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COMMENTARY

Human Ether-a-gogo Related Gene (*HERG*) K^+ Channels as Pharmacological Targets

PRESENT AND FUTURE IMPLICATIONS

Maurizio Taglialatela, Pasqualina Castaldo, Anna Pannaccione, Giovanna Giorgio and Lucio Annunziato*

SECTION OF PHARMACOLOGY, DEPARTMENT OF NEUROSCIENCE, SCHOOL OF MEDICINE, UNIVERSITY OF NAPLES FEDERICO II, NAPLES, ITALY

ABSTRACT. Electrophysiological and molecular biology techniques have widely expanded our knowledge of the diverse functions where K^+ channels are implicated as potential and proven pharmacological targets. The aim of the present commentary is to review the recent progress in the understanding of the functional role of the K^+ channels encoded by the human ether-a-gogo related gene (*HERG*), with particular emphasis on their direct pharmacological modulation by drugs, or on their regulation by pharmacologically relevant phenomena. About 3 years have passed since the cloning, expression, and description of the pathophysiological role of *HERG* K^+ channels in human cardiac repolarization. Despite this short lapse of time, these K^+ channels have already gained considerable attention as pharmacological targets. In fact, interference with *HERG* K^+ channels seems to be the main mechanism explaining both the therapeutic actions of the class III antiarrhythmics and the potential cardiotoxicity of second-generation H_1 receptor antagonists such as terfenadine and astemizole, as well as of psychotropic drugs such as some antidepressants and neuroleptics. It seems possible to anticipate that the main tasks for future investigation will be, on the one side, the better understanding of the intimate mechanism of action of *HERG* K^+ channel-blocking drugs in order to elucidate the conditions regulating the delicate balance between antiarrhythmic and proarrhythmic potential and, on the other, to unravel the pathophysiological role of this K^+ channel in the function of the brain and of other excitable tissues. BIOCHEM PHARMACOL **55**;11:1741–1746, 1998. © 1998 Elsevier Science Inc.

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ROLE OF *HERG*† K^+ CHANNELS IN THE PHYSIOLOGY AND PATHOLOGY OF EXCITABLE TISSUES

The Heart

The duration of the cardiac action potential is controlled by a fine equilibrium between inward and outward currents [1]. Among the channels and transporters allowing ion fluxes across cardiac cell membranes during the plateau phase of the action potential, K^+ channels have a prominent role in the repolarization process. Under physiological conditions, several classes of K^+ currents shape the action potential in cardiac cells: the transient outward current (I_{to}), the delayed rectifier repolarizing current made up of both rapid (I_{Kr}) and slow (I_{Ks}) components [2], and the inward rectifier I_{K1} current, which participates in the final phases of repolarization. Other K^+ channels are also active during the action potential plateau in selective heart regions, such as the acetylcholine-activated K^+ channel ($I_{K(Ach)}$) in the atria and the Purkinje fibers, or under specific pathological conditions, such as the ATP-dependent K^+ channel ($I_{K(ATP)}$), the Na^+-dependent K^+ current ($I_{K(Na)}$), and the fatty-acid-activated K^+ current ($I_{K(FA)}$).

A major leap toward a better understanding of the cardiac repolarizing mechanisms has been made during the last few years by the study of the molecular genetics of the LQTS. This life-threatening, genetically transmitted disease is characterized by a marked prolongation of the QT interval on the ECG and by frequent episodes of syncope or cardiac arrest, usually occurring during conditions of psychological or physical stress [3]. These syncopal episodes are due to "torsade de pointes" that often degenerate into ventricular fibrillation and eventually result in the sudden death of most of the affected patients. Linkage analysis has led to the conclusion that LQTS presents a genetic locus heterogeneity; several forms of LQTS have, in fact, been recognized, and a growing number of genetic defects underlying each form are now emerging (Fig. 1A) [4–6], although it seems likely that more are yet to be discovered

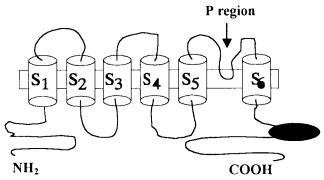
* Corresponding author: Dr. Maurizio Taglialatela, Section of Pharmacology, Department of Neuroscience, School of Medicine, University of Naples Federico II, Via S. Pansini 5, 80131 Naples, Italy. Tel. 39-81-7463318; FAX 39-81-7463323; E-mail: mtaglial@unina.it.

† *Abbreviations:* EADS, early after-depolarizations; EAG, ether-a-gogo; *HERG*, human ether-a-gogo related gene; LQTS, long QT syndrome; and ROS, reactive oxygen species.

A. Forms of Inherited LQTS

LQTS	Chromosome linkage	Current affected	Gene	Mechanism of the disease
1	11 (11p15.5)	I_{Ks}	K_vLQT1	↓ repolarizing K^+ current
2	7 (7q35-36)	I_{Kr}	HERG	↓ repolarizing K^+ current
3	3 (3p21-24)	I_{Na}	SCN5A	↑ depolarizing Na^+ current
4	4 (4q25-27)	?	?	?

B. Predicted topology of the K^+ channel subunit encoded by *HERG*



C. *HERG* mutations causing LQTS

Mutation	Localization	Functional effect	Reference
Δbp1261 (frameshift)	S_1	Decreased expression	[5,11,12]
N470D	S_2	Decreased expression Altered deactivation	[5,12]
T474I	S_2/ S_3	?	[13]
ΔI500-F508	S_3	No expression	[5,12]
A561V	S_5	?	[5,12]
A561T	S_5	?	[14]
I593R	P region	?	[15]
Y611H	S_5-P region	?	[13]
A614V	S_5-P region	?	[13]
G628S	P region	No expression	[5,12]
V630L	P region	?	[13]
V822M	CNB domain	?	[16]
c/g substitution (splice donor site alteration)	CNB domain	?	[5,12]

FIG. 1. Relevance of *HERG* K^+ channels for human LQTS. (B) The putative topological arrangement of the K^+ channel subunit encoded by *HERG* is shown. The transmembrane domains (from S_1 to S_6) are indicated with cylinders. The dark region shown in the C-terminus corresponds to the putative cyclic nucleotide binding (CNB) domain. Four subunits are thought to assemble to form a functional tetrameric channel.

[7, 8]. One of the genes that is altered in LQTS (LQTS2) is *HERG* [9]. The predicted protein encoded by this gene shares considerable sequence similarity with other K^+ channels [10], displaying a putative topological arrangement with six α-helical transmembrane domains, the N- and C-termini located in the cytoplasm, a cyclic nucleotide-binding domain in the C-terminus, and a pore-forming region between the transmembrane segments S_5 and S_6 (Fig. 1B). Several mutations in *HERG* affecting different regions of the expressed protein have been described (Fig. 1C), although the functional consequences of the mutations for most of them are as of yet unknown.

Heterologous expression of the *HERG* product confirmed the hypothesis that *HERG* encoded for a K^+-selective channel, and expanded this observation to suggest that *HERG* represents the molecular basis of the cardiac repolarizing current I_{Kr} [17]. In fact, the main biophysical features of I_{Kr}, namely its peculiar inward rectification that decreases the conductance at positive potentials despite being activated by depolarization [18, 19], its modulation by the extracellular concentration of K^+, along with its peculiar pharmacological profile (see below), were clearly evident in the K^+ currents elicited upon *HERG* expression.

The Central Nervous System

The previously mentioned results clearly identified *HERG* K^+ channels as fundamental elements for cardiac repolarization. However, *HERG* K^+ channels are not exclusively expressed in the heart; their presence has also been revealed in other excitable tissues such as the brain and the skeletal muscle [20]. In neublastoma cells, *HERG* K^+ channels have, in fact, been implicated in the changes of the resting membrane potential associated with the cell cycle [21], in the control of neuritogenesis and differentiation [22], and in the spike-frequency adaptation [23]. Furthermore, the relevance of *HERG* K^+ channels in neuronal function has been further reinforced by recent evidence showing that the *seizure* locus in *Drosophila* encodes for the fly homologue of *HERG*. Mutations in the *seizure* locus cause a temperature-sensitive paralytic phenotype associated with hyperreactivity of the flight motor pathway [24, 25].

HERG K^+ CHANNELS AS TARGETS FOR ANTIARRHYTHMIC DRUGS

The understanding of the molecular basis of the K^+ currents involved in cardiac repolarization is of crucial importance, not only to provide molecular tools that could help to dissect the relative contribution of each K^+ current in the regulation of the action potential, but also to allow the therapeutic control of disturbances of the cardiac rhythm. In fact, the increasing concerns regarding the shortcomings of "classic" antiarrhythmic agents such as quinidine and "quinidine-like" drugs (i.e. encainide and flecainide) in adequately controlling potentially lethal ventricular arrhythmias [26], has boosted research in the so-called "pure" class III antiarrhythmic agents [27, 28]. These compounds have the ability to lengthen the duration of the action potential and to increase the myocardial refractoriness in the absence of significant changes in conduction velocity. These pharmacodynamic properties have proven to be desirable in controlling cardiac arrhythmias [27], although it should be underlined that class III antiarrhythmics, such as amiodarone and *d*-sotalol, also exerted significant proarrhythmic actions in specific clinical settings [29]. In fact, these compounds may cause the occurrence of EADs, one of the pathogenetic mechanisms responsible for paroxysmal ventricular fibrillation or "torsade de pointes" [30].

One of the hallmarks of I_{Kr} is its modulation by class III antiarrhythmic drugs. In fact, the original distinction between I_{Kr} and I_{Ks} was based on the differential sensitivity of these two components to class III antiarrhythmics, with I_{Kr} being much more sensitive to blockade than I_{Ks} [2]. Although no direct pharmacological comparison between

heterologously expressed *HERG* K^+ channels, possibly underlying I_{Kr}, and the K^+ channel subunits, possibly underlying I_{Ks} (minK + K_vLQT1) [31, 32], is yet available, several studies have already been performed on the blockade of *HERG* K^+ channels by class III antiarrhythmics. These include the methanesulfonanilides dofetilide [33, 34], E-4031 [35], and ibutilide [36], and RP 58866 and its active enantiomer terikalant [37]. The results obtained show that these drugs behaved as very potent inhibitors of *HERG* K^+ channels expressed either in *Xenopus* oocytes or in transfected mammalian cells, confirming the idea that *HERG* encodes for a K^+ current having the pharmacological properties of native cardiac I_{Kr}. Although it should be remembered that K^+ channels different from *HERG* are also effectively blocked by these compounds [38], a direct comparison of class III antiarrhythmic blockade of *HERG* with other K^+ currents suggests that I_{Ks} and I_{Kl} are affected only at concentrations at least 100 times higher than those effective in inhibiting *HERG* [37]. This raises the important question of whether I_{Ks} and I_{Kl} specific blockers could be useful approaches in antiarrhythmic therapy [39, 40].

The studies showing that class III antiarrhythmics effectively block *HERG* K^+ channels also clarified several crucial points regarding their mechanism of action. The observation that the blocking potency of the methanesulfonanilides dofetilide [33, 34] and MK-499 [41] was higher when applied on the intracellular side of the membrane as compared with extracellular perfusion suggests that the drugs diffuse through the membrane to reach their intracellular site of action. Furthermore, the biophysical properties of drug blockade are consistent with a state-dependent blocking mechanism, with the open state of the channel having the highest sensitivity to the drugs, and the closed and inactivated states displaying lower drug affinities [33, 34].

Given the well-characterized property of class III antiarrhythmics to be more effective at lower stimulation frequencies ("reverse use-dependence") [28], it appears rather interesting to investigate whether the binding site for these drugs on *HERG* K^+ channels is located inside or outside of the membrane electric field. However, the study of the voltage-dependence of the blocking process by class III antiarrhythmics has been hampered in most cases by their rather slow blocking and unblocking kinetics. These kinetic properties explain the conflicting results reported and prompt further investigation on the voltage-dependence and the frequency-dependence of the blockade of *HERG* K^+ channels by class III antiarrhythmics.

HERG K^+ CHANNELS AS TARGETS FOR PROARRHYTHMIC DRUGS

Antagonists of the histamine type 1 (H_1) receptors are widely prescribed to relieve the symptoms of allergic reactions [42]. Second-generation antihistamines such as terfenadine, astemizole, loratadine, cetirizine, acrivastine, and ebastine have been developed during the last 20 years in order to overcome the marked antimuscarinic and sedative properties displayed by the compounds of the first generation [43]. Because of this novel pharmacological profile, second-generation antihistamines have been progressively replacing the older molecules present in the market, becoming one of the most prescribed drug families in developed countries [44]. Despite this enormous success, several reports have appeared in the literature of the last 10 years indicating the rare occurrence of "torsade de pointes" with a marked prolongation of the QT interval on the surface ECG after the consumption of astemizole or terfenadine [45]. These ventricular arrhythmias occurred either in patients who took intentional overdoses of astemizole or terfenadine [46, 47] or in subjects suffering from several predisposing factors to the development of cardiac arrhythmias [48], such as a reduced drug-metabolizing capacity, congenital prolongation of the QT interval, ischemic heart disease, congestive heart failure, or electrolyte unbalance such as hypokalemia and hypomagnesemia [44].

It has been suggested that the QT prolongation and ventricular arrhythmia caused by terfenadine and astemizole may be secondary to their ability to interfere with cardiac K^+ channels involved in action potential repolarization [49], and particularly with the I_{Kr} repolarizing current [2, 50, 51]. The discovery that *HERG* encodes for I_{Kr} has prompted investigation on the possible blocking effect of second-generation antihistamines on the K^+ channels encoded by *HERG*. The results of these studies have elegantly shown that both terfenadine [52] and astemizole [53] blocked *HERG* K^+ channels in a concentration range similar to that found in the plasma of subjects with cardiotoxic manifestations [43]. Similar results have been obtained recently with the piperidinic second-generation antihistamine ebastine [54]. However, it seems likely that *HERG* blockade and cardiotoxic potential are not class properties of second-generation antihistamines. In fact, loratadine, despite having similar antihistaminic potency *in vivo* with respect to other antihistamines [55], has been shown to lack significant *HERG*-inhibitory properties [54]. This raises the intriguing question of whether other nonsedating antihistamines that are widely prescribed also display cardiotoxic potential.

Second-generation antihistamines are not the only drugs displaying potential cardiotoxicity. For example, antipsychotics such as the butyrophenone haloperidol and the piperidinic phenothiazine thioridazine can induce, in rare cases, the occurrence of major ventricular arrhythmias, QT prolongation, and sudden death [42]. The recent description of the high-affinity blockade of *HERG* K^+ channels by haloperidol and its reduced metabolite [56] seems to suggest that *HERG* K^+ channels have to be regarded as important pharmacological targets for those drugs displaying marked proclivities to proarrhythmic manifestations.

The observation that drugs belonging to different chemical classes and provided with different therapeutic indications are able to determine cardiotoxic effects by means of an interference with *HERG* K^+ channels emphasizes the

importance of an early evaluation of the possible blockade of *HERG* K^+ channels, either constitutively present or heterologously expressed in *Xenopus* oocytes or in mammalian transfected cells, during the developmental phases of novel drugs that could display cardiotoxic potential.

HERG K^+ CHANNELS AS TARGETS FOR FREE OXYGEN RADICALS

A growing body of evidence suggests that oxidative damage may profoundly influence cell excitability in both physiological and pathological states. Several ion channels and transporters have been shown to be sensitive to the modulation by oxidative stress [57]. Recent evidence has shown that ROS can specifically modulate the function of *HERG* K^+ channels [58]: an enhancement of ROS production increases *HERG* outward K^+ currents, whereas a decrease in ROS levels can inhibit the resting outward K^+ currents and prevent their increase induced by oxidizing conditions. The modulation of *HERG* K^+ channels by ROS, occurring in both resting and stimulated conditions, may represent an important functional mechanism linking the changes in the levels of O_2 and ROS in heart tissue with the electrophysiological modifications occurring during ischemia–reperfusion phenomena. In fact, it seems possible to speculate that the increase of the outward currents mediated by *HERG* caused by the burst of ROS production that follows the reperfusion of the tissue after an ischemic period would tend to repolarize the membrane potential and shorten the action potential duration. This seems to be confirmed by the consistent shortening of the action potential induced by oxidative damage recorded in Purkinje fibers and ventricular myocytes after longer times of exposure to ischemia–reperfusion conditions. Therefore, although species-specific differences as well as regional differences in the effects of ischemia–reperfusion conditions on the action potential in cardiac cells have been documented, the modulation of *HERG* K^+ channels by ROS could represent a crucial target for pharmacological intervention in ROS-induced arrhythmias [59].

Furthermore, although the functional importance of *HERG* K^+ channels in the brain is not yet elucidated, the major role played by ROS in neurodegenerative disorders, excitotoxic damage, and ischemia–reperfusion conditions [60–62], along with the ROS-induced alteration in the membrane potential control in neuronal cells [63], suggests that the modulation of *HERG* K^+ channels by ROS may represent a relevant pathophysiological mechanism deserving further pharmacological evaluation.

In addition, the finding that the K^+ currents mediated by *HERG* channels are enhanced under conditions of oxidative damage, and, in general, that ROS modulate the action potential repolarization process, has considerable pharmacological implications. Differences in the duration of the QT interval have been known for many years, with females having longer QTs than males. Therefore, females are at a greater risk than males of developing ventricular tachyarrhythmias in response to antiarrhythmic drug treatment [64]. This evidence raises the intriguing possibility that estrogens, by means of their *in vitro* and *in vivo* antioxidant properties [65], may prevent this modulation, thereby explaining the prolonged QT interval observed in females, along with their increased propensity to the development of drug-induced arrhythmias.

SOME OPEN QUESTIONS FOR THE FUTURE

The recognition of the fundamental role played by the K^+ channels encoded by *HERG* in cardiac pathophysiology has greatly improved the knowledge of the mechanism of action of several drug classes having both antiarrhythmic and proarrhythmic potentials. It is possible to anticipate that this knowledge will be of invaluable help for treating not only those individuals suffering from congenital disturbances of the cardiac rhythm [66], but also to achieve better control of the large number of patients affected by acquired arrhythmias. In addition, the described action of proarrhythmic drugs on *HERG* K^+ channels should represent a warning bell for cardiologists, immunologists, psychiatrists, and general practitioners, since it increases the level of awareness of the potentially lethal drug-induced cardiotoxic manifestations, and stimulates a more careful evaluation of the predisposing factors of each individual patient.

Furthermore, an important question deserving investigation is the apparent contradiction in the ability of two distinct pharmacological categories of *HERG* K^+ channel-blocking compounds to exert opposite effects on cardiac function, some being endowed with antiarrhythmic properties and others displaying proarrhythmic potential.

In addition, due to the well-known role of the autonomic nervous system in triggering syncopal phenomena [3], it will be challenging for cardiac pathophysiology to investigate whether an increased sympathetic discharge could modulate *HERG* K^+ channel function. Because a binding domain for cyclic nucleotides is present in the *HERG* amino acid sequence [9], and divalent cations have been described to permeate through EAG [67], a K^+ channel with marked sequence similarities to the *HERG* product [9], it seems possible to speculate that changes in cyclic nucleotide levels or Ca_i^{2+} triggered by cardiac β-adrenoceptor activation could modulate *HERG* K^+ channel activity.

Finally, although the regional distribution of *HERG*-encoded K^+ channels in the brain is yet unknown, it will be of fundamental importance to verify whether, in analogy to its role in cardiac function, *HERG* K^+ channels could be a potential target for mutation- or drug-induced disturbances of human neuronal excitability, such as those associated with the development of epileptic episodes. It is possible to foresee that the future elucidation of the possible pathophysiological role of *HERG* K^+ channels in tissues other than the heart could reveal an even more exciting picture than the one currently available.

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