

PERSPECTIVE

# Activators of Cation Channels: Potential in Treatment of Channelopathies

Guiscard Seeböhm

*Institute of Physiology I, University of Tuebingen, Tuebingen, Germany*

Received December 9, 2004; accepted December 16, 2004

## ABSTRACT

Cation channels are membrane proteins that provide controlled pathways for ion passage through cellular membranes. They play important roles in physiological processes such as secretory transduction, control of ion homeostasis, cell volume, vesicle cycling, and electrical control of excitable tissues. In a variety of channelopathies, ion channel function is reduced, and activators of cation channels are promising candidates to regain channel function in acquired or inherited channelopathies. Shortage in cation channel activators prevents testing of efficiency of activators in a variety of indications. This shortage might result from the

relative incapability of modern drug screening methods, but increasing knowledge about cation channel activator binding and action might enable us in the future to use in silico-guided drug design of channel modulators. New compounds such as the HERG channel activator (3*R*,4*R*)-4-[3-(6-methoxy-quinolin-4-yl)-3-oxo-propyl]-1-[3-(2,3,5-trifluoro-phenyl)-prop-2-ynyl]-piperidine-3-carboxylic acid (RPR260243) will enable us to increase our understanding in cation channel modulation and to test the concept of channel activation as a clinically relevant principle in treatment of channelopathies.

Ion channels are integral membrane proteins that provide controlled pathways for ion passage through cellular membranes. Cation selective channels play important roles in physiological processes such as secretory transduction, control of ion homeostasis, cell volume, vesicle cycling, and electrical control of excitable tissues. The importance of cation channels is also amplified by the fact that many therapeutic drugs mediate their effects by targeting these proteins. Po-

tassium-selective channels are the most genetically diverse of all cation channels. Starting with the first cloned potassium selective ion channel from *Drosophila melanogaster*, Shaker, more than 100 potassium channels have been identified. The number of functionally distinct channels in native tissues is further increased by heteromultimeric assembly of potassium channel  $\alpha$ -subunits with other  $\alpha$ - and  $\beta$ -subunits and other modifications such as alternative splicing of mRNAs, glycosylation, and phosphorylation. In light of the broad range of physiological roles of cation channels, it is not surprising that channel impairment results in a variety of pathophysiological conditions. Channels might lose or gain function as a result of mutations in the promotor or coding

This work was supported by the Deutsche Forschungsgemeinschaft. Article, publication date, and citation information can be found at <http://molpharm.aspetjournals.org>. doi:10.1124/mol.104.010173. Please see the related article on page 827.

**ABBREVIATIONS:** LQTS, long QT syndrome; aLQTS, acquired LQTS; Bay K 8644, (S)-(-)-1,4-dihydro-2,6-dimethyl-5-nitro-4-(2-[trifluoromethyl]phenyl)-3-pyridine carboxylic acid methyl ester; BMS-180448, (3*S*-trans)-*N*-(4-chlorophenyl)-*N'*-cyano-*N''*-(6-cyano-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-4-yl); JTV-506, ((-)-(3*S*,4*R*)-2,2-bis(methoxymethyl)-4-[(1,6-dihydro-1-methyl-6-oxo-3-pyridazinyl)amino]-3-hydroxychroman-6-carbonitrile hemihydrate (CAS 170148-29-5); KR-30450, (-)-(2*R*)-2-[(1,3]-dioxolan-2-yl)-2-methyl-4-(2-oxopyrrolidin-1-yl)-6-nitro-2*H*-1-benzopyran; P1075, *N*-cyano-*N'*-(1,1-dimethylpropyl)-*N''*-3-pyridylguanidine; SKP-450, 2-(2''(1'',3''-dioxolan-2-yl)-2-methyl-4-(2'-oxopyrrolidin-1-yl)-6-nitro-2*H*-1-benzopyran; WAY-133537, (R)-4-[3,4-dioxo-2-(1,2,2-trimethyl-propylamino)cyclobut-1-enylamino]-3-ethyl-benzonitrile; ZD6169, (S)-*N*-(4-benzoylphenyl)3,3,3-trifluoro-2-hydroxy-2-methyl-priopionamide; Y 26763, (-)-(3*S*,4*R*)-4-*N*-acetyl-*N*-hydroxyamino-6-cyano-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-3-ol; ZD6169, *N*-(4-benzoyl phenyl)-3,3,3-trifluoro-2-hydroxy-2-methylpropionamide; ZM-244085, 9-(3 cyanophenyl)hexahydro-1,8 acridinedione; NS-004, 1-(5-chloro-2-hydroxyphenyl)-5-trifluoromethyl-1,3-dihydro-2-benzimidazol-2-one; BRL-55834, (3*S*,4*R*)-3,4-dihydro-2,2-dimethyl-4-(2-oxopiperidin-1-yl)-6-pentafluoroethyl-2*H*-1-benzopyran-3-ol; DHS-I, triterpenoid glycosides dehydrosayasaponin I (DHS-I) (also known as soyasaponin I and soyasaponin III); BDF 9148, 4-[3'-(1''-benzhydryl-azetidine-3''-oxy)-2''-hydroxypropoxy]-1*H*-indole-2-carbonitrile; FPL 64176, methyl-2,5-dimethyl-4-(2-phenylmethyl)benzoyl-[1*H*]pyrrole-3-carboxylate.

region of a gene. Likewise, functional impairment of channel function might result from regulatory derangements or by autoantibodies. The diseases based on altered ion channel function are called channelopathies and include Bartter's syndrome type 2 [KCNJ1, Kir1.1 or RomK (Derst et al., 1997)] persistent hyperinsulinemic hypoglycemia of infancy [Kir 6.2 and Sur (Thomas et al., 1995; Nestorowicz et al., 1996)] and episodic ataxia type 1 [KCNA1 or Kv1.1, (Adelman et al., 1995)].

Inherited long QT syndrome (LQTS) is a disorder that can occur by mutations in the coding region of the cardiac Na<sup>+</sup>-channel (SCN5A, LQT syndrome 3) (Wang et al., 1995) or Ankyrin-B (LQTS4) (Mohler et al., 2003). However, in most cases of LQTS, the potassium channel  $\alpha$ -subunits KCNQ1 (LQTS1) and HERG (human *ether-a-go-go*-related gene, LQTS2) or their  $\beta$ -subunits (KCNE1, 2; LQT 5, 6) are affected. A second group of patients develop LQTS in response to clinically used drugs. This type of LQTS is called acquired LQTS (aLQTS) or drug-induced LQTS and is far more common than the inherited forms of LQTS. Drugs associated with increased risk of aLQTS include antiarrhythmics (amiodarone, disopyramide, dofetilide, ibutilide, procainamide, quinidine, sotalol), antibiotics (clarithromycin, erythromycin, sparfloxacin), psychoactive drugs (chlorpromazine, droperidol, haloperidol, levomethadyl, mesoridazine, methadone, pimozide, thioridazine), antimalarial drugs (halofantrine, chloroquine) and others. A more complete list of drugs with risk of prolonging the QT interval and inducing torsade-de-pointes arrhythmias is available at <http://www.torsades.org/medical-pros/drug-lists/drug-lists.htm#>. Most of the listed drugs are highly potent HERG blockers, and some are comparably weak KCNQ1/KCNE1 blockers. The inherited LQTS associated KCNQ1/KCNE1 and HERG/KCNE2 channel mutations cause a decrease in net repolarizing current  $I_K$  by reducing potassium currents through "dominant-negative" or "loss-of-function" mechanisms, whereas the aLQTS is the result of  $I_K$ -blockade. In both cases, the reduced repolarizing  $I_K$  results in lengthened action potentials, reduced repolarization reserve, increased Ca<sup>2+</sup>-inflow, likelihood of early afterdepolarizations, and prolongation of the QT interval on the electrocardiogram. These alterations predispose affected persons to syncope, seizures, aborted cardiac arrests, and, sometimes, sudden cardiac death. However, block of  $I_{Kr}$  or  $I_{Ks}$  could be beneficial under special conditions and highly selective blockade of HERG/KCNE2- or KCNQ1/KCNE1-channels were formerly considered promising antiarrhythmic approaches and several companies developed selective blockers (Gerlach, 2003; Lee et al., 2003). Indeed, one of the most effective antiarrhythmic drugs, amiodarone, has several targets, including HERG/KCNE2-channels. The block of HERG channels is regarded as a severe problem for pharmaceutical compounds intended for clinical trials. Therefore, testing for HERG blockade has become an integral part of drug development and safety pharmacology. Treatment for inherited LQTS includes high thoracic left sympathectomy and implantation of cardioverter-defibrillators (Schwartz et al., 2000). These invasive therapies are cost-intensive and not favored by patients for obvious reasons. The primary drug therapy of LQTS is the blockade of  $\beta$ -adrenergic receptors.  $\beta$ -Blocker therapy has been shown to be beneficial in symptomatic LQTS patients (Ward, 1964; Schwartz et al., 1975). However, in about 20 to 35% of LQTS patients,

$\beta$ -blockers are not effective (Ackerman, 1998; Moss et al., 2000). These high-risk patients continue to have breakthrough cardiac events like aborted cardiac arrest, syncope, and even sudden death. The failure rate of  $\beta$ -blocker therapy might be higher in patients carrying mutations in the potassium channel genes KCNQ1 and HERG than in SCN5A, as indicated by a recent study on 28 genotyped patients (Chatrath et al., 2004). Although the known therapies have considerably reduced mortality in inherited LQTS, there is a well recognized need for improved treatments of inherited LQTS. The aLQTS forces the discontinuation of drug use and proscribes usage of certain drugs in predisposed patients. The situation is complicated because the aLQTS might not always be apparent. During aLQTS and inherited LQTS, a drug activating  $I_K$  is supposed to be beneficial.

HERG channels somehow act as "magnets" for small hydrophobic drugs with aromatic ring systems. For some time, it remained elusive why so many drugs bind to and block the HERG channel. Then, Mitcheson et al. (2000) determined the putative binding site of the high-potency blockers MK-499, terfenadine, and cisapride. The main determinants for the "sticky" binding site for highly potent blockers are aromatic residues pointing toward the central cavity. These aromatic residues form hydrophobic and/or  $\pi$ -stacking interactions with lipophilic and aromatic constituents of drug molecules (Chen et al., 2002). Knowing that many clinically relevant drugs contain aromatic ring systems and are lipophilic to enable membrane passage the high incidence of interactions of the HERG/KCNE2 channels with drugs becomes understandable. Several academic groups and companies are currently trying to combine pharmacophore models of HERG blockers with structural constraints for the HERG channel derived from homology to the bacterial channels KcsA and MthK (Bains et al., 2004) with the goal of generating in silico tools for the prediction of potential HERG-blocking reagents. In any case, for acute aLQTS, a drug counteracting the  $I_{Kr}$ -block would be very desirable.

Channelopathies most often result from a loss of channel function. It would be an attractive approach to activate channels to regain channel function. Channel activation could arise from augmented currents without marked alterations of kinetics or, in the case of gating modifiers, as a result of faster activation, slower deactivation, altered voltage-dependence, or a combination of these mechanisms. To test the potential clinical benefit of this approach, specific activator compounds would be needed. In most cases, the lack of activator molecules has made testing this hypothesis impossible. Only a few highly potent cation channel activators are known. Several of these activators, such as chlorzoxazone, stilbenes, and fenamates, are not specific and are of relatively low potency. Examples include a variety of K<sub>ATP</sub>-channel agonists (BMS-180448, cromakalim, celikalim, diazoxide, JTV-506, KR-30450, Lemakalim, levosimendan, minoxidil sulfate, nicorandil, P1075, pinacidil, rilmakalim, SKP-450, WAY-133537, Y 26763, ZD6169, ZM-244085), seven classes of Ca<sup>2+</sup>-activated K<sup>+</sup>-channel [Big (large) conductance and small conductance K<sup>+</sup> channel] activators (BMS-204352, chlorzoxazone, DHS-I, 1-EBIO/DC-EBIO, maxiKdiol, NS-004/analogs, BRL-55834), two KCNQ2–5 activators (BMS-204352/MaxiPost, retigabine), one KCNK-activator (Riluzole), 3 KCNQ1/KCNE1 activators (fenamates, R-L3, stilbenes), one G protein-coupled inwardly rectifying potas-

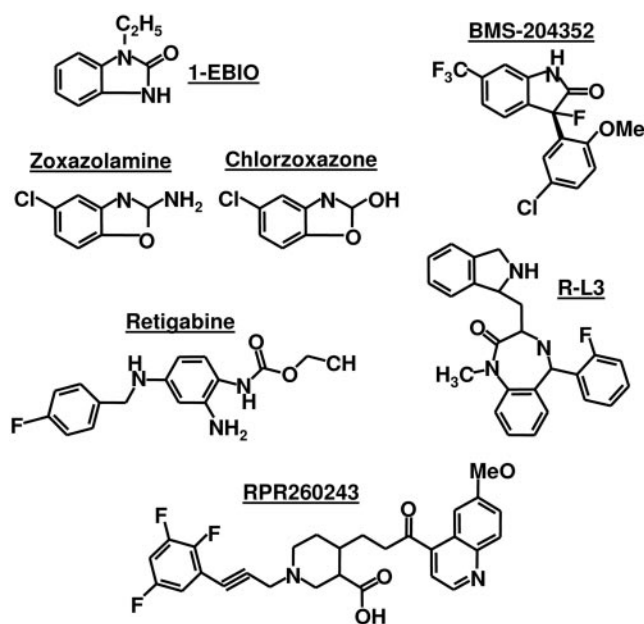
sium channel activator (flupirtine), one sodium channel activator (BDF 9148/analogs), and several L-type Ca-channel activators of dihydropyridine type (Bay K 8644, FPL 64176). The  $K_{ATP}$ -channel activators are indicated in hypertension and to stimulate hair regrowth. Possible further indications might be asthma and hyperactive bladder disorders. Bay K 8644 remained a tool for basic research but was recently tested for its potential in verapamil intoxication (Magdalan, 2003). The KCNQ2–5 channels form the classic M-channels and the activators retigabine and BMS-204352 might have a future for the treatment of incontinence or epilepsy. Large conductance channel activators could become important in treatment of stroke, hypertension and overactive bladder disorders (Malysz et al., 2004). The activation of KCNK channels by Riluzole exerts significant antiseizure properties (Borowicz et al., 2004). Other possible indications for specific channel openers are reviewed (Cooper and Jan, 1999; Lawson and Dunne, 2001). Thus, cation channel activators are very rare but hold a broad variety of potential applications.

The pharmaceutical industry searches for ion channel modulators using high capacity screening methods and huge compound libraries. Robust assays can easily screen 10,000 compounds per day on a single target. However, only a few cation channel activator lead structures have been reported. Why is it so difficult to discover activators? A glimpse of an idea arises when studying data on interactions of cation channel activators with their binding site on the channel. The variety of  $K_{ATP}$ -channel activators might arise from the fact that all  $K_{ATP}$ -channel activators bind to one of two binding sites in the sulfonylurea subunit of the channel and that this subunit provides a relatively easy accessible drug target. In the case of the other activators, very little is known about their binding sites. Bay K 8644 is believed to interact with the S5–S6 pore module and probably the III/IV interface of L-type  $Ca^{2+}$  channels (Zhorov et al., 2001; Yamaguchi et al., 2003). We have identified the binding site of an activator for a voltage-dependent potassium channel. Alanine-scanning methods combined with three-dimensional modeling techniques were used to determine the putative binding site of a benzodiazepine R-L3 that activates KCNQ1 channels (Seeböhm et al., 2003b). The binding site is located deep in the potassium channel protein among pore helix, S5, S6, and possibly S4. In theory, this position and the small size of the binding pocket might not allow even closely related chemicals to enter and bind to this position. If substances have to bind to binding sites deep inside channel proteins as suggested for BAY K 8644 and R-L3, then even minor structural changes in the activator molecules could disrupt correct binding, and the chemical optimization process might be highly challenging.

Activators are often found by accident when screening for compounds intended for other targets. Possibly there is a technical problem unaddressed by modern screening techniques: pharmaceutical companies screen for lead structures. Then they modify these leads to explore the structure in detail to find the molecule with the best combination of  $EC_{50}$ , bioavailability, selectivity, and drug stability. This concept works well for binding sites on the surface or in large cavities. An example is the search for small molecule cation channel blockers for which the preferential binding site is the large central cavity of cation channels. Thus, in the conventional screen, lead structures and analogs binding to surface-accessible

binding sites are preferentially identified. It is therefore not surprising that activators are often found randomly when working with compounds intended for distinct targets but not by systematic screens for channel activators. The screening methods used to identify channel blockers are possibly not well suited for the identification of activators. Most often, voltage-dependent fluorescence and cell-based assays with potassium depolarizations are used to identify channel blockers. The assays might identify blockers of  $K^{+}$ -permeation easily, but whether they are sensitive enough to identify the effects of a gating modifying activator such as RPR260243 or R-L3 is questionable. Thus, classic screening methods might not be very effective in the development of activators. Alternative screening methods such as automated patch clamp or automated two-electrode voltage clamp should help, but these methods are relatively slow [100–500 compounds per day (Xu et al., 2001)]. Gathering of structural data and functional modification of channel features by drugs could allow us in future to use computer aided approaches for putative channel activators. Such in silico approach could be combined with the relatively slow electrophysiologic screening methods.

In this issue of *Molecular Pharmacology*, Kang et al. (2005) report the initial characterization of the first known HERG channel activator, RPR260243. They find that RPR260243 markedly slows HERG deactivation in single cells and functionally counteracts blockade by dofetilide in retrogradely perfused hearts (Langendorf heart). The only high-potency selective KCNQ1/KCNE1 activator R-L3 known today was reported by Salata et al. (1998). R-L3 also shortens action potential duration, suppresses early afterdepolarization in ventricular myocytes isolated from hypertrophied rabbit hearts, reverses action potential lengthening, and suppresses early afterdepolarizations in rabbit myocytes treated with the  $I_{Kr}$  (HERG)-blocker dofetilide mimicking LQTS (Xu et al., 2002). These studies provided the first hint that activation of cardiac  $I_{Kr}$  and  $I_{Ks}$  could be beneficial in LQTS. Like RPR260243, R-L3 has the potential to activate most of the



**Fig. 1.** Structure of potassium channel activators. Cation channel activators are small lipophilic molecules.



LQTS-associated mutant channels. However, one mutation disrupted the activating effect, probably by disrupting the R-L3 binding site (Seeböhm et al., 2003a). Increasing resting membrane stabilization by  $K_{ATP}$ -channel opening was proposed to be beneficial in LQTS (Tan et al., 1999; Shimizu and Antzelevitch, 2000); therefore, activation of  $K^+$ -channels in LQTS might be a therapeutic principle. Activators can increase channel currents by increasing open probability or by propagation of membrane insertion by affecting channel trafficking. Compounds acting by these mechanisms do not change macroscopic gating of channels. RPR260243 markedly slows HERG channel deactivation, thereby increasing net HERG currents. The compound seems to increase the energy barrier for open-state to closed-state transitions. This slowing of deactivation identifies RPR260243 as a gating modifier. The effect of RPR260243 on HERG channel deactivation is comparable with the effect of R-L3 on KCNQ1 channels. Maybe the binding sites share similarity. Analysis of the binding site and the effects of the drug in vivo could be the next steps in the study of this new compound. RPR260243 provides us with a new tool for the exploration of gating modifier action and could prove to be clinically relevant.

In summary, activators of cation channels are promising candidates to regain channel function in acquired or inherited channelopathies. However, a shortage in cation channel activators prevents testing of efficiency of activators in a variety of indications. This shortage might result from the relative incapability of modern drug screening methods. An increased knowledge about cation channel activator binding and action might enable us to use in silico-guided drug design of channel modulators. The new RPR260243 will enable us to increase our understanding in cation channel modulation and to test the concept of  $I_{K_r}$ -activation as a clinically relevant principle in cardiac repolarization disorders.

#### Acknowledgments

I am grateful to Dr. N. Strutz-Seeböhm (University of Tuebingen, Germany), Dr. M. C. Sanguinetti (University of Utah, Salt Lake City, UT), Dr. K. Steinmeyer (Sanofi-Aventis, Frankfurt, Germany) for much helpful discussion and for comments on the manuscript. I thank Dr. W. Scholz (Merck, Darmstadt, Germany) and Dr. U. Quast (University of Tuebingen, Tuebingen, Germany) for expert information.

#### References

- Ackerman MJ (1998) The long QT syndrome: ion channel diseases of the heart. *Mayo Clin Proc* **73**:250–269.
- Adelman JP, Bond CT, Pessia M, and Maylie J (1995) Episodic ataxia results from voltage-dependent potassium channels with altered functions. *Neuron* **15**:1449–1454.
- Bains W, Basman A, and White C (2004) HERG binding specificity and binding site structure: evidence from a fragment-based evolutionary computing SAR study. *Prog Biophys Mol Biol* **86**:205–233.
- Borowicz KK, Rwiader M, Drelewska E, and Czuczwar SJ (2004) Interactions between riluzole and conventional antiepileptic drugs—a comparison of results obtained in the subthreshold method and isobolographic analysis. *J Neural Transm* **111**:1511–1522.
- Chatrath R, Bell CM, and Ackerman MJ (2004) Beta-blocker therapy failures in symptomatic probands with genotyped long-QT syndrome. *Pediatr Cardiol* **25**:459–465.
- Chen J, Seeböhm G, and Sanguinetti MC (2002) Position of aromatic residues in the S6 domain, not inactivation, dictates cisapride sensitivity of HERG and eag potassium channels. *Proc Natl Acad Sci USA* **99**:12461–12466.

- Cooper EC and Jan LY (1999) Ion channel genes and human neurological disease: recent progress, prospects and challenges. *Proc Natl Acad Sci USA* **96**:4759–4766.
- Derst C, Konrad M, Kockerling A, Karolyi L, Deschenes G, Daut J, Karschin A, and Seyberth HW (1997) Mutations in the ROMK gene in antenatal Bartter syndrome are associated with impaired  $K^+$  channel function. *Biochem Biophys Res Commun* **230**:641–645.
- Gerlach U (2003) Blockers of the slowly delayed rectifier potassium  $I_K$  channel: potential antiarrhythmic agents. *Curr Med Chem Cardiovasc Hematol Agents* **1**:243–252.
- Kang J, Chen XL, Wang H, Ji J, Cheng H, Incardona J, Reynolds W, Viviani F, Tabart M, and Rampe D (2005) Discovery of a small molecule activator of the human ether-a-go-go-related gene (HERG) cardiac  $K^+$  channel. *Mol Pharmacol* **10**:1345–1359.
- Lawson K and Dunne MJ (2001) Peripheral channelopathies as targets for potassium channel openers. *Expert Opin Investig Drugs* **10**:1345–1359.
- Lee K, Park JY, Ryu PD, Kwon LS, and Kim HY (2003) IKr channel blockers: novel antiarrhythmic agents. *Curr Med Chem Cardiovasc Hematol Agents* **1**:203–223.
- Magdalan J (2003) New treatment methods in verapamil poisoning: experimental studies. *Pol J Pharmacol* **55**:425–432.
- Malysz J, Buckner SA, Daza AV, Milicic I, Perez-Medrano A, and Gopalakrishnan M (2004) Functional characterization of large conductance calcium-activated  $K^+$  channel openers in bladder and vascular smooth muscle. *Naunyn-Schmiedeberg Arch Pharmacol* **369**:481–489.
- Mitcheson JS, Chen J, Lin M, Culberson C, and Sanguinetti MC (2000) A structural basis for drug-induced long QT syndrome. *Proc Natl Acad Sci USA* **97**:12329–12333.
- Mohler PJ, Schott JJ, Gramolini AO, Dilly KW, Guatimosim S, duBell WH, Song LS, Haurogne K, Kyndt F, Ali ME, et al. (2003) Ankyrin-B mutation causes type 4 long-QT cardiac arrhythmia and sudden cardiac death. *Nature (Lond)* **421**:634–639.
- Moss AJ, Zareba W, Hall WJ, Schwartz PJ, Crampton RS, Benhorin J, Vincent GM, Locati EH, Priori SG, Napolitano C, et al. (2000) Effectiveness and limitations of beta-blocker therapy in congenital long-QT syndrome. *Circulation* **101**:616–623.
- Nestorowicz A, Wilson BA, Schoor KP, Inoue H, Glaser B, Landau H, Stanley CA, Thornton PS, Clement JP, Bryan J, et al. (1996) Mutations in the sulfonylurea receptor gene are associated with familial hyperinsulinism in Ashkenazi Jews. *Hum Mol Genet* **5**:1813–1822.
- Salata JJ, Jurkiewicz NK, Wang J, Evans BE, Orme HT, and Sanguinetti MC (1998) A novel benzodiazepine that activates cardiac slow delayed rectifier  $K^+$  currents. *Mol Pharmacol* **54**:220–230.
- Schwartz PJ, Periti M, and Malliani A (1975) The long Q-T syndrome. *Am Heart J* **89**:378–390.
- Schwartz PJ, Priori SG, Dumaine R, Napolitano C, Antzelevitch C, Stramba-Badiale M, Richard TA, Berti MR, and Bloise R (2000) A molecular link between the sudden infant death syndrome and the long-QT syndrome. *N Engl J Med* **343**:262–267.
- Seeböhm G, Pusch M, Chen J, and Sanguinetti MC (2003a) Pharmacological activation of normal and arrhythmia-associated mutant KCNQ1 potassium channels. *Circ Res* **93**:941–947.
- Seeböhm G, Pusch M, Chen J, and Sanguinetti MC (2003b) Pharmacological activation of normal and arrhythmia-associated mutant KCNQ1 potassium channels. *Circ Res* **93**:941–947.
- Shimizu W and Antzelevitch C (2000) Effects of a  $K^+$  channel opener to reduce transmural dispersion of repolarization and prevent torsade de pointes in LQT1, LQT2 and LQT3 models of the long-QT syndrome. *Circulation* **102**:706–712.
- Tan HL, Alings M, Van Olden RW, and Wilde AA (1999) Long-term (subacute) potassium treatment in congenital HERG-related long QT syndrome (LQTS2). *J Cardiovasc Electrophysiol* **10**:229–233.
- Thomas PM, Cote GJ, Wohlk N, Haddad B, Mathew PM, Rabl W, Aguilar-Bryan L, Gagel RF, and Bryan J (1995) Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. *Science (Wash DC)* **268**:426–429.
- Wang Q, Shen J, Splawski I, Atkinson D, Li Z, Robinson JL, Moss AJ, Towbin JA, and Keating MT (1995) SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. *Cell* **80**:805–811.
- Ward OC (1964) A new familial cardiac syndrome in children. *J Ir Med Assoc* **54**:103–106.
- Xu J, Wang X, Ensign B, Li M, Wu L, Guia A, and Xu J (2001) Ion-channel assay technologies: quo vadis? *Drug Discov Today* **6**:1278–1287.
- Xu X, Salata JJ, Wang J, Wu Y, Yan GX, Liu T, Marinchak RA, and Kowey PR (2002) Increasing  $I(K_s)$  corrects abnormal repolarization in rabbit models of acquired LQT2 and ventricular hypertrophy. *Am J Physiol* **283**:H664–H670.
- Yamaguchi S, Zhorov BS, Yoshioka K, Nagao T, Ichijo H, and Adachi-Akahane S (2003) Key roles of Phe1112 and Ser1115 in the pore-forming IIIIS5–S6 linker of L-type  $Ca^{2+}$  channel  $\alpha_1C$  subunit (CaV 1.2) in binding of dihydropyridines and action of  $Ca^{2+}$  channel agonists. *Mol Pharmacol* **64**:235–248.
- Zhorov BS, Folkman EV, and Ananthanarayanan VS (2001) Homology model of dihydropyridine receptor: implications for L-type  $Ca^{2+}$  channel modulation by agonists and antagonists. *Arch Biochem Biophys* **393**:22–41.

**Address correspondence to:** Dr. Guiscard Seeböhm, Physiologisches Institut 1, Universität Tuebingen, Gmelinstr. 5, D-72076 Tuebingen, Germany. E-mail: guiscard.seeböhm@gmx.de