads to changes in cardiac excitability.

Keywords: endocytosis, voltage-dependent potassium channel, cardiac excitability, clathrin, caveolin

1. Introduction

Excitable cells adjust their biophysical properties to the functional demand by modulating ion channel activity. Macroscopic channel activity is determined by the number of surface ion channels and the single channel properties. Thus, cells can modulate their channel activity either quantitatively (changing the number) or a qualitatively (changing the property). Modulation of voltage-dependent ion channels has a profound effect on the activity of excitable cells. The most extensively studied mechanism of voltage-dependent ion channel modulation is the qualitative regulation by direct serine/threonine phosphorylation of the channel proteins. For example, β -adrenergic receptor stimulation phosphory-

lates the α -subunit of cardiac voltage-dependent Ca^{2+} channels, which enhances Ca^{2+} currents and produces positive chronotropic and inotropic effects (1). At the same time, β -adrenergic receptor stimulation enhances the cardiac slow delayed rectifier K^+ current via phosphorylation of the α -subunit, which acts to prevent the excessive prolongation of action potential duration (APD) due to increased inward Ca^{2+} currents (2). In addition to serine/threonine phosphorylation, tyrosine phosphorylation can also modulate ion channel activity. The Kv1.2 channel was the first voltage-dependent potassium channel reported to be regulated by tyrosine phosphorylation (3). It was later found that the tyrosine phosphorylation-induced suppression of Kv1.2 currents was not due to changes in biophysical properties of the channel, but rather due to endocytosis of the channel protein, which was the first report that voltage-dependent potassium channels could be modulated by endocytosis (4).

There are multiple types of voltage-dependent potassium channels in the heart that contribute to cardiac ac-

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Published online in J-STAGE on November 20, 2012 (in advance)
doi: 10.1254/jphs.12R12CP

tion potential repolarization. Based primarily on electrophysiological properties, the potassium channels are divided into two major classes: transient outward potassium channels (I_{to}) and delayed outwardly rectifying potassium channels (I_K). Kv1.2 is the ultra-rapid delayed rectifier potassium channel that plays a particularly important role in rodent hearts. In the human heart, the major voltage-dependent potassium channels are Kv1.5 (the ultra-rapid delayed rectifier), hERG (the rapid delayed rectifier), KCNQ1/KCNE1 (the slow delayed rectifier), and Kv4.3 (the transient outward) (5). Of these, three delayed rectifier potassium channels (Kv1.5, hERG, and KCNQ1/KCNE1) play essential roles in terminating cardiac action potentials. It is now known that many cardiac voltage-dependent potassium channels undergo endocytic regulation. In this brief article, we review recent studies on endocytosis of potassium channels, with a particular focus on stimulus-induced endocytosis of the human cardiac delayed rectifier potassium channel (Fig. 1).

2. Two major pathways involved in potassium channel endocytosis

Endocytic pathways can be largely divided into clathrin-dependent and clathrin-independent (6). The clathrin-dependent pathway is the most studied and has provided a large body of knowledge on endocytosis. By contrast, the clathrin-independent pathway has been less well characterized, although the presence of many different clathrin-independent pathways has recently been established. Of these, caveolin-dependent endocytosis is the most widely studied. There is now strong evidence that cardiac voltage-dependent potassium channels can be internalized by clathrin-dependent or caveolin-dependent pathways.

2.1. Clathrin-dependent endocytosis

Clathrin-dependent endocytosis involves formation of clathrin-coated pits, invagination of the coated pits, and pinching-off of the pits to form the coated vesicles (7).

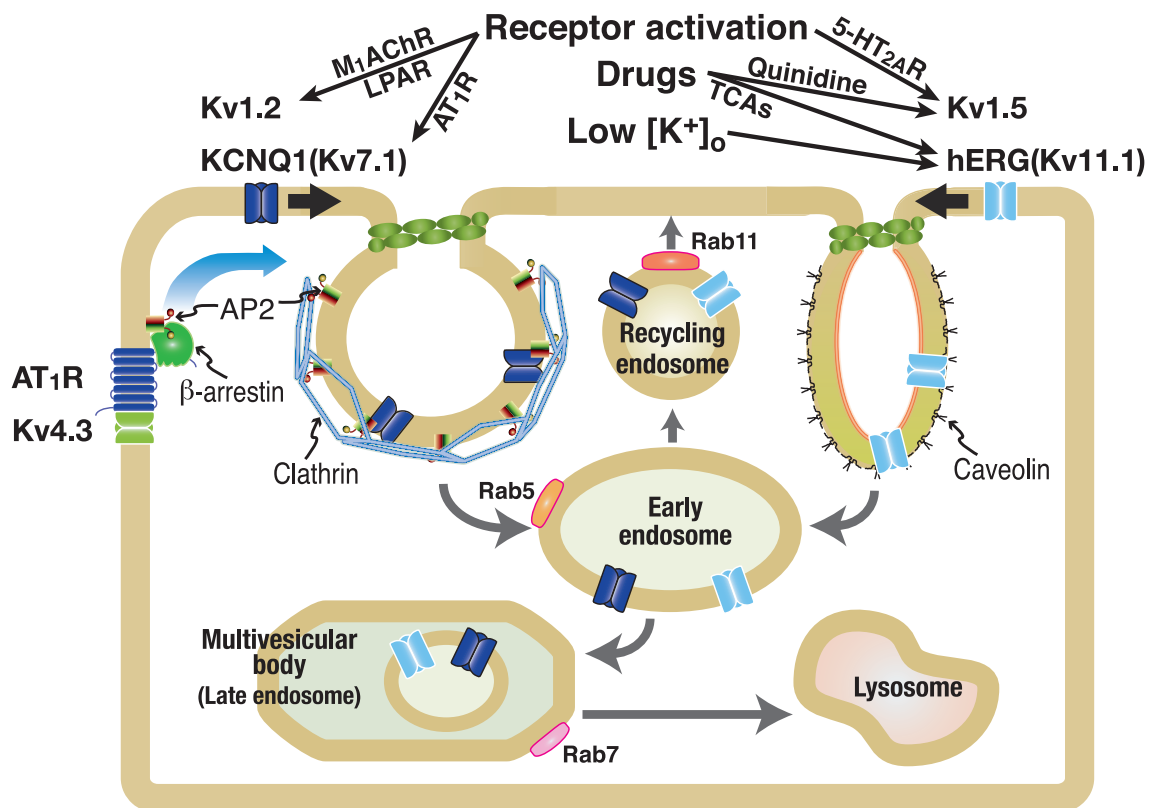


Fig. 1. Stimulus-induced endocytosis of cardiac voltage-dependent potassium channels. Kv1.2 and KCNQ1 are internalized via clathrin-dependent endocytosis by receptor activation and quinidine; quinidine-induced Kv1.5 internalization has not been clearly demonstrated to be caveolin-dependent. hERG is internalized via caveolin-dependent endocytosis by low [K⁺]_o and some tricyclic antidepressants: desipramine- and amoxapine-induced hERG internalization has not been clearly demonstrated to be caveolin-dependent. Kv4.3 is indirectly internalized with AT₁R via clathrin-dependent endocytosis. M₁AChR, M₁ muscarinic ACh receptor; LPAR, lysophosphatidic acid receptor; AT₁R, angiotensin II receptor type 1; 5-HT_{2A}R, 5-HT_{2A} receptor; TCAs, tricyclic antidepressants.

Many factors are involved in clathrin-dependent endocytosis, however the major players are three proteins, clathrin, adaptor protein 2 (AP2), and dynamin. Clathrin in the form of a three-legged triskelion assembles into a polygonal lattice, which binds to the plasma membrane via adaptor proteins including AP2 to form clathrin-coated pits. Transmembrane cargos are recruited to the clathrin-coated pits by direct or indirect interactions with the clathrin-associated adaptor complex AP2. The cargo selection is achieved by recognition of internalization motifs in the cytoplasmic regions of cargo by adaptor proteins. The most well-known internalization signals are the tyrosine-based YXX Φ and dileucine-based [DE]XXXL[LI] motifs. The clathrin-coated pits invaginate inward with the help of various accessory proteins until they form a neck structure that connects the forming vesicle to the plasma membrane. The connected vesicles are pinched-off at the neck by the large GTPase, dynamin, to form free clathrin-coated vesicles.

2.2. Caveolin-dependent endocytosis

Caveolae are a specialized form of lipid rafts, with a flask-shaped morphology that is generated by assembly of caveolins, the coat components. Caveolins insert into the cytoplasmic leaflet of the membrane lipid bilayer to form a hairpin structure, exposing both the N- and C-termini to the cytoplasmic phase of the membrane. Caveolins directly bind with cholesterol, and loss in membrane cholesterol leads to disassembly of the caveolae structures. There are three caveolin subtypes, caveolin-1 and caveolin-2, which are found in non-muscle cells, and caveolin-3, found in muscle cells. Unlike clathrin-dependent endocytosis, the mechanism by which cargo is recruited in caveolae is not understood. However, several proteins including ion channels are known to interact with caveolins, which may result in concentration of the cargo proteins in caveolae. Although the importance of caveolins has been well established, their precise function and regulation in caveolae formation remains widely studied. Like clathrin-dependent endocytosis, dynamin is required to pinch-off the caveolae vesicles (8).

3. Stimulus-induced endocytosis of potassium channels

3.1. Receptor-mediated endocytosis

3.1.1. Kv1.2

The Kv1.2 channel is directly tyrosine phosphorylated and suppressed by several stimuli that include activation of G-protein-coupled receptors. Suppression of Kv1.2 by M₁ muscarinic ACh receptor activation was the first example of tyrosine phosphorylation-dependent modulation of voltage-dependent ion channels (3). The suppression

of Kv1.2 current is due to endocytosis of the Kv1.2 channel from the cell surface via a clathrin-dependent mechanism. The actin-binding protein cortactin and the small GTPase RhoA play important roles in Kv1.2 channel endocytosis. Cortactin links Kv1.2 to the actin cytoskeleton, stabilizing Kv1.2 expression on the cell surface. Tyrosine phosphorylation of Kv1.2 reduces the interaction between cortactin and Kv1.2, which leads to endocytosis of the Kv1.2 channel (9). RhoA is a key modulator of actin dynamics and its activity is required for Kv1.2 endocytosis. ROCK, a downstream effector of RhoA, affects Kv1.2 trafficking through LIM kinase / cofilin (10). LIM kinase activated by ROCK phosphorylates cofilin (a major actin depolymerizing factor), thereby inactivating it, which is thought to modulate endocytosis by altering actin dynamics. The lysophosphatidic acid receptor (LPAR) has been shown to elicit Kv1.2 endocytosis through the RhoA/ROCK signaling pathway (10). In addition to the tyrosine kinase-dependent endocytosis, trafficking of Kv1.2 has been reported to be regulated by cAMP (11). Further, in contrast to stimulus-induced endocytosis, steady-state endocytosis of Kv1.2 has been suggested to be caveolin-dependent (10). Internalization of Kv1.2 has been recently shown in native neuronal cells (12).

3.1.2. Kv1.5

Kv1.5 is the best-studied cardiac voltage-dependent potassium channel for endocytic regulation, and it is responsible for the ultra-rapid delayed rectifier potassium currents (I_{Kur}) in the human atrium. Kv1.5 is also expressed in the pulmonary artery where serotonin (5-HT) and Kv1.5 are thought to have an etiological role in pulmonary hypertension (PH). Activation of the 5-HT_{2A} receptor by 5-HT was reported to cause internalization of the Kv1.5 channel through activation of tyrosine kinase (13). Kv1.5 contains proline-rich regions that bind to the Src homology 3 (SH3) domain, and Src tyrosine kinases were reported to bind and phosphorylate Kv1.5, leading to suppression of the current (14). However, unlike Kv1.2, direct tyrosine phosphorylation of the channel has not been clearly demonstrated as a mechanism of receptor-mediated Kv1.5 endocytosis. Although 5-HT-induced Kv1.5 endocytosis was shown to be caveolin-dependent in vascular myocytes, the localization of Kv1.5 in caveolae in the heart remains controversial, with some reports demonstrating the direct interaction of Kv1.5 and caveolin, while others showing no interaction (15, 16). One of the positive reports has shown that a membrane-associated guanylate kinase (MAGUK), SAP97, and caveolin-3 form a scaffolding complex that can recruit Kv1.5 to a tripartite complex in the heart (16). Rab GTPases are intimately involved in vesicular trafficking in eukaryotic

cells. Among them, Rab5 is known to play an important role in endocytosis and early endosomal formation. However, consensus has not been reached on the involvement of Rab5 in Kv1.5 endocytosis. Several studies have suggested dynein motor involvement in the retrograde trafficking of Kv1.5 from the cell surface (17). Although the mechanisms of Kv1.5 endocytosis have not been fully elucidated, it is likely that at least some of the receptor-mediated Kv1.5 endocytosis is caveolin-dependent.

3.1.3. KCNQ1

KCNQ1 (Kv7.1) is the α -subunit of the slow delayed rectifier potassium channel (I_{Ks}), and loss-of-function mutation of KCNQ1 causes long QT syndrome (LQTS) type 1. Reduction of outward potassium currents prolongs ventricular APD, which can result in LQTS. KCNQ1 together with KCNE1 (β -subunit) forms the I_{Ks} channel. In contrast to Kv1.2 and Kv1.5, which belong to the Shaker subfamily of Kv channels, activation of tyrosine kinase enhances, rather than suppresses, KCNQ1 currents (18). Accordingly, it has not been demonstrated that tyrosine kinase activation internalizes KCNQ1. However, activation of angiotensin II receptor type 1 (AT_1R) causes internalization of KCNQ1, which is likely via a clathrin- and dynamin-dependent pathway (M. Okazaki et al., unpublished data). AT_1R -mediated endocytosis of KCNQ1 does not require KCNE1 and is completely blocked by PKC inhibition. PKC phosphorylation sites on the C-terminal of KCNQ1 seem to be responsible for the endocytosis (M. Okazaki et al., unpublished data). Recently, PKC activation was reported to cause internalization of KCNQ1/KCNE1 channels via a dynamin-dependent pathway, in which the presence of KCNE1 is essential (19). Further, the downstream effector of PKC activation was shown to be AMP-activated protein kinase (AMPK), which internalizes KCNQ1 (without KCNE1) through an E3 ubiquitin ligase, Nedd4-2, in epithelial Madin-Darby canine kidney (MDCK) cells (20). It has been also shown that Nedd4-2 directly ubiquitinates KCNQ1 protein to internalize KCNQ1 (without KCNE1) in a heterologous expression system (21). Thus, although the KCNQ1/KCNE1 channel is definitely internalized by PKC activation, the underlying mechanisms are complicated and require further investigation. As for Kv1.5, Rab5-dependent endocytosis was reported for KCNQ1 endocytosis, although its involvement in the receptor-mediated endocytosis is not clear (22).

3.1.4. Kv4.3

In the human and canine heart, the molecular counterpart of the transient outward potassium current (I_{to}) that

contributes to phase 1 repolarization is thought to be Kv4.3. Direct interaction of c-Src tyrosine kinase and Kv4.3 was reported to increase the Kv4.3 current (23). Enhancement of the Kv4.3 current by tyrosine kinase is similar to KCNQ1 and opposite to Kv1.2 and Kv1.5. With respect to endocytosis, activation of AT_1R was reported to suppress the Kv4.3 current through internalization of the receptor and channel complex (24). Kv4.3 has a β -subunit KChIP2 that enhances delivery of Kv4.3 to the cell surface. The components of the complex that were internalized by AT_1R activation were AT_1R , Kv4.3, and KChIP2. AT_1R -mediated Kv4.3 internalization is probably indirect and occurs via the well-known mechanism of G-protein-coupled receptor endocytosis, which involves receptor phosphorylation and binding of β -arrestin.

3.2. Drug-induced endocytosis

3.2.1. Kv1.5

Quinidine, a class Ia antiarrhythmic drug, has class I activity (sodium channel blocking) and class III activity (potassium channel blocking), and it is used for atrial and ventricular tachyarrhythmias. As previously described, Kv1.5 is the molecular counterpart of I_{Kur} in human atrial myocytes. In addition to the well-known direct pore-blocking effect of quinidine on the Kv1.5 channel, a novel mechanism for Kv1.5 suppression was recently reported, which is quinidine-induced endocytosis of Kv1.5 (25). Interestingly, the Kv1.5 current suppressed by this mechanism was recovered upon washout of quinidine, which was driven by a population of the internalized Kv1.5 channels that recycled back to the plasma membrane with the same time constant as constitutive recycling. By contrast, chronic treatment of quinidine decreased the total number of Kv1.5 channels on the cell surface by diverting the internalized channel from recycling to the proteasomal degradation pathway. Quinidine-induced internalization of Kv1.5 is dynein-dependent and calcium-dependent. Thus, previous electrophysiological experiments would not have detected the internalization as the internal solution of the patch pipette generally contains a calcium-chelating agent. Importantly, quinidine-induced endocytosis of Kv1.5 was demonstrated in both HL-1 atrial myocyte cell lines and in native cardiac myocytes. It is possible that other antiarrhythmic drugs besides quinidine may exert their effect on Kv1.5 channel through a similar endocytic mechanism. In addition, in a clinical setting, antiarrhythmic drugs are usually administered chronically, which, in the case of quinidine, is likely to cause an irreversible decrease of cell surface Kv1.5 channels.

3.2.2. hERG

hERG (Kv11.1) forms the rapid delayed rectifier potassium channel (I_{Kr}), whose loss-of-function mutation causes LQTS type 2. Although more than 10 types of congenital LQTS have been reported, acquired LQTS typified by drug-induced LQTS is much more common than congenital LQTS. The mechanism of drug-induced LQTS that can lead to life-threatening arrhythmia is considered to involve (1) blocking of the hERG channel and (2) impairment of forward trafficking of the hERG protein to the cell surface. In addition to these two mechanisms, drug-induced endocytosis of hERG protein was recently reported (26, 27). Amoxapine and desipramine, the tricyclic antidepressants, can cause acquired LQTS through inhibition of I_{Kr} , and they were reported to cause internalization of hERG protein when acutely applied to the HEK cells expressing the hERG channel. Desipramine was also shown to cause poly-ubiquitination of hERG protein, leading to its degradation (26), which is in contrast to the mono-ubiquitination observed when hERG is internalized by low extracellular potassium (see below). In addition, their chronic treatment can disturb forward trafficking of the hERG protein. As such, the drug-induced changes in ion channel density should be considered with respect to both therapeutic and side effects. Considering the drug-induced endocytosis of hERG and Kv1.5, it is possible that endocytosis of ion channels can represent a novel target for drug discovery.

3.3. Low potassium-induced endocytosis

3.3.1. hERG

Hypokalemia is a known risk factor for LQTS and sudden cardiac death, while elevation of potassium concentration corrects LQTS in certain cases. A potential explanation for the deleterious effect of low extracellular potassium on LQTS was recently presented (28). In that study, when HEK cells expressing hERG were subjected to 0 mM $[K^+]_o$, the hERG channel was internalized over several hours. The internalized hERG channel was subsequently degraded through the multivesicular body (MVB) / lysosomal pathway, and overnight treatment with 0 mM $[K^+]_o$ resulted in almost complete loss of the mature hERG protein on the cell surface. The hERG endocytosis by low $[K^+]_o$ was triggered by mono-ubiquitination of the channel protein and was caveolin-dependent, and the ubiquitin molecule was removed from the internalized hERG channel before entering into lysosomes (29, 30). The conformation change into the non-conducting state induced by low $[K^+]_o$ is thought to make the channel susceptible to mono-ubiquitination, resulting in channel endocytosis (30). Although the condition of 0 mM extracellular K^+ is extreme, the authors also demon-

strated that low, but physiological, levels of $[K^+]_o$ prolonged cardiac APD and decreased hERG channel on the cell surface using low- K^+ diet-fed rabbits (28). Thus, it is likely that low $[K^+]_o$ -induced internalization of hERG channel occurs in vivo, which leads to APD prolongation and exacerbation of LQTS.

4. Conclusions

Major cardiac voltage-dependent potassium channels are regulated in a quantitative manner via endocytosis. However, the mechanisms responsible for endocytosis are complicated and are not fully understood. Accumulating evidence suggests that there are fundamental differences in the mechanisms used by each potassium channel to undergo endocytosis (Fig. 1). For example, some channels are internalized via clathrin-dependent endocytosis, while others are internalized via caveolin-dependent endocytosis. This research field is still at an early stage, and future studies are required to examine the role of endocytic regulation of ion channels in physiological and pathophysiological contexts. Since ion channel activity can be regulated qualitatively and quantitatively, it is important to consider the quantitative regulation of voltage-dependent ion channels in addition to the well-known qualitative regulation when examining alterations in cellular excitability.

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