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PERSPECTIVE

Activators of Cation Channels: Potential in Treatment of Channelopathies

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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. Potassium-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 α-subunits with other α- and β-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

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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-trifluro-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-trifluro-2-hydroxy-2-methylpropionamine; ZM-244085, 9-(3 cyanophenyl)hexahydro-1,8 acridinedione; NS-004, 1-(5-chloro-2-hydroxyphenyl)-5-trifluromethyl-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 dehydrosoyasaponin 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^+-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 α-subunits KCNQ1 (LQTS1) and HERG (human *ether-a-go-go*-related gene, LQTS2) or their β-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^{2+}-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 β-adrenergic receptors. β-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, β-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 β-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 π-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 areof relatively low potency. Examples include a variety of K_{ATP}-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^{2+}-activated K^+-channel [Big (large) conductance and small conductance K^+ 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 (Seebohm 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

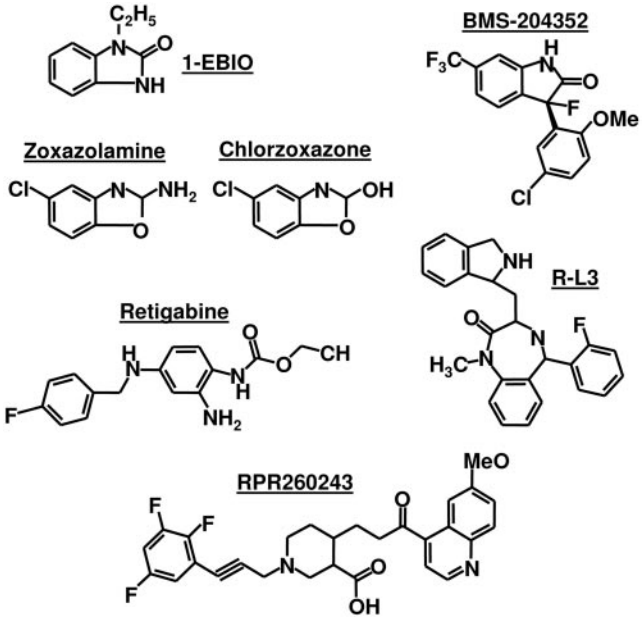

Fig. 1. Structure of potassium channel activators. Cation channel activators are small lipophilic molecules.

LQTS-associated mutant channels. However, one mutation disrupted the activating effect, probably by disrupting the R-L3 binding site (Seebohm 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_{Kr}-activation as a clinically relevant principle in cardiac repolarization disorders.

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