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Review

Impaired Adrenergic/Protein Kinase A Response of Slow Delayed Rectifier Potassium Channels as a Long QT Syndrome Motif: Importance and Unknowns

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ABSTRACT

The slow delayed rectifier potassium current (I_{Ks}) significantly contributes to cardiac repolarization under specific conditions, particularly at stimulation by the protein kinase A (PKA) during increased sympathetic tone. Impaired PKA-mediated stimulation of I_{Ks} channels may considerably aggravate dysfunction of the channels induced by mutations in the *KCNQ1* gene that encodes the structure of the α-subunit of I_{Ks} channels. These mutations are associated with several subtypes of inherited arrhythmias, mainly long QT syndrome type 1, less commonly short QT syndrome type 2, and atrial fibrillation. The impaired PKA reactivity of I_{Ks} channels may significantly increase the risk of arrhythmia in these patients. Unfortunately, only approximately 2.7% of the *KCNQ1* variants identified as putatively clinically significant have been studied with respect to this problem. This review summarizes the current knowledge in the field to stress the importance of the PKA-mediated regulation of I_{Ks} channels, and to appeal for further

RÉSUMÉ

La composante lente du courant potassique à rectification retardée (I_{Ks}) contribue significativement à la repolarisation cardiaque dans des conditions particulières, notamment lors de la stimulation par la protéine kinase A (PKA) au cours de l'augmentation du tonus sympathique. La dégradation de la stimulation médiée par la PKA des canaux I_{Ks} peut considérablement aggraver la dysfonction des canaux induits par les mutations dans le gène *KCNQ1* qui code la structure de la sous-unité α des canaux I_{Ks}. Ces mutations sont associées à de nombreux sous-types d'arythmies héréditaires, principalement le syndrome du QT long de type 1, moins fréquemment le syndrome du QT court de type 2 et la fibrillation auriculaire. La dégradation de la réactivité de la PKA des canaux I_{Ks} peut augmenter significativement le risque d'arythmie chez ces patients. Malheureusement, seuls environ 2,7 % des variants *KCNQ1* considérés prétendument importants sur le plan clinique ont fait l'objet d'études sur ce problème. La

Cardiac repolarization is guided by a fine balance between various depolarizing and repolarizing currents. The slow delayed rectifier potassium current (I_{Ks}) is one of the currents that contributes to the repolarizing phase of cardiac action potential (AP), especially during increased β-adrenergic stimulation through activation of the protein kinase A (PKA). Mutations in the *KCNQ1* gene associated with inherited arrhythmogenic syndromes may alter not only the channel trafficking and gating, but also the PKA-mediated stimulation of the I_{Ks} channel. This may result in an aggravated clinical phenotype. Unfortunately, only a minority of the published studies provide data on the impact of particular *KCNQ1* mutations on the PKA regulation of the I_{Ks} channel. In this review, we summarize the current knowledge of this topic, focusing especially on the analysis of altered PKA regulation in previously studied *KCNQ1* mutations.

The Cardiac I_{Ks} Channel and Regulation of Its Function

The cardiac I_{Ks} channel is a heteromeric protein complex composed of 2 different proteins, the pore-forming α-subunit Kv7.1 and a regulatory β-subunit KCNE (most often the KCNE1, minK), which significantly modulates the gating properties of the channel. The structure of the Kv7.1 protein, encoded by the *KCNQ1* gene, resembles that of other voltage-gated potassium channels (Fig. 1). The C-terminal region comprises 4 helices: helices A and B serve as a binding site for regulatory molecules (calmodulin and phosphatidylinositol 4,5-bisphosphate [PIP_2]), whereas helices C and D participate especially in the channel trafficking and assembly of the Kv7.1 subunits into tetramers.[1] Helix D is also known to be involved in the PKA regulation of the channel (see later).

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analysis of this regulation in *KCNQ1* mutations associated with inherited arrhythmogenic syndromes. On the basis of the facts summarized in our review, we suggest several new regions of the α-subunit of the I_{Ks} channels as potential contributors to PKA stimulation, namely the S4 and S5 segments, and the S2-S3 and S4-S5 linkers. Deeper knowledge of mechanisms of the impaired PKA response in mutated I_{Ks} channels may help to better understand this regulation, and may improve risk stratification and management of patients suffering from related pathologies.

présente revue résume les connaissances actuelles dans le domaine pour souligner l'importance de la régulation médiée par la PKA des canaux I_{Ks} et pour demander d'autres analyses sur cette régulation dans les mutations *KCNQ1* associées aux syndromes arythmogènes héréditaires. Selon les faits résumés dans notre revue, nous montrons que plusieurs nouvelles régions de la sous-unité α des canaux I_{Ks} contribuent potentiellement à la stimulation de la PKA, à savoir les segments S4 et S5, et les lieurs S2-S3 et S4-S5. Des connaissances approfondies des mécanismes de la dégradation de la réponse de la PKA dans les canaux I_{Ks} mutés peuvent aider à mieux comprendre cette régulation et à améliorer la stratification du risque et la prise en charge des patients souffrant de pathologies associées.

The exact stoichiometry of Kv7.1 and KCNE1 has been debated. According to Nakajo et al.,[2] the ratio Kv7.1:KCNE1 seems to vary between 4:1 and 4:4 depending on the relative expression levels of these proteins. However, other authors have argued that a 4:2 ratio is the only correct stoichiometry of Kv7.1 and KCNE1 required for the proper channel formation.[3] A recent robust structural KCNQ1/KCNE1 model used this 4:2 stoichiometry as well.[4]

The Kv7.1-KCNE1 interaction sites seem to be located at several places within the proteins. The N-terminus and the first half of the transmembrane domain (positions 44-55) of KCNE1 were demonstrated to interact extensively with the Kv7.1 voltage-sensing domain (the extracellular ends of S1, and S1-S2 and S3-S4 linkers) and the Kv7.1 pore-domain (S5-P loop and P loop-S6 linkers, and the extracellular ends of S5 and S6).[5-7] More limited contacts were apparent between the second half of the KCNE1 transmembrane domain (positions 56-71), and the Kv7.1 S5 segment and S4-S5 linker.[7] The KCNE1 C-terminus was observed to interact with the C-terminus of KCNQ1,[8] particularly with its part below the S6 activation gate,[9] and with the S4-S5 linker of KCNQ1.[9] Recently, Jalily Hasani et al.[4] showed the importance of the interaction of the KCNE1 N-terminus with Kv7.1, particularly with its S1-S2, S3-S4, and S5-S6 linkers, and S3 segment. However, as Xu et al.[7] have suggested, other Kv7.1 regions can be modulated by KCNE1 as well, via allosteric interactions that may considerably affect the I_{Ks} channel function.

The I_{Ks} channel is regulated in many ways, including binding cofactors and activation of various signalling pathways. For example, PIP_2, adenosintrisphosphate, calmodulin in complex with Ca^{2+} (Ca^{2+}/CaM complex), phospholipase C, and protein kinase C play important roles. All the various ways of regulation of the I_{Ks} channel function are important, but we will concentrate on the regulation mediated by the PKA in this review. This regulation is schematically illustrated in Figure 1. PKA is a tetrameric enzyme dependent on the cyclic adenosine monophosphate (cAMP). As is well known, cAMP binds to the regulatory subunits of PKA, and this interaction causes their dissociation from the catalytic subunits, leading to PKA activation. The activated PKA phosphorylates various amino acid residues, especially those located in the N-terminal region of the Kv7.1 protein. Besides the generally accepted phosphorylation of serine at position 27 (S27),[10] phosphorylation of serine at position 92 (S92) seems to be also involved. Artificial phosphomimetic missense mutations S92A and S92D resulted in a partially preserved increase of I_{Ks} during PKA stimulation, and PKA regulation of the double mutant S27D/S92D was completely disrupted.[10,11] Recent papers by Thompson et al.[12,13] have confirmed this hypothesis on the single-channel level. The channel dephosphorylation is mediated by another enzyme, protein phosphatase 1 (PP1). The intracellular level of cAMP increases at the stimulation of β-adrenergic receptors coupled with G_s-proteins due to activation of the adenylyl cyclase (AC). Another enzyme, phosphodiesterase (PDE), terminates the PKA response by splitting the cAMP molecule to 5′-AMP.

The PKA-dependent phosphorylation of I_{Ks} channels requires a macromolecular complex, which includes the Kv7.1 tetramer, the KCNE1 modulatory subunits, the enzymes PKA and PP1, and a protein called Yotiao (Fig. 1). Regarding KCNE1 and PKA stimulation of I_{Ks} channels, KCNE1 was demonstrated to play a key role in transduction of Kv7.1 phosphorylation into the increased channel function by Kurokawa et al.[14] In this study, no increase of the current was apparent in the absence of KCNE1 despite the fact that Kv7.1 was phosphorylated.[14] The intracellular C-terminus of KCNE1 was shown to be necessary for the channel response.[15] The interaction between the C-terminal regions of KCNE1 and Kv7.1 leads to conformational changes in the Kv7.1 structure that were suggested to be responsible for shortening the distance between the N- and C-terminal regions of Kv7.1.[8] As discussed by Dvir et al.,[16] this interaction is likely a crucial step in the Yotiao-mediated transfer of PKA toward the N-terminal phosphorylation sites of Kv7.1. The impact of KCNE1 on the PKA-mediated I_{Ks} response was supported by observations that KCNE1 mutations located in the C-terminus of the protein impaired PKA stimulation of the channel, either by reducing phosphorylation of Kv7.1 (due to impaired interaction between Kv7.1 helix C and KCNE1 C-terminus-P127T mutation),[16] or by disturbing the following channel response (but with preserved Kv7.1 phosphorylation-D76N mutation).[14] In contrast, the response to PKA stimulation was not impaired in 2 other C-terminal KCNE1 mutations, W87R and V109I.[14,16]

Yotiao is an A-kinase-anchoring protein (AKAP) encoded by the *AKAP9* gene, which mediates the interaction between the Kv7.1 protein and PKA. Two binding sites for Yotiao are located within Kv7.1. The first one is a part of the N-terminal region, and the second one is found in the C-terminal region—the leucine zipper motif in the helix D. All the above-mentioned enzymes (PKA, PP1, AC, and PDE) associate with

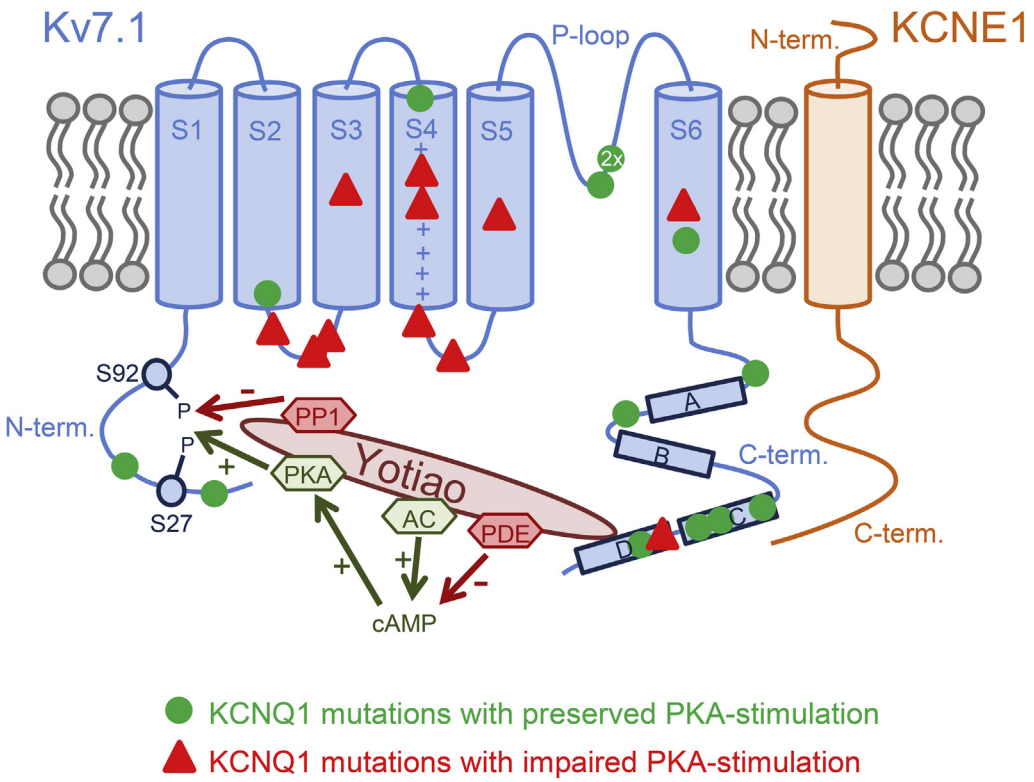

Figure 1. Scheme of the macromolecular complex formed by the pore-forming α-subunit Kv7.1, modulatory β-subunit KCNE1, and anchoring protein Yotiao. The green circles/red triangles indicate mutations with preserved/impaired reactivity on the PKA stimulation. For details, see the text. A, B, C, and D, helices A, B, C, and D; AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; PDE, phosphodiesterase; PKA, protein kinase A; PP1, protein phosphatase 1.

Yotiao. Yotiao itself also serves as a substrate for PKA and directly participates in the modulation of the I_{Ks} channel activity.[17,18] It was also suggested to be directly involved in transduction of the Kv7.1 phosphorylation into the enhanced activity of the I_{Ks} channel.[19] The importance of Yotiao is emphasized by the fact that mutations located in the *AKAP9* gene may be responsible for inherited arrhythmias, just as mutations in other components of the macromolecular complex forming the functional I_{Ks} channel are. For instance, the S1570L mutation in Yotiao significantly impaired PKA-mediated phosphorylation of Kv7.1 and, consequently, completely abolished I_{Ks} reactivity to PKA stimulation.[20]

A direct interaction of the I_{Ks} channel complex with microtubules (specifically the interaction of the N-terminus of Kv7.1 with β-tubulin) was also confirmed to play a major role in the coupling of the channel phosphorylation by PKA to I_{Ks} activation.[21]

Besides the direct effect of PKA on the I_{Ks} channel phosphorylation, its stimulation also increases the affinity of the channel to PIP_2,[10,22] which plays a role in I_{Ks} channel regulation as well.[23] Crosstalk of different signaling pathways is common and may result in modified effects.[24] As another key example, both Ca^{2+}/CaM complex (mediating Ca^{2+}-sensitive stimulation of the I_{Ks} channel) and PKA participate, independently and additively, in I_{Ks} enhancement during increased β-adrenergic stimulation.[25] Such crosstalk is clinically relevant, because a purely separate activation of the particular signalling pathways is unlikely in the complex environment of a living organism. For the purpose of this review, the situation was simplified, and the review focuses merely on the PKA regulation of the I_{Ks} channel.

Cardiac I_{Ks} Channel Function and the Impact of Its Dysfunction on Cardiac Repolarization

The duration of plateau and final repolarization phases of cardiac AP is dependent on a fine balance between depolarizing and repolarizing currents. I_{Ks} is one of the key repolarizing currents in the heart. However, its contribution to cardiac AP repolarization at rest is rather weak[26-31] due to very slow activation, particularly at membrane voltages relevant for AP plateau in control conditions (ie, approximately 0 mV).[26,32] The contribution of I_{Ks} to cardiac repolarization is slightly increased at higher stimulation rates (Fig. 2, C-E, baseline).[27,33,34] It results from incomplete deactivation of I_{Ks} channels and their accumulation in the closed states near to the open state, which facilitates rapid reactivation of the channels (Fig. 3, left vs right panel).[35,36] At rest, however, I_{Kr} is the most powerful repolarizing current, especially in human cardiomyocytes, and I_{Ks} is considered to be a pivotal player in so-called repolarization reserve.[37,38]

Under conditions when PKA is activated, for example, during a stimulation of β-adrenergic receptors, I_{Ks} is considerably increased because of an altered gating of the channels. The channel activation is accelerated (Fig. 3)[39] and its voltage dependence is shifted to more negative membrane

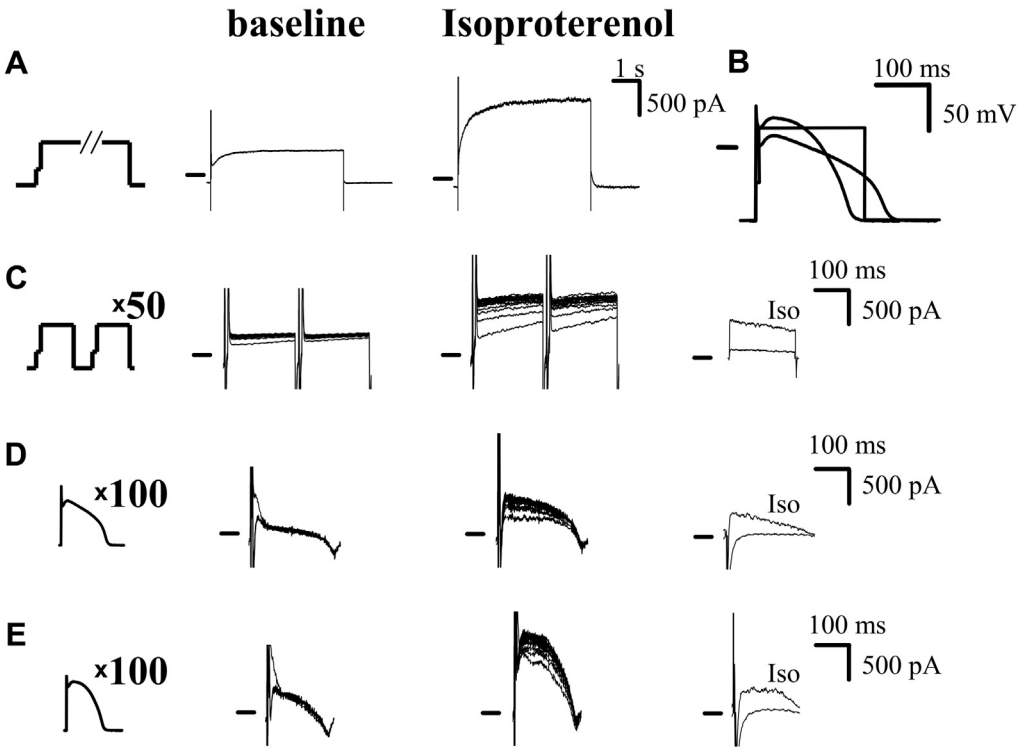

Figure 2. Effect of the shape of depolarization pulse, stimulation frequency, and application of isoproterenol on I_{Ks} accumulation in canine ventricular myocytes. (**A**) Depolarizing 5-s pulse from −80 to +20 mV (1st panel), activated I_{Ks} under basal conditions (2nd panel), and in the presence of 100 nM isoproterenol (3rd panel); stimulation frequency 0.1 Hz. (**B**) Scheme of voltage pulses used for stimulation at fast frequency in parts **C-E**, namely square 200-ms pulse from −80 to +20 mV, baseline action potential (AP) (long, low plateau), AP under isoproterenol (high, short plateau) used in parts **C**, **D**, and **E**, respectively. APs were measured using high-resistance microelectrodes filled with 3 M KCl at the cycle length of 500 ms. (**C-E**) Square pulses (**C**), baseline AP pulses (**D**), isoproterenol-shaped AP pulses (**E**), all with 20-ms interpulse interval (1st panel), induced I_{Ks} accumulation at baseline (2nd panel), and after application of isoproterenol (3rd panel). The 4th panels show accumulated currents measured as the difference between the 100th and the 1st traces in the previous 2 panels. Modified from Stengl et al.[27] with permission from John Wiley and Sons.

voltages.[17,28] On the single-channel level, the cAMP-dependent I_{Ks} increase was shown to be caused by an increased probability of the channel opening, accompanied by a faster kinetics of the openings and a greater occupancy of higher subconductance levels.[12] These changes were mediated preferentially through the changed kinetics of the voltage sensor rather than those of the pore.[12] Moreover, as well known, the membrane voltage during AP plateau is more depolarized and the Ca^{2+} transient is higher under PKA activation.[28,40] All these changes result in a considerable increase of I_{Ks} during AP repolarization, even at slow stimulation rates (Figs. 2A and 3, right panel).[28,39,40-42] Because I_{Ks} deactivation is decelerated under PKA activation, and the accelerated onset of I_{Ks} is significantly faster at the higher rate (Fig. 3, left vs right panel),[39] I_{Ks} accumulates even more at faster stimulation (Fig. 2, C-E).[27-29,39] The increase in function of the I_{Ks} channel under PKA-mediated activation described above notably contributes to accommodating the AP length to an increased heart rate at higher β-adrenergic activation, when calcium influx through the phosphorylated (and thus more active) L-type calcium ($I_{Ca\text{-}L}$) channels can promote formation of early afterdepolarizations. The same changes of I_{Ks} control the atrial repolarization at increased PKA activation.[43] The accumulated I_{Ks} thus reduces the so-called beat-to-beat variability of AP duration (APD), which prevents the potential occurrence of arrhythmias under these conditions.[29,44,45]

Considering all this, it is not surprising that a dysfunction of I_{Ks} channels usually expresses itself in one of 2 conditions: either in the case of a reduced repolarization reserve (eg, an inhibition of I_{Kr} by drugs), and/or during an increased β-adrenergic stimulation (eg, physical or emotional exertion). This was shown in several experimental studies on human and canine ventricular myocytes,[26,28-31,46] and it is also well known from clinical practice. Inherited arrhythmias associated with mutations in the gene-encoding structure of I_{Ks} channels are a typical example of I_{Ks} dysfunction in humans. Mutations in the *KCNQ1* gene are connected mainly with the long QT syndrome (LQTS) type 1 (LQT1), with the short QT syndrome type 2, and with atrial fibrillation (AF) (http://triad.fsm.it/cardmoc/). Although gain-of-function *KCNQ1* mutations are responsible for short QT syndrome type 2 and AF, LQT1 is typically associated with loss-of-function heterozygous *KCNQ1* mutations. Two basic types of the heterozygous loss-of-function mutations may be distinguished. The first type is represented by dominant-negative mutations, that is, mutations leading to a > 50% decrease in I_{Ks} (both the mutated and wild-type [WT] subunits assemble to form the

tetramers; the mutated subunits negatively affect the WT subunits). The second type is haploinsufficiency mutations resulting in a < 50% decrease in I_{Ks}. These mutations are usually caused by a defective transport or coassembly of the KCNQ1 subunits. Symptomatic carriers of the dominant-negative mutations show a longer QT interval and a higher frequency of cardiac events in comparison with carriers of the haploinsufficient mutations.[47] As we might assume, given the important role of PKA-stimulated I_{Ks} in cardiac repolarization, symptoms of LQT1 are mostly observed under an increased sympathetic tone; physical exercise, swimming in particular, often triggers arrhythmic events in these patients.[48,49]

KCNQ1 Mutations and PKA Stimulation

As stated above, physical exercise is a common trigger of cardiac events in LQT1,[48,49] and PKA stimulation is a key way of regulation of the I_{Ks} channel function.[27-29,39] Hence, we would expect that the analysis of PKA stimulation would be an essential aspect of studies dealing with a biophysical analysis of mutations in the gene-encoding structure of the I_{Ks} channel (including the *KCNQ1* gene).

Overview of Current Knowledge

Currently, there are 916 putatively clinically significant variants of the human *KCNQ1* gene reported in the NCBI database (https://www.ncbi.nlm.nih.gov/search/?term=KCNQ1; October 16, 2018). We found data on biophysical testing in only 143 of these variants (Supplemental Table S1). Hence, the functional impact has been studied in approximately 16% of the identified *KCNQ1* mutations.

An unexpectedly high frequency of genetic variation within many Mendelian disease-susceptibility genes has been revealed with the help of next-generation sequencing technologies. This applies to the *KCNQ1* gene as well. At least 10% to 15% of *KCNQ1* rare variants are false-positive, that is, they do not cause any pathology.[50] This phenomenon has led to a high level of uncertainty as to how rare variants should be interpreted, with many being labelled as variants of uncertain significance.[50,51] In an effort to add clarity and uniformity to the classification and reporting of genetic variants in LQTS and other Mendelian disorders, the American College of Medical Genetics and Genomics and the Association for Molecular Pathology released a new set of interpretation and reporting standards in 2015.[52] In these guidelines, results of functional studies play an important role in the classification algorithm of genetic variants. Because of the time and financial demands of complex functional analysis, it is unlikely to be done routinely in each mutation. On the other hand, information gained from such analysis may be crucial for risk stratification and proper therapeutic approach, especially in small families where genotype-phenotype correlations are not possible.

Loss-of-function mutations were identified in 124 of 143 variants, whereas gain-of-function mutations were detected in 17 cases. Both the loss-of-function and gain-of-function mutations were localized throughout the whole protein. Two mutations, Q147R and R231C, localized in the S1-S2 linker and in the S4 segment, respectively, were considered to exert both the loss-of-function and gain-of-function defects.

The PKA response was investigated in only 25 of the 143 biophysically tested mutations (approximately 17.5%) in spite of the fact that PKA stimulation considerably regulates the I_{Ks} channel function (as discussed above).

Characteristics of PKA Stimulation in KCNQ1 Mutations

We investigated in detail reactivity to the PKA stimulation in all *KCNQ1* mutations with known data. The main facts are summarized in Table 1.

Various agents were used to induce PKA stimulation in the studies, depending on the cell type used. A membrane-permeable activator of AC forskolin (1-50 μM) was used most often, either alone or in combination with a nonspecific inhibitor of cAMP (and cyclic guanosine monophosphate [cGMP]) PDEs 3-isobutyl-1-methylxanthine (15-200 μM). Alternatively, cAMP (200-400 μM) was applied through the pipette solution in combination with okadaic acid (0.2 μM), an inhibitor of serine/threonine phosphatases, chiefly PP1 and protein phosphatase 2A.

When we looked at the model systems used in the surveyed studies, we found that the human I_{Ks} channels (KCNQ1/KCNE1/Yotiao) heterologously expressed on a cell line (usually on the human embryonic kidney 293 or the Chinese hamster ovary cells) were the models most often used for the analysis of PKA reactivity. In the study by Matavel et al.,[22] the channel cRNA was injected into the Xenopus oocytes. Although these cell models were all noncardiac, all the models satisfactorily reproduced the PKA-mediated response of I_{Ks} channels (both the current increase and consequent changes of gating properties—Table 1, and Supplemental Table S2), in agreement with the response of native I_{Ks} channels in cardiomyocytes.[28,39] Considering the technical and financial aspects, these heterologous models should not be rejected despite the newly available alternatives. Two other studies used cardiac cell models. In the first study, Li et al.[53] infected neonatal mouse and adult guinea-pig ventricular cardiomyocytes by an adenovirus vector over-expressing human I_{Ks} channels with dominant-negative G306R mutation previously characterized in a heterologous system. The resulting data confirmed the dominant-negative character of the mutation reacting substantially on PKA stimulation. Unfortunately, a deeper analysis was not performed, and to our knowledge the technique was not repeated in any further paper dealing with a *KCNQ1* mutation. It seems, then, that this technique did not show any clear benefit. In the second study, Moretti et al.[54] performed a unique functional analysis of R190Q mutation in patient-specific derived cardiomyocytes. They provided complex data including the PKA response of I_{Ks} and action potential configuration, clearly showing proarrhythmic phenotype of the cells. It is ideal to see signs of arrhythmogenesis directly in patient-specific derived cardiomyocytes. On the other hand, differentiation of such cardiomyocytes is challenging, both technically and financially. Moreover, currently available human derived cardiomyocytes show immature phenotype,

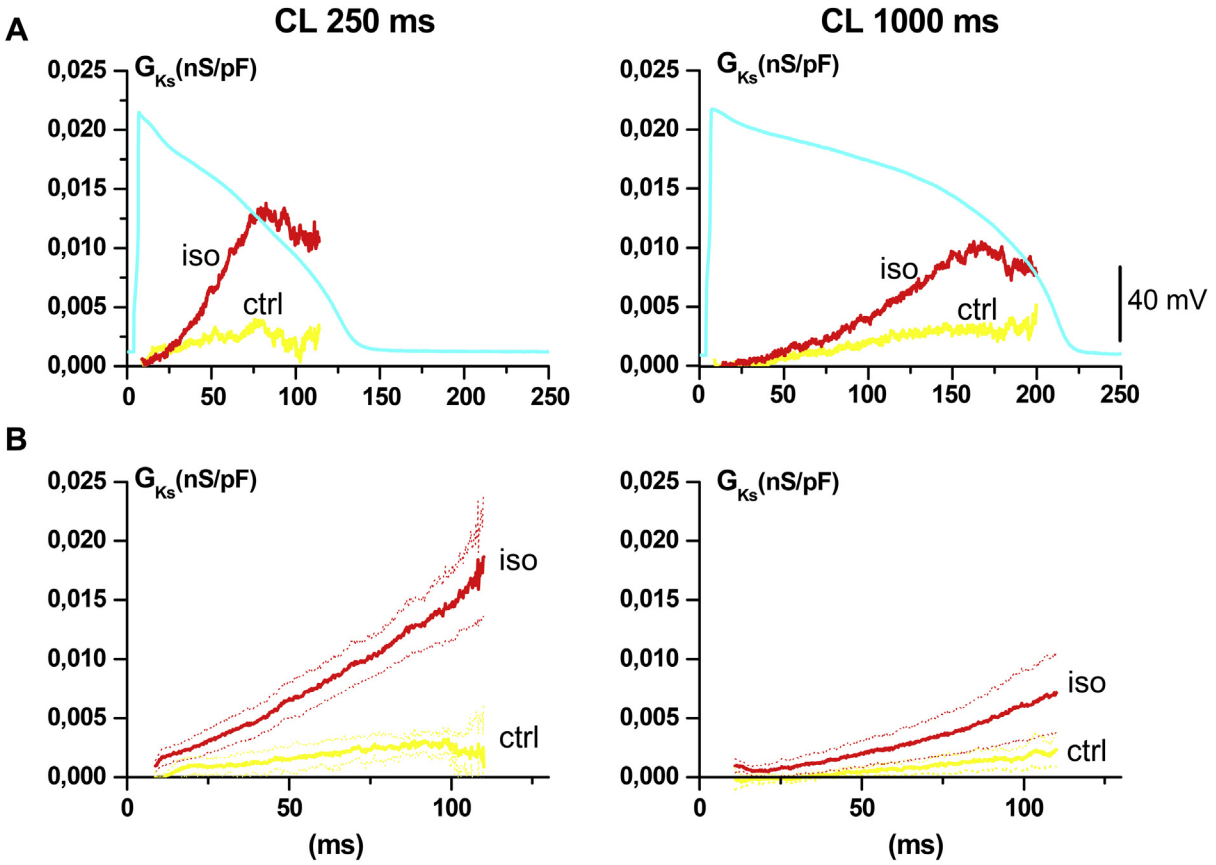

Figure 3. Dependence of I_{Ks} conductance on the cycle length and its modulation by β-adrenergic stimulation in guinea-pig ventricular myocytes. (**A**) Representative example of I_{Ks} conductance (G_{Ks}) changes at cycle length (CL) 250 and 1000 ms during action potential waveform in control (ctrl) and under the effect of isoprenaline (iso). (**B**) Average G_{Ks} profiles (**full lines**) and their standard error of the mean (**dotted lines**); n = 6. Modified from Rocchetti et al.[39] with permission from John Wiley and Sons.

which may considerably limit their use in functional studies. To summarize, we consider the above-mentioned heterologous systems as suitable models for biophysical analysis of the functional impact of *KCNQ1* (and also other cardiac channel genes) mutations. If possible, more complex, clinically relevant analysis should be subsequently performed on human derived cardiomyocytes with mutations selected based on data from the heterologous systems.

As expected, a considerable increase of I_{Ks} was observed after cAMP-level-increasing agents interacted with WT channels. The increase ranged between 30% and 250%, with a mean I_{Ks} activation of approximately 87% (n = 18; Table 1). More detailed characteristics of the PKA reactivity were available in 10 mutations (7 studies; Supplemental Table S2). The I_{Ks} increase was accompanied by a leftward shift of the voltage dependence of activation of approximately 13 mV (7 studies), an accelerated activation (time constant of activation τ_a shorter by approximately 19% on average; 4 studies), and a decelerated deactivation (time constant of deactivation τ_d longer by approximately 21% on average; 4 studies) in WT channels. All of these changes were qualitatively in agreement with cardiac cell data in the literature.[28,39] The responses of mutated channels varied, as discussed below.

Mutations With Preserved or Increased PKA Response

PKA stimulation was not impaired in 14 of the 25 *KCNQ1* mutations analyzed (for the approximate location of these mutations, see the green circles in Fig. 1). The channel activation varied between 30% and 150% in the homozygously expressed mutated channels, with a mean I_{Ks} activation of approximately 84% (n = 7; Table 1; note that the excessive value in K557E mutation was omitted—it likely resulted from the very small absolute I_{Ks} magnitude in the homozygous K557E channels that made the evaluated current amplitude very sensitive to noise). The respective heterozygously expressed mutated channels were activated at an average by approximately 100%, with the values ranging from 28% to 170% (n = 9; Table 1). These relative responses to PKA stimulation were comparable with the responses of WT channels. If available, other relevant functional characteristics were also changed in agreement with WT channels in most cases (the upper part of Supplemental Table S2).

The I_{Ks} increment was comparable with WT channels in most of the mutations (eg, mutations R14C, A46T, T312I, and K422T); however, it was even greater in others (eg, G168R, A344V, R366Q, and R555C). All the latter

Table 1. Overview of *KCNQ1* mutations reactivity on stimulation by protein kinase A

Mutation	Location	Function/phenotype	PKA stimulation					Ref
			Induced by	+/−	WT	mut-hom	mut-het	
R14C	N-terminus	GOF/AF	fors, 1 μM	+	60%	60%	-	63
A46T	N-terminus	GOF/LQT1, AF	iso, 1 μM	+	111%	107%	-	64
G168R	S2	LOF/LQT1	fors, 10 μM	+	80%	-	160%	55
R174C	S2-S3	LOF/LQT1	fors, 50 μM	−	38%	20%	-	22
G189R	S2-S3	LOF/LQT1	fors, 10 μM	−	80%	-	10%	55
R190Q	S2-S3	LOF/LQT1	fors, 10 μM	−	80%	-	25%	55
			epi, 1 μM	−	197%	-	11%	54
S209F	S3	GOF/LQT1	8CPT, 200 μM + OA, 0.2 μM	−	250%	0%	-	65
S225L	S4	LOF/LQT1	fors, 10 μM	+	80%	-	60%	55
R231H	S4	GOF/AF	fors, 10 μM + IBMX, 200 μM	−	100%	22%	-	62
I235N	S4	LOF/LQT1	fors, 10 μM + IBMX, 200 μM	−	52%	-	20%	61
R243C	S4-S5	LOF/LQT1	fors, 10 μM	−	80%	-	20%	55
			fors, 50 μM	−	38%	21%	-	22
V254M	S4-S5	LOF/LQT1	fors, 10 μM	−	80%	-	-10%	55
G269S	S5	LOF/LQT1	fors, 5 μM + IBMX, 15 μM	−	95%	-	40%	59
			iso, 0.1 μM	−	102%	35%	37%	
G306R	P-loop	LOF/LQT1	fors, 10 μM + IBMX, 100 μM	+	45%	NA	NA	53
T312I	P-loop	LOF/LQT1	fors, 10 μM	+	80%	-	80%	55
T312del	P-loop	LOF/LQT1	fors, 10 μM + IBMX, 100 μM	+	51%	-	50%	66
A341V	S6	LOF/LQT1	cAMP, 300 μM + OA, 0.2 μM	−	63%	3%	3%	56
A344V	S6	LOF/LQT1	cAMP, 300 μM + OA, 0.2 μM	+	63%	150%	107%	56
R366Q	C-terminus	LOF/LQT1	fors, 50 μM	+	38%	111%	-	22
K422T	C-terminus	LOF/LQT1	fors, 10 μM	+	30%	-	28%	67
R539W	C-terminus	LOF/LQT1	cAMP, 400 μM + OA, 0.2 μM + fors, 10 μM	+	45%	63%	-	68
R555C	C-terminus	LOF/LQT1	fors, 10 μM	+	80%	-	170%	55
			fors, 50 μM	+	38%	68%	-	22
K557E	C-terminus	LOF/LQT1	cAMP, 200 μM + OA, 0.2 μM	+	55%	450%	106%	42
			8CPT, 250 μM + OA, 0.2 μM	+	154%	-	143%	16
G589D	C-terminus	LOF/LQT1	cAMP, 300 μM + OA, 0.2 μM	−	63%	-	12%	56
A590T	C-terminus	LOF/*	8CPT, 300 μM + OA, 0.2 μM	+	43%	30%	-	69

The mixture of cAMP and OA was present in the pipette solution whereas others PKA-stimulators were applied to the outer membrane surface.

+/−, preserved/absent PKA activation, respectively; 8CPT, 8-(4-chlorophenylthio) adenosine 3′,5′-cyclic monophosphate; AF, atrial fibrillation; cAMP, cyclic adenosine monophosphate; epi, epinephrine; fors, forskolin; GOF, gain-of-function; IBMX, 3-isobutyl-1-methylxanthine; iso, isoproterenol; het, heterozygous; hom, homozygous; LOF, loss-of-function; LQT1, long QT syndrome type 1; mut, mutation; NA, not available in the paper, a substantial increase mentioned; OA, okadaic acid; PKA, protein kinase A; WT, wild-type.

* Mild/borderline QT prolongation.

mutations were characterized as loss-of-function mutations. The preserved or even increased PKA stimulation in these mutated I_{Ks} channels may prevent excessive dysfunction of the channels during exertion in carriers of the loss-of-function *KCNQ1* mutations.

Unfortunately, the preserved response of mutated I_{Ks} channels to PKA stimulation is obviously insufficient for preserved proper cardiac repolarization, because many carriers of these mutations showed clinical symptoms. This view is supported by the findings of Spätjens et al.,[42] who analysed K557E mutation with preserved PKA stimulation (Table 1). They demonstrated (Fig. 4) that the increase of I_{Ks} under 1 μM isoproterenol (in the absolute values) was negligible in channels formed by coexpressed WT and K557E subunits ($K557E_{het}$ channels) in comparison with the increase of I_{Ks} seen in WT channels (Fig. 4A; due to small baseline current in $K557E_{het}$ channels). Therefore, accommodation of APD to a higher stimulation frequency under β-adrenergic stimulation was impaired in $K557E_{het}$ channels (Fig. 4B). This clearly demonstrates that the functional impact of *KCNQ1* mutations should always be tested not only in the basal conditions but also in the conditions relevant for clinical situations known to be connected with an increased risk of arrhythmias in mutation carriers. Such complex analysis is required to determine the full extent of the functional impact of a specific mutation.

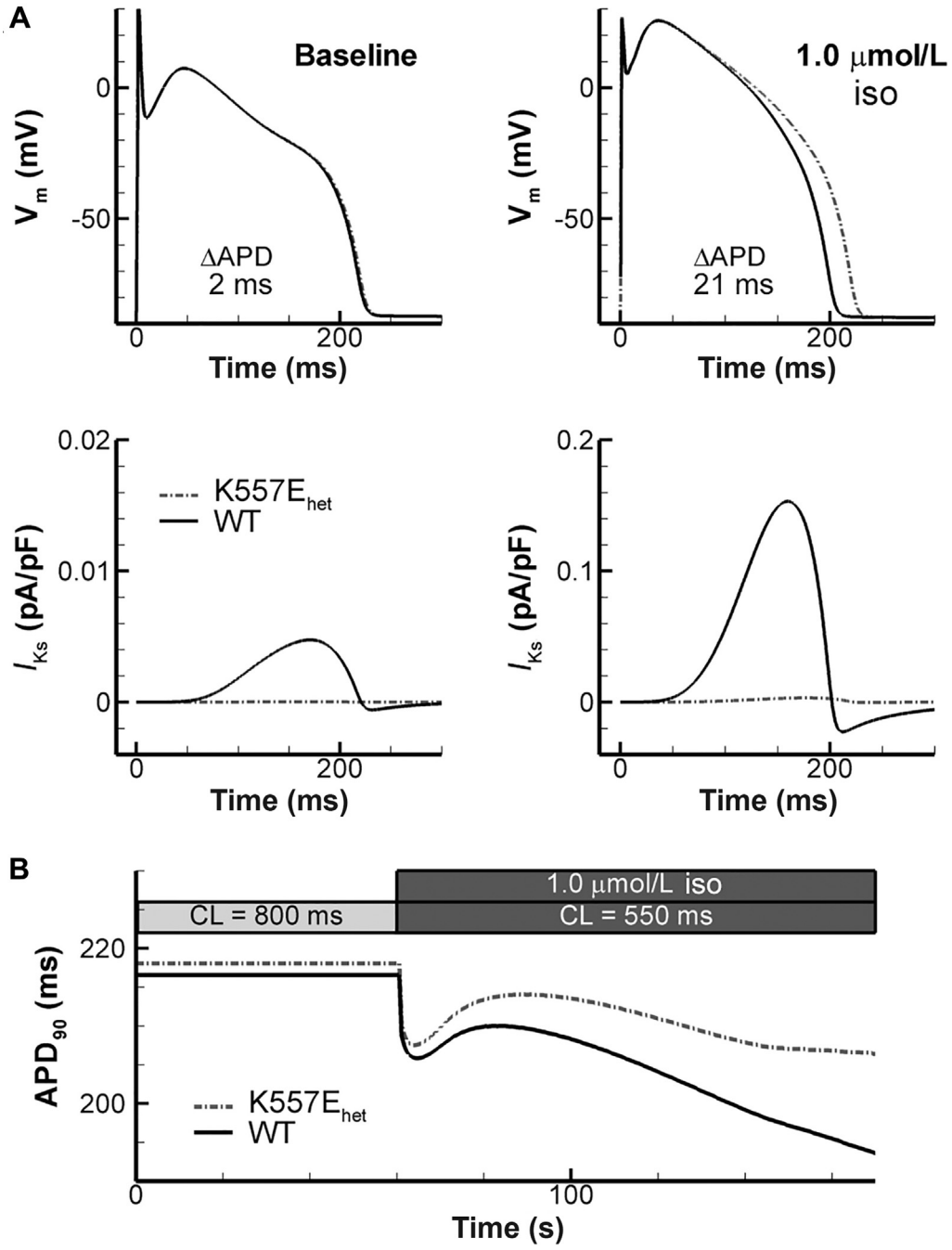

Figure 4. Changes of cardiac AP duration (APD) caused by I_{Ks} channels with K557E mutation that was identified in patients with LQT1. (**A**) AP and I_{Ks} traces simulated using a model of canine ventricular myocyte under basal conditions (*left panels*) and after protein kinase A (PKA) stimulation mediated by 1 μM isoproterenol (iso; *right panels*); cycle length 1000 ms; V_m, the membrane voltage. The solid lines stand for wild-type (WT) I_{Ks} channels, whereas the dash-dotted lines represent channels with heterozygous coexpression of WT and mutated K557E subunits ($K557E_{het}$). Despite preserved PKA stimulation in $K557E_{het}$ channels (Table 1), the increase of I_{Ks} under 1 μM isoproterenol (in the absolute values) was negligible in these channels in comparison with WT channels, and therefore cannot significantly affect APD. (**B**) To mimic exercise, the steady cycle length of 800 ms was abruptly changed to 550 ms and isoproterenol was added. An impaired accommodation of APD to these changes is obvious in $K557E_{het}$ channels. Modified from Spätjens et al.[42] with permission from Oxford University Press.

Mutations With Absent PKA Response

The remaining 11 mutations (among them R190Q, I235N, A341V, and G589D; for the approximate location of these mutations, see the red triangles in Fig. 1) disrupted I_{Ks} channel response to PKA stimulation, resulting in an insufficient current increase (on average only by approximately

17% in both the heterozygously and homozygously expressed mutated channels; n = 10 and 6, respectively; Table 1). This increment was considerably lower than in WT channels in the same studies. In the case of V254M mutation, even a reduction of the current amplitude by 10% in comparison with WT channels was observed during PKA stimulation.[55] The typical leftward shift of the voltage dependence of the activation (usually observed under PKA stimulation in WT channels and also in the mutated channels with preserved PKA response—see above) was not apparent in most of the mutated channels with absent PKA reactivity (the lower part of Supplemental Table S2). Data describing time dependence of activation and deactivation were mostly missing. These data were only available in the study by Heijman et al.,[56] where they were surprisingly changed under PKA stimulation (Supplemental Table S2). However, the significance of these observed changes was not commented on in the paper.

The majority of PKA-insensitive mutations were connected with a loss of the I_{Ks} function. The clinical phenotype of the mutation carriers ranged from asymptomatic patients to those who had experienced sudden death.[57,58] For example, most carriers of G269S mutation remained asymptomatic, with a normal or borderline resting QT interval and a prolonged QT interval under an increased sympathetic tone.[59] In contrast, the A341V mutation resulted in a severe clinical phenotype with a high risk of cardiac events.[60] According to mathematical simulations performed by Bartos et al.,[61] even mutations with impaired PKA regulation that do not reduce the basal I_{Ks} current might affect APD during β-adrenergic stimulation just as much as the dominant negative mutations do. The simulated mutated I_{Ks} with a physiological current under the basal conditions but insensitive to PKA prolonged APD_{90} by 6% to 8% for the cycle lengths between 300 and 1000 ms, whereas the dominant negative I_{Ks} channel caused an APD_{90} increase by 3% to 4% under the basal conditions, and by 6% to 8 % under PKA stimulation. A combination of the dominant negative effect and absent PKA response resulted in 8% to 12% APD_{90} prolongation.[61] Moretti et al.[54] reported a 30% reduction in the ratio of APD_{90} to AP interval and occurrence of early afterdepolarizations in the presence of 100 nM isoproterenol in their study dealing with the loss-of-function PKA-insensitive R190Q mutation, which was performed on patient-specific derived cardiomyocytes.

The gain-of-function mutation R231H increases the I_{Ks} current at the negative membrane voltages and is associated with the familial AF.[62] Mathematical simulations confirmed this, showing a shortening of the atrial APD_{90} at all tested cycle lengths in the model with this mutation. On the other hand, the ventricular APD_{90} was not significantly altered at the same cycle lengths in the simulations.[62] Nevertheless, an abnormal ventricular excitability has been noticed in some R231H carriers, suggesting that this mutation may result in an abnormal ventricular repolarization due to the impaired PKA stimulation in the mutated channels.[62]

The comparable level of I_{Ks} activation by PKA in homo- and heterozygous mutated channels with absent PKA response (by 17% in both cases—see above) implies that the PKA-dependent upregulation shows strong dominant negativity. A similar observation was reported by Heijman et al.,[56] who concluded that phosphorylation of all 4 KCNQ1 subunits was required to produce the PKA-dependent increase.

Interestingly, the mutations disrupting the PKA stimulation of the I_{Ks} channel are diffused over the whole Kv7.1 protein rather than concentrated in particular regions (see the red triangles in Fig. 1), in spite of the fact that only specific regions of this protein have been observed to play a role in the PKA regulation of the channel (see the section "The cardiac I_{Ks} channel and regulation of its function" and the related Fig. 1). Surprisingly, the majority of mutations located in the C-terminal region did not cause a loss of channel ability to react to PKA stimulation, even though the C-terminus is known to be involved in this regulation.[17] Other regions, namely the S4 and S5 segments, and the S2-S3 and S4-S5 linkers, have not previously been considered to play a role in PKA stimulation, but almost all the mutations located in these regions disrupted PKA stimulation of the I_{Ks} channels in the reviewed studies. Hence, we suggest that these regions may in fact play a role in the regulation of I_{Ks} channels by PKA. We hypothesize that mutations located in these regions may result in a conformational change of the Kv7.1 protein that distances the N- and C-terminal regions, thereby disabling phosphorylation of the N-terminal serine residues. Alternatively, the channel function might be affected by an impaired Kv7.1-KCNE1 interaction, because KCNE1 is known to play an important role in the PKA response of the I_{Ks} channel, and mutations with absent PKA reactivity might impair the protein-protein interaction via allosteric changes (for a more thorough explanation, see the section "The cardiac I_{Ks} channel and regulation of its function"). The interaction between the particular signalling pathways (as shortly reviewed in the section "The cardiac I_{Ks} channel and regulation of its function") cannot be excluded as an explanation either. The real role of the regions newly suggested to be involved in the PKA stimulation of I_{Ks} channels should be studied further.

Relevance of PKA Regulation of I_{Ks} Channels and Its Analysis

As already discussed, the PKA-dependent phosphorylation is a key way of regulation of the I_{Ks} channel function.[27-29,39] An elevated sympathetic tone is associated with an increased risk of arrhythmia in patients carrying mutations in genes encoding subunits of the I_{Ks} channel macromolecular complex, especially in patients with LQTS.[48,49]

Further study and clarification of the underlying molecular mechanisms will help us better understand the pathophysiology of channelopaties. If the regions that we have newly proposed as potential players in the PKAregulation of the I_{Ks} channel function are confirmed and interconnected with the known components of the regulatory pathway, it might promote a more complex analysis of regulatory pathways in general, including even less directly involved channel regions. Detailed information about the dysfunction of I_{Ks} channels associated with a particular mutation (including PKA reactivity of the channel) may also considerably improve the clinical management of patients, in 2 important ways. First, functional confirmation of the pathogenity of a mutation can confirm the diagnosis, especially in clinically borderline individuals, helping the physician decide whether to initiate therapy. Knowing the level of channel dysfunction may contribute to a better prediction of proarrhythmogenic risk in carriers of a particular mutation. Second, deeper knowledge of

the pathophysiology of the disease may help physicians develop novel therapeutic options in the future.

Conclusions

The cardiac I_{Ks} serves as a repolarization reserve and contributes to APD modulation, especially under an increased sympathetic tone. Mutations in the *KCNQ1* gene may modify the response of I_{Ks} channels to β-adrenergic stimulation. This may considerably influence the clinical phenotype in mutation carriers. However, only a small fraction of the known *KCNQ1* mutations (approximately 2.7%) have been characterized with regard to their PKA sensitivity so far. This review suggests that a complex analysis, including further investigation of the PKA regulation of the I_{Ks} channel, is required to fully understand the functional impact of a specific *KCNQ1* mutation. We also suggest that several Kv7.1 regions that have not been considered so far (namely the S4 and S5 segments, and the S2-S3 and S4-S5 linkers) may actually play a role in the PKA stimulation of I_{Ks} channels. Their significance should be reconsidered.

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Disclosures

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Supplementary Material

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