Drugs of the Future 2001, 26(5): 473-484

CCC: 0377-8282/2001

Review Article

I_{Ks} channel blockers: potential antiarrhythmic agents

U. Gerlach

Aventis Pharma Deutschland GmbH, Medicinal Chemistry, G838, D-65926 Frankfurt/Main, Germany.
e-mail: uwe.gerlach@aventis.com

CONTENTS

Introduction

The cell membrane is made up of an electrically isolating material known as the bilipid layer. To ensure the exchange of ions, channels are inserted. Most channel proteins are specialized in the transport of inorganic ions such as sodium, potassium, calcium and chloride, and connect the intracellular with the extracellular space. Every second more than 10 million ions can pass through an ion channel (1) enabling inorganic ions to diffuse rapidly along the electrochemical gradient through the membrane. However, this does not mean that channels are just water-filled pores in the membrane since they normally exhibit a high selectivity for certain ions. In addition, they are not permanently open but controlled by so-called gates, which are open for a short period of time and then close again. The opening of the gates is controlled by a specific stimulus such as a change in the membrane potential (*i.e.*, voltage-dependent ion channels), mechanical stress (*i.e.*, mechanically controlled ion channels) or binding of a messenger molecule (*i.e.*, ligand-coupled ion channels). These ion channels are responsible for the electrical excitability of nerve and muscle cells (2), the secretion of hormones (3), volume regulation of cells (4), as well as other important life-supporting activities.

Ion channels in the heart

Electrical impulses in cardiac and other excitable cells are generated by local changes in the relative permeability of the surface membrane to various ions. An outward flow of positively charged ions across the cell membrane results in a more negative potential (hyperpolarization) and the inward flow of positive ions, in contrast, makes the intracellular environment positive (depolarization). In the heart, during the normal cardiac cycle, a regular rhythmic pattern must be established in time-dependent changes of cellular permeability to ensure the cycle of events that underlies normal cardiac function. During the cardiac cycle, impulses that originate at the sinoatrial (SA) node are conducted via specialized conducting tracts throughout the atria until they converge at the atrioventricular (AV) node, pass through the bundle of His and the Purkinje fiber-conducting system, and excite the working myocardial cells in the ventricles. Coordination of these cellular events is ensured by the unique electrical properties of the different cell types.

The clinical test of electrical activity in the heart, the ECG (electrocardiogram) gives important information about events during the cardiac cycle, but cannot provide a detailed picture about the possible underlying mechanisms. Impulse spread can be tracked via the QRS complex and ventricular action potential duration can be evaluated by the QT interval. However, changes in either of these waveforms can be due to a complex interaction between perturbations in several ionic channels, thus making extrapolation to a specific ionic channel misleading.

The shape of the action potential of heart cells (Fig. 1) is strongly controlled by the correct interplay of ion channels (5). The main ion channels contributing to the action potential are the sodium, calcium and potassium channels. Potassium channels form the most diverse family and they are responsible for inward rectification (I_{K1}, I_{KACh}, I_{KATP}) and sustained outward (I_{Kur}, I_{Kr}, I_{Ks}) and transient outward (I_{to}) rectification. The depolarization (upstroke) of the membrane potential caused by an inward sodium current is followed by a partial early repolarization due to an outward potassium current through rapidly activating and inactivating K^+ channels. The extent of this early repolarization (notch) affects the time course of the other voltage-gated currents and, therefore, indirectly controls the action potential duration (APD). The plateau phase depends on a balance of inward (depolarizing) and outward (repolarizing) currents. The depolarizing force is mainly due to Ca^{2+} influx that slowly declines as L-type

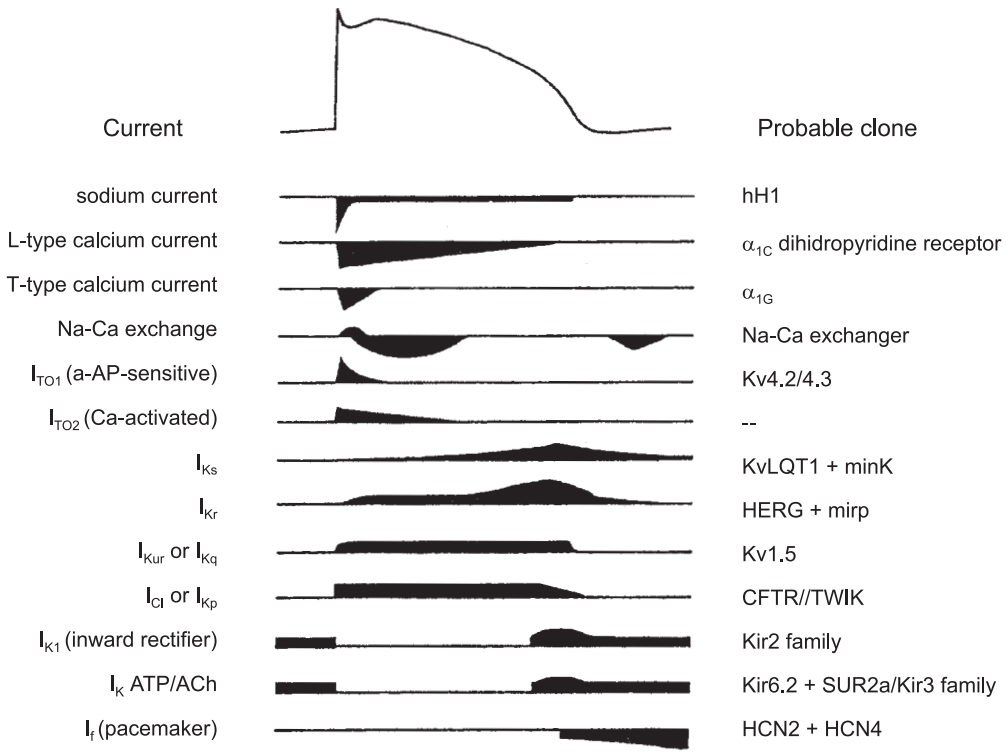


Fig. 1. Cardiac ion currents and their corresponding probable clones.

Ca^{2+} channels inactivate, but a noninactivating Na^+ current can also support the plateau phase. The repolarizing action depends on K^+ efflux due to activation of several voltage-gated potassium channels, mainly the rapid delayed rectifier (I_{Kr}) and the slowly delayed rectifier (I_{Ks}) potassium currents (Fig. 1). Several other conductances maintain or modulate the resting potential, most of which are inwardly rectifying K^+ channels which carry (almost) no current during the plateau phase.

The ATP-sensitive K^+ current, I_{KATP}, is important under some metabolic conditions. ATP-sensitive K^+ channels are inhibited by increased levels of intracellular ATP and are activated during ischemia, when ATP falls and ADP rises. Therefore, this channel has received attention because of the considerable role it plays in pathological conditions in which cellular ATP is likely to fall. These channels are probably responsible for shortening the action potential in severe ischemia (6). Most of these ion currents and their ion channels have been a target of antiarrhythmic therapy.

Ion channel blockers as antiarrhythmic agents

Cardiac arrhythmias are still a major cause of death in the Western world, especially in patients with ischemic heart disease. These irregularities are caused by abnormalities in electrical activity which may result from excessive sympathetic stimulation and or changes in the ionic mechanism responsible for the generation and propagation of the normal action potential.

The existing antiarrhythmic agents have been classified mechanistically into 4 main classes: class I (sodium channel blockade), class II (β-adrenergic blockade), class III (potassium channel blockade) and class IV (calcium channel blockade) (7). Some antiarrhythmic drugs, however, are not specific to one mechanism or ion channel, but instead act on several systems. A prominent example is the frequently used antiarrhythmic drug, amiodarone. It exerts a multiplicity of pharmacological effects which could be associated in part with each of the class I-IV antiarrhythmic agents and is an effective agent in the control of ventricular tachyarrhythmias and fibrillation (VT/VF) (8, 9).

Each of the antiarrhythmic classes have undergone several clinical trials in recent years which resulted in surprising and, in part, disappointing outcomes. In the 1980s, CAST (Cardiac Arrhythmic Suppression Trial) involving encainide and flecainide uncovered the inefficacy and even proarrhythmic risk of sodium channel blockers in patients with a relatively low long-term risk for cardiac death (10-13). The elevated mortality in the treated group was associated with agent-induced blockade of the sodium channel and the study was terminated.

To circumvent the problems associated with class I antiarrhythmics, pharmacological and clinical research shifted toward class III agents which act on the delayed rectifier potassium current and therefore prolong the AP. Dofetilide, *d*-sotalol, E-4031 and MK-499 are examples of

so-called pure class III compounds (14). All of these compounds act selectively on the rapid form of the delayed rectifier potassium channel (I_{Kr}), but not on the slowly activating potassium channel I_{Ks}. This class of agents also underwent a series of clinical trials which were quite disastrous for the patients and the drugs. The clinical study ESVEM (Electrophysiological Study versus Electrocardiographic Monitoring) provided positive evidence for the racemate *d,l*-sotalol which, in addition to I_{Kr} activity also possesses β-blocking properties (15). In contrast, the SWORD-trial (Survival With Oral *d*-sotalol) was terminated because of an increase in mortality in the treated patients using the pure I_{Kr} channel blocker *d*-sotalol (16). In the recent DIAMOND (Danish Investigation of Mortality on Dofetilide) study (17) conducted in patients with a higher risk of mortality than the SWORD population, the outcome was neutral at the best. The negative outcome of these clinical trials has resulted in the discontinuation of some developmental compounds (*e.g.*, MK-499).

These disappointing results have been attributed to what is referred to as negative use dependence which has been observed preclinically with various I_{Kr} blockers (18). Negative use dependence has two effects. First, an extended prolongation of the AP of heart cells at low heart rate frequencies and in a low β-adrenergic state. This prolongation of the AP may lead to early after depolarizations (EADs) and, under certain circumstances, to fatal torsades de pointes (TdP) (19, 20). The second effect is a weak prolongation of the AP under high heart rate frequency (21) and high β-adrenergic stimulation (22, 23). This unsatisfactory activity reduces the antiarrhythmic effect of these drugs and the positive effect of the AP prolongation expected from the activity on the AP at a normal heart frequency. Negative use dependency has been attributed to an increased contribution of the I_{Ks} channel on the repolarization under an elevated heart rate (21) and β-adrenergic activation (22).

The antiarrhythmic benefit afforded by class III agents is proposed to be the result of a sufficient prolongation of myocardial refractoriness such that the wavelength of activation exceeds the path length of the reentrant circuit, thereby preventing the initiation or maintenance of reentrant excitation (24). On the other hand, there is evidence that β-adrenergic sympathetic activity is an important factor in the genesis of malignant ventricular tachyarrhythmias (25-27). It has been demonstrated that β-adrenergic stimulation attenuates or even reverses the electrophysiological and antiarrhythmic effects of various class I antiarrhythmic agents (28) and class III antiarrhythmics such as *d*-sotalol (29), E-4031 (22) and sematilide (30). Since this may be due to an increase of I_{Ks} under these conditions, I_{Ks} channel blockers seem to offer a more desirable class III antiarrhythmic profile than I_{Kr} channel blockers.

Molecular structure and function of the I_{Ks} channel

The prominent features of the I_{Ks} current are the slow activation upon depolarization and no inactivation even under a long period of depolarization (31). This slow opening of the delayed rectifier channels is believed to be mainly responsible for the repolarization of the excitable cells and therefore for the duration of the plateau phase of the cardiac AP. This ensures optimal cardiac function by regulating the relationship between systolic ejection and diastolic filling (32). It was proposed as early as 1969 with the first quantitative description of time-dependent outward currents, that the delayed rectifier current I_K in Purkinje fibers of sheep consists of more than one component (33). This was the first description of the I_{Ks} current. Later, the I_K of guinea pigs myocytes was found to be composed of two currents (34-36) and the slow component was called I_{Ks}.

Ever since the 1990s, the molecular basis of I_{Ks} has been controversial. When the I_{sK} (also called minK) protein, which was originally cloned from rat kidney (31) and expressed in *Xenopus* oocytes, was isolated, a potassium current with a striking similarity to the native current was found (31). This is the reason for considering the I_{sK} a channel protein on which the I_{Ks} should be based (37). On the other hand, I_{sK} encoded a protein of 130 amino acids (129 in humans) and with a pore domain unique from other voltage-dependent channels and only one transmembrane domain. The question as to whether I_{sK} alone may create a voltage-gated potassium channel was raised particularly because functional I_{sK} expression in a number of eukaryotic cell lines failed (38-40). Very early it was suggested that I_{sK} is not a channel by itself, but a regulator of a dormant endogenous *Xenopus* oocyte potassium channel (41). Soon after the discovery of KvLQT1 gene by positional cloning (42), it was shown that the KvLQT1 gene product was a potassium channel subunit that generated a potassium current similar to an I_{Ks} current when expressed together with the I_{sK} protein in different systems (43, 44). However the interaction of I_{sK} with KvLQT1 subunits is unusual. It has been shown that there is a direct interaction of the C-terminus domain of the I_{sK} protein (residues 67-129) with the pore region of the KvLQT1 protein (45). These data explain both the increase in amplitude of the current when I_{sK} is coexpressed with KvLQT1 as well as the slowing down of the activation kinetics. The ratio of I_{sK} to KvLQT1 subunits is still unknown although it was proposed that a tetramer of KvLQT1 proteins could associate with 1-4 I_{sK} proteins (34). It was shown that the I_{sK} protein has a significant influence on the inhibitory potency of I_{Ks} channel blockers like the chromanoles (see below) (40, 46, 47). Recently, KCNQ1 is the name used to replace KvLQT1 and KCNE has substituted minK (I_{sK}) (48).

Tissue and species distribution of the I_{Ks} channel

The I_{Ks} channel and its underlying proteins KvLQT1 and I_{sK} have been found in several species and tissues using molecular biology and electrophysiological techniques. Table I gives a representative overview of these findings. For the sake of simplicity, no differentiation has

Table I: Species and tissue distribution of the slowly delayed rectifier current (I_{Ks}).

Species	Tissue	Ref.
Human	Heart	93, 94
Dog	Heart	95, 96
Rabbit	Heart	97, 98
Guinea pig	Heart	34-36
Mouse	Heart (not adult)	99-101
Rat	Heart (neonatal)	37
Frog	Heart (atrium)	102
Gerbil	Inner ear	103, 104
Rat	Uterus	37, 105
Rat	Colon	49, 50
Rat	Tracheal epithelia	106
Rat	Kidney	107
Rat	Pancreas	108

been made as to whether the channel is functional or just one of the proteins.

Selective I_{Ks} channel blockers

The first selective blockers of the I_{Ks} channel were published in 1995 and 1996 by two groups who employed two different approaches and found two different chemical structures to be active (49-52). One group modified benzodiazepines from MSD (52) while the other modified chromanols (49, 50, 51) from Hoechst. Both the structures of these compounds and the information obtained from their use concerning the I_{Ks} channel will be discussed in detail, with special attention given to the widely distributed chromanol 293B. Just recently, a third highly potent I_{Ks} channel blocker was described by BMS although little data is available (53).

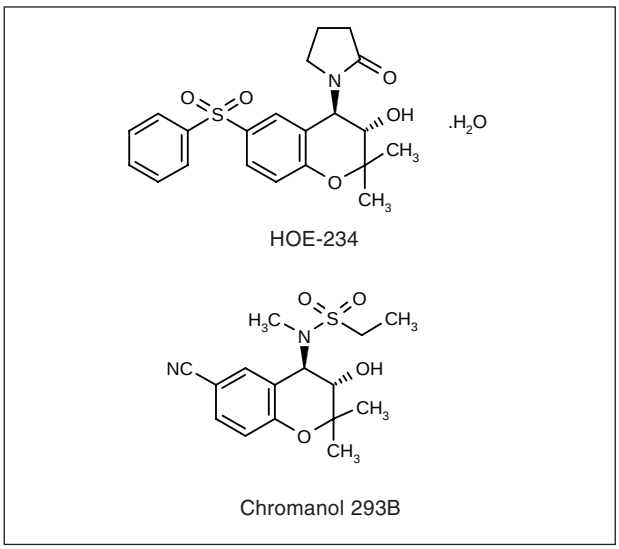


Fig. 2. K_{ATP} channel opener (HOE-234) and I_{Ks} channel blocker (chromanol 293B).

Chromanols

The I_{Ks} channel blocking ability of 293B was found to be a side activity in another research program. In the 1980s, many K_{ATP} channel openers such as HOE-234 were synthesized (54); sulfonamide analogs were also prepared (Fig. 2). Although these structures were inactive on the intended target K_{ATP} channel, they were tested for their activity on chloride transport. Surprisingly, high activity on Cl^- transport was discovered (50). A prominent compound in this series was chromanol 293B. The first publication proposed that the compounds act only indirectly on the Cl^- transport by blocking an associated cAMP-regulated potassium channel (50). This potassium conductance was responsible for the secondary phase of the voltage and conductance transit. The exact nature of this potassium channel was not clear in this early publication. Subsequent studies on cloned potassium channels from the guinea pig demonstrated that chromanol 293B specifically blocks I_{sK} channels expressed in *Xenopus* oocytes with an IC_{50} value of 6.2 μM (51). The (3*R*,4*S*)-enantiomer was found to be more potent than the (3*S*,4*R*)-enantiomer (IC_{50} = 5 and 39 μM, respectively) (49). In contrast, 293B had negligible effects on cloned potassium channels (Kv1.1, Kir2.1, HERG) expressed in *Xenopus* oocytes, proving the selectivity of the compound on I_{sK} channels (49, 51) and other closely related potassium channels (55).

Several studies have been conducted to elucidate the site of blockade at the molecular level. There are conflicting data concerning the discrepancy in block of homomeric KvLQT1 and heteromeric KvLQT1/minK channels by chromanol 293B (49). While I_{Ks} and KvLQT1 were blocked in a similar manner in COS-7 cells (56), significantly different values were obtained for the two channels in *Xenopus* oocytes using both the racemate and single enantiomers of 293B (40, 57). This finding in oocytes was later supported by investigations using other chromanols which had significantly different effects on I_{Ks} and KvLQT1 (47). In this context, the binding region for chromanols was identified to be within the H5/S6 region of KvLQT1 (58).

The activity of 293B on the native I_{Ks} channel in guinea pig cardiomyocytes was comparable (IC_{50} = 2.1 μM) to that measured in the *Xenopus* oocyte, indicating a link with cardiac activity. Furthermore, the selectivity to I_{Kr} in guinea pig cardiomyocytes was proven. Thus, these novel structures were found to be selective I_{Ks} channel blockers. The activity and selectivity of 293B on native channels of guinea pig and human cardiomyocytes was investigated using the patch clamp technique on single cells. 293B strongly inhibited I_{Ks} although other ion currents such as the inward rectifier K^+, Na^+ and L-type Ca^{2+} currents were unaffected. The I_{to} in human ventricular myocytes was inhibited by 293B at an EC_{50} of 24 μM, showing 20-fold selectivity for I_{Ks}. 293B prolonged repolarization (APD_{90}) in guinea pig and human ventricular myocytes to a similar fractional extent (~33%, 1 μM) and independent of the stimulation frequency (59). This was

in contrast to the measured reverse use-dependent AP prolongation observed with the I_{Kr} blocker dofetilide (59).

The state-, frequency- and enantiomer-dependent block of the potassium channel I_{Ks} by 293B in *Xenopus* oocytes (60) and on the AP of rabbit ventricular myocytes (61) were investigated. The positive use dependency found in guinea pig (59) was not seen in the rabbit (61), possibly due to species differences. A further study using guinea pig ventricular myocytes investigated the time-dependent block of 293B (62). The IC_{50} value obtained was comparable to previously reported results (59) and a mathematical model for the 293B block was constructed. The blocking rate constant showed a linear function with 293B concentration, indicating a 1:1 binding stoichiometry (62). At a membrane potential of +80 mV, the blocking rate was 4×10^4 $M^{-1}s^{-1}$ and the unblocking rate was 0.2 s^{-1}.

The activity of 293B on rabbit and porcine SA nodes was also investigated. In both cases, 293B blocked the I_{Ks} in SA node with an IC_{50} value of ~5 μM in the rabbit and 8.8 μM in pig. However, in the pig, the effect was much more pronounced as the I_K in the porcine SA node is largely derived from I_{Ks}. This seems to be in agreement with the marked effect of HMR-1556 on ventricular and atrial effective refractory period (ERP) found in the anesthetized pig. The effect on the SA node and, therefore, on rabbit heart rate, seems negligible (63-65).

The activity of chromanol 293B was also shown in a multicellular preparation. The transmembrane APD of guinea pig papillary muscles was measured after equilibration with 293B (10 μM), dofetilide and ambasilide and addition of isoproterenol (10 and 100 nM) (23). While the AP prolonging effect of dofetilide was reduced in the presence of isoproterenol, the effect of 293B was clearly increased (23). Measurement of the effect of 293B (1-100 μM) on transmural ECG and transmembrane APs from epi-, myo- and endocardial cells from arterially perfused wedges of canine left ventricle showed a dose-dependent prolongation of QT interval and APD_{90} in the three cell types (66). There was no widening of the T wave, increase in the transmural dispersion of repolarization or TdP. In both experiments during the setting of enforced slow pacing and isoproterenol, the drug showed EADs which may simulate the conditions of LQT1-syndrome (23, 38, 66). These signs for proarrhythmia could be leveled off by additional application of β-blockers (66, 67).

An experiment in anesthetized dogs using a highly sophisticated mapping system demonstrated the interesting properties of these I_{Ks} channel blockers *in vivo* (68, 69). Measurement of the regional differences of ERP in the canine heart via needle electrodes inserted in the left ventricle showed that chromanol 293B (10 mg/kg) led to a regional, uniform increase in local ERP while still preserving the transmural homogenicity of refractoriness (68). Furthermore, 293B had a more pronounced effect under faster rather than slower pacing rates and had a positive use-dependent effect on ERP, in contrast to the I_{Kr} blocker dofetilide which showed negative use dependence in the same model (68, 69).

The electrophysiological effects of 293B in the postinfarcted canine heart were investigated using the same method (70, 71). 293B ubiquitously prolonged local ERP in the infarcted, border and normal zones of these hearts, with the most marked effects seen in the infarct zone. A drug-induced transmural dispersion was not observed and again a positive use dependency could be measured (71). The prolongation of refractoriness in the ischemic myocardium was even more pronounced at a faster rather than slower heart rate and was homogenous throughout the intact ventricular wall (70, 71).

Comparison of 293B, L-735,821 (an I_{Ks} channel blocker from MSD) and two I_{Kr} channel blockers in dog ventricular myocytes, multicellular right ventricular papillary muscle and Purkinje fiber preparations showed a slight frequency-independent increase in APD (~7%) following addition of 293B (10 μM) and L-735,821 (100 nM). However, a marked (20-80%) lengthening of APD was observed with the I_{Kr} channel blockers which also showed a reverse frequency dependency (72). *In vivo* ECG recordings from intact anesthetized dogs indicated no significant effect of 293B (1 mg/kg i.v.), but this may be due to an insufficient dosage (72).

293B was further used to characterize the cardiac repolarization in Purkinje fibers of German shepherd dogs with the inherited syndrome of sudden death (73). The results of these investigations showed that I_{Ks} may be involved in this anomaly.

In the meantime, a chemical optimization program revealed novel and more potent compounds. Surprisingly, removal of the hydroxy group increased the activity of the chroman structures as demonstrated by IKS-142, which had an IC_{50} value of 22 nM (74). A more potent compound with the chromanol structure, HMR-1556, was also found and profiled as a developmental compound at Aventis. HMR-1556 or (3*R*,4*S*)-(+)-*N*-[-3-hydroxy-2,2-dimethyl-6-(4,4,4-trifluorobutoxy)chroman-4-yl]-*N*-methylmethanesulfonamide is a close analog of 293B although with improved activity due to the trifluoro-butoxy side chain on the aromatic ring (74, 75) (Fig. 3).

Comparison of this prototype compound with the starting point, the K_{ATP} channel opener HOE-234, showed some remarkable differences. In the agonist series, electron withdrawing substituents on the aromatic ring such as cyano or sulfonyl groups were the most potent, while in the case of the I_{Ks} channel blockers, electron donating groups such as alkoxy or benzyloxy were the most potent. Furthermore, for the I_{Ks} channel blockers, the stereochemistry on the C-4 nitrogen and on the C-3 hydroxy was opposite to the stereochemistry of the more active enantiomer for the K_{ATP} agonist (54, 74, 75).

HMR-1556 inhibits the potassium current of I_{Ks} channels expressed in *Xenopus* oocytes with an IC_{50} value of 120 nM (76). In contrast, 10 μM HMR-1556 had no effect or only slightly blocked the currents of the expressed K^+ channels HERG, Kv1.5, Kv1.3 and Kir2.1, and the cationic current HCN2, indicating the selectivity of the compound (76). The opposite enantiomer (S-5557) was

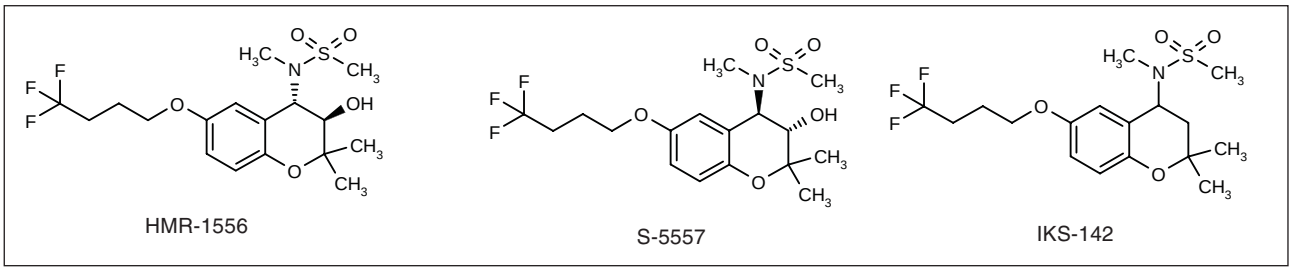


Fig. 3. Chroman and chromanol I_{Ks} channel blockers.

about 5 times less active, inhibiting the I_{Ks} channel with an IC_{50} value of 0.56 μM (74, 75).

Studies using whole-cell patch clamping of isolated guinea pig ventricular myocytes revealed that HMR-1556 induced inhibition of the I_{Ks} current with an IC_{50} of 34 nM; other currents such as I_{Kr} and I_{K1}, were only slightly blocked at a concentration of 10 μM. However, 10 μM HMR-1556 inhibited the transient outward current (I_{to}) and the sustained outward current (I_{sus}) in rat ventricular myocytes by 25 and 36%, respectively. The L-type Ca^{2+} channel in guinea pig cardiomyocytes was blocked by 31% with 10 μM HMR-1556 (76), indicating a selectivity of about 300-fold over other ion channels.

Another study examining guinea pig right papillary muscles using the micropuncture technique at various pacing rates showed that in the frequency range of 0.5-7 Hz, HMR-1556 (1 μM) caused a 19-27% prolongation of the APD_{90}. The prolongation of APD_{90} was even more pronounced in the presence of isoproterenol (10 μM), and was more pronounced at low pacing rates (47 and 35% at 0.5 and 1 Hz, respectively, *vs.* 25% at 7 Hz) (76).

Results from studies recording the monophasic AP in Langendorff-perfused guinea pig hearts showed that in spontaneously beating preparations, HMR-1556 (0.1 and 1 μM) prolonged the mean APD_{90} by 3 and 10%, respectively, with no further prolongation seen at concentrations of 10 μM. The prolongation was much greater at low pacing rates (25% at 100 beats/min [bpm] and 13% at 150 bpm) than at fast pacing rates (9% at 350 bpm). The left ventricular pressure (LVP_{max}) was not affected by 1 μM HMR-1556, but was decreased by 15% with a concentration of 10 μM. Other parameters such as heart rate and coronary flow were only slightly decreased with 1 μM HMR-1556 (76).

Since safety is always an obvious concern when dealing with antiarrhythmic agents, HMR-1556 was investigated in an *in vitro* test model for TdP in isolated rabbit hearts (77). Results showed that HMR-1556 (10 μM) prolonged the mean AP by 11-18%. Under low potassium/low magnesium and low pacing conditions, no EADs, a predictor of TdP, occurred. This is in clear contrast to the I_{Kr} channel blockers which show a high incidence of TdP in this model (78). Addition of isoproterenol also did not lead to EADs.

The effect of HMR-1556 (1 mg/kg i.v.) on the refractory period of the atrium and ventricle at various frequencies (100-180 bpm) in chloralose-anesthetized pigs was investigated. A pronounced prolongation was observed in the atrium (33-36%) and the ventricle (35-40%) which was independent of frequency. The prolongation could be described as neutral use-dependent (65).

Class III activity of HMR-1556 was further demonstrated *in vivo* in studies reporting dose-dependent prolongation of QTc in conscious telemetric dogs following oral treatment (3, 10, 30 mg/kg) (74).

Benzodiazepines

The second chemical class of I_{Ks} channel blockers reported are the benzodiazepines (Fig. 4). This class was discovered in a completely different way than the chromanols. L-365,260 was under development as a cholecystokinin-B (CCK-B) antagonist (79). Following a high oral dose (100 mg/kg), the QT time in dogs was markedly increased, prompting further investigation of this side effect (80). It was found that L-365,260 was a potent inhibitor of the I_{Ks} channel (IC_{50} = 214 nM). The selectivity against I_{Kr} channel blockade (IC_{50} = 5000 nM) was good, but the main activity was the CCK-B antagonism with an IC_{50} value of 2 nM (80). This was the starting point for a chemical optimization program with the goal of preserving or even enhancing I_{Ks} channel activity and minimizing the activity on the CCK-B receptor. The main variations were done on the substituent of the C-3 amine and replacement of the urea group with amides resulted in a new series of compounds with activity on the potassium channel. The prototypes were the phenyl-acrylic derivative (L-735,821) which was the first I_{Ks} channel blocker published from this series (52), and the phenylacetyl derivative L-768,673, which was further investigated *in vivo* (80).

L-735,821 blocked the I_{Ks} channel in guinea pig cardiomyocytes with an IC_{50} value of 6 nM and was 250-fold more selective for this channel over I_{Kr} (IC_{50} = ~1.5 μM) (52). Furthermore, it had no effect on I_{K1} at concentrations < 10 μM although it blocked the L-type calcium current at > 0.3 μM. A special prepulsing protocol revealed that the block by the compound was time-, voltage- and use-dependent, suggesting that it affects the channel preferentially in the open state. L-735,821 increased the APD_{90}

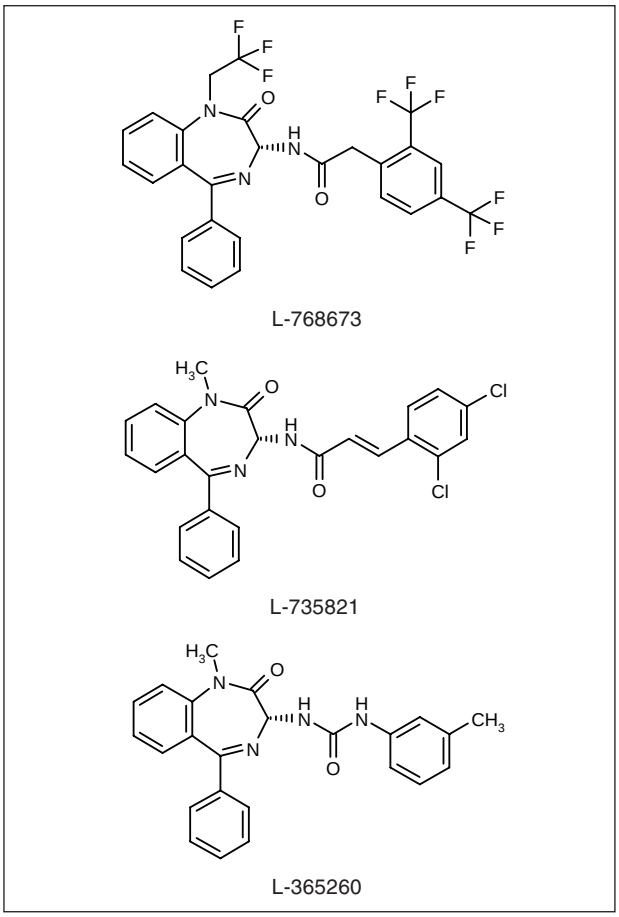


Fig. 4. Benzodiazepine I_{Ks} channel blockers.

in a dose- and frequency-independent manner (52).

An interesting comparison of the selective I_{Ks} channel blocker L-735,821 (20 nM) with the selective I_{Kr} channel blocker MK-499 (200 nM) was performed in a study using rabbit Purkinje cells (81). Both compounds significantly lengthened the AP, but prolonged exposure of the cells to MK-499 resulted in the appearance of EADs, an indication of proarrhythmic effects. These negative effects were not seen with L-735,821.

In addition to enhanced activity, increased bioavailability and a longer half-life were also desirable. Under these premises, L-768,673 was chosen as a developmental candidate (82, 83). L-768,673 blocked the I_{Ks} channel in guinea pig cardiomyocytes with an IC_{50} value of 6 nM and was highly selective for this channel over I_{Kr} (IC_{50} = ~6 μM). In addition, the activity at the CCK-B receptor was eliminated (IC_{50} > 1000 nM). The bioavailability was 27% (methocel suspension) and 76% (oil-gelcap) and the half-life was an extraordinarily long 43 h in dogs and 47 h in rats (80).

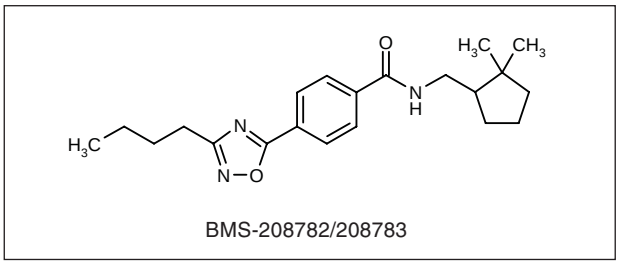
Several *in vivo* experiments have been performed using L-768,673 with results further demonstrating the compound's I_{Ks} channel blocking properties. Class III activity was demonstrated following oral L-768,673 dosing in conscious dogs which resulted in dose-dependent prolongation of the QT interval of up to 15% (80). Investigations on hemodynamic and electrocardiographic effects of the agent after i.v. administration to chloralose-anesthetized dogs revealed no significant effects of the agent on blood pressure, left ventricular pressure, PR time or QRS time (80).

In addition to investigations of the electrophysiological effects of the agent, the antiarrhythmic activity was also examined (84). In an anesthetized canine model of recent (about 8 days) anterior myocardial infarction, L-768,673 (0.003 and 0.03 mg/kg i.v.) significantly suppressed electrically induced ventricular tachyarrhythmias (VT) and reduced the incidence of lethal arrhythmias induced by myocardial ischemia (80 and 90% survival, respectively) (84). Remarkably, this efficacy was associated with only a modest increase in the QTc interval of 4-6%. I_{Kr} blockers require a 12-17% increase in QTc time to achieve a more than 50% reduction in the incidence of malignant ventricular arrhythmias.

In a conscious canine model of healed (approx. 4 weeks) anterior myocardial infarction, ventricular fibrillation (VF) was provoked by transient occlusion of the left circumflex coronary artery during exercise. Pretreatment with L-768,673 (0.03 mg/kg i.v.) prevented the ischemia-provoked arrhythmias during exercise-induced high sympathetic tone in 5 of 6 animals previously susceptible to VF. Efficacy in this experiment was also accompanied by only a slight increase in the QTc interval of 7% (84). In this model, I_{Kr} blockers like *d*-sotalol were ineffective in preventing arrhythmias, presumably due to an attenuation of the effects of I_{Kr} blockade in the setting of high heart rate and high sympathetic tone. (29, 30, 85, 86). L-768,673 has, therefore, shown activity as an antiarrhythmic drug for the treatment of ventricular arrhythmia and the prevention of sudden cardiac death (SCD). Reports of multikilogram synthesis for this agent (82, 83) suggest further development.

Benzamides

Recently, the structure of a novel I_{Ks} channel blocker was published (53). The molecular target I_{Ks} was expressed in *Xenopus* oocytes and compounds from a screen which blocked the K^+ current in these oocytes by > 50% were used as initial leads. These tetralones were represented by SQ-23791, with an IC_{50} value at I_{Ks} of 5 μM. SAR studies led to a compound with a 40-fold selectivity for I_{Ks} although it also had affinity for other cardiac ion channels and certain CNS receptors. This effect was shown to be due to the pharmacophore essential to I_{Ks} affinity and as a result this series was unavoidably abandoned. SAR studies of SQ-23791 revealed two benzamides that had good activity at I_{Ks} but which were without activity at I_{Kr}, I_{Na} or I_{Ca}. Modification of the amine portion of the molecule revealed that branched alkyl amines were preferred and stereoselectivity was not high. Variation of the linker modifications showed that isosteric



BMS-208782/208783

Fig. 5. Benzamide I_{Ks} channel blocker.

ureas and oxazoles were inactive, but further modification of aryl substituents led to the synthesis of BMS-208782/208783 (IC_{50} = 2 and 9 nM for the (*R*)- and (*S*)-enantiomers, respectively) (Fig. 5). Both had IC_{50} values for inhibition of I_{Kr} of > 30 μM, inhibition of I_{Ca} and I_{Na} of > 10 μM, and no activity in CNS screens. BMS-208782 had a half-life of 3.5 h in dogs, with oral bioavailability of only 5% (53). Metabolism studies revealed that the butyl chain attached to the heterocyclic ring was a primary site for inactivation. To partially solve this problem, the chain was shortened and capped with a trifluoromethyl group. This improved bioavailability in dogs to 23%, but reduced affinity for the I_{Ks} channel to 40 nM. This program was said to be abandoned although a convincing reason was not given (53).

Drugs with I_{Ks} blocking activity as an additional effect

In addition to the structures described above which represent highly active and selective I_{Ks} channel blockers, there are also reports of compounds showing antagonistic activity on I_{Ks} as an additional effect. These compounds are shown in Table II but only a few will be mentioned. The most well-known compound with additional effects on I_{Ks} is azimilide (87). Although it blocks the I_{Ks} channel in guinea pig ventricular myocytes with an IC_{50} value of 3 μM (88), it is 7.5-fold more potent in blocking the I_{Kr} channel (IC_{50} = 0.4 μM) (88). At first glance, this dual action on both delayed rectifier channels seems intriguing. However, studies with azimilide showed that a combined blockade of I_{Kr} and I_{Ks} enhanced the arrhythmogenic effect of sympathetic stimulation (89).

Various barbiturates are also antagonists of the I_{Ks} channel with very high concentrations required to reduce the potassium flow half-maximally (90, 91). These concentrations may be achieved during anesthesia in the clinic.

Conclusions

During the last several years, three different structural classes have been found to be potent and selective blockers of the I_{Ks} channel. The respective prototypes of these three classes are HMR-1556, L-768,673 and BMS-208782. L-768,673 and HMR-1556 have exhibited activity in several *in vitro* and *in vivo* studies and L-768,673 in particular, which was active in a model of clinical SCD, proved the concept that I_{Ks} channel blockade is an antiarrhythmic principle (92). It was also remarkable that the I_{Kr} blockers failed in this model (85, 86). Furthermore, L-768,673 significantly reduced the incidence of lethal arrhythmias induced by myocardial ischemia in a canine model of recent myocardial infarction (84). Interestingly, this antiarrhythmic effect was seen in both cases with only a moderate increase in QTc (84). The electrophysiological mode of action of dose-dependently prolonging ERP or QTc was demonstrated *in vivo* with HMR-1556 in anesthetized pigs (65) and with both compounds in conscious telemetric dogs (74, 84).

As with all antiarrhythmic agents, there is always the concern of proarrhythmia. However, when HMR-1556 was investigated in a TdP model of isolated rabbit heart, it showed no negative effects and positively distinguished

Table II: Compounds showing antagonistic activity on I_{Ks} as an additional effect.

Compound	IC_{50} (I_{Ks})	Main activity	Ref.
Ambasilide	32 μM	Various ion channels	109
Azimilide	1-3 μM	I_{Kr} channel blockade	88, 110
Barbiturates	0.26-0.56 μM	Anesthetic	90, 91
Cetirizine	1 mM	Antihistamine	111, 112
Clofilium	50 μM	Various ion channels	113, 114
Diphenhydramine	130 μM	Antihistamine	112, 115
Ebastine	0.8 μM	Antihistamine	112
Haloperidol	2.6 μM	Antipsychotic	116
Indapamine	100 μM	Diuretic	117
Propofol	250 μM	Anesthetic	90
Quinidine	~50 μM	Various ion channels	118, 119
Tedisamil	2.5 μM	Various ion channels	120
Terfenadine	10 μM	Antihistamine	112, 115
Thiopentone	56 μM	Anesthetic	90
Triamterene	100 μM	Diuretic	121
Trioridazine	14 μM	Psychotropic	122

itself from the I_{Kr} channel blocker (77, 78). *In vivo* safety studies are still ongoing.

As mentioned above, there is evidence that I_{Ks} channel activity is increased under β-adrenergic stimulation (22) and increased β-adrenergic activity leads to malignant ventricular tachyarrhythmias (25-27). Thus, a blocker of these properties is highly attractive. An increase or preservation of AP prolongation during β-adrenergic activation is included in the class III antiarrhythmic profile and appears to be more desirable than the classic class III antiarrhythmic (I_{Kr}) compounds.

The collected data suggest that selective I_{Ks} channel blockers may be well suited to be new antiarrhythmic agents and we look forward to the first clinical trials with these agents.

References

1. Hille, B. *Membrane excitability: Action potential propagation.* In: Textbook of Physiology. Patton, H.D. et al. (Eds.). W.B. Saunders: Philadelphia 1989, 46-79.

2. Hodgkin, A.L., Huxley, A.F., Katz, B. *Measurement of current-voltage relations in the membrane of the giant axon of Loligo.* J Physiol (Lond) 1952, 116: 424-48.

3. Dean, P.M., Matthews, E.K. *Electrical activity in pancreatic islet cells.* Nature 1968, 219: 389-90.

4. Grinstein, S., Dupre, A., Rothstein, A. *Volume regulation by lymphocytes. Role of calcium.* J Gen Physiol 1982, 79: 849-68.

5. Roden, D.M. *Antiarrhythmic drugs.* In: The Pharmacological Basis of Therapeutics. Hardman, J.G., Limbird, L.E., Molinoff, P.B., Ruddon, R.W., Gilman, A.G. (Eds.). McGraw-Hill: New York 1996, 839-74.

6. Billman, G.E. *Role of ATP sensitive potassium channel in extracellular potassium accumulation and cardiac arrhythmias during myocardial ischaemia.* Cardiovasc Res 1994, 28: 762-9.

7. Vaughan Williams, E.M. *Classifying antiarrhythmic actions: By fact or speculation.* J Clin Pharmacol 1992, 32: 964-77.

8. Charlier, R., Deltour, G., Baudine, A., Chaillet, F. *Pharmacology of amiodarone, an antianginal drug with a new biological profile.* Arzneim-Forsch 1968, 18: 1408-17.

9. Mason, J.W. *Amiodarone.* N Engl J Med 1987, 316: 455-66.

10. *Preliminary report: Effect of encainide and flecainide on mortality in a randomized trial of arrhythmia suppression after myocardial infarction. The Cardiac Arrhythmia Suppression Trial (CAST) Investigators.* N Engl J Med 1989, 321: 406-12.

11. Echt, D.S., Liebson, M.D., Mitchell, L.B. et al. *Mortality and morbidity in patients receiving encainide, flecainide, or placebo. The Cardiac Arrhythmia Suppression Trial.* N Engl J Med 1991, 324: 781-8.

12. Woosley, R.L. *CAST: Implications for drug development.* Clin Pharmacol Ther 1990, 47: 553-6.

13. Pratt, C.M., Moye, L.A. *The cardiac arrhythmia suppression trial: Background, interim results and implications.* Am J Cardiol 1990, 65: 20-9B.

14. Singh, B.N. *The coming of age of the class III antiarrhythmic principle: Retrospective and future trends.* Am J Cardiol 1996, 78(Suppl. 4A): 17-27.

15. Mason, J.W. *A comparison of seven antiarrhythmic drugs in patients with ventricular tachyarrhythmias. Electrophysiologic Study versus Electrocardiographic Monitoring Investigators.* N Engl J Med 1993, 329: 452-8.

16. Waldo, A.L. Camm, A.J., DeRuyter, H. et al. *Effect of d-sotalol on mortality in patients with left ventricular dysfunction after recent and remote myocardial infarction. The SWORD Investigators. Survival With Oral d-Sotalol.* Lancet 1996, 348: 7-12.

17. Torp-Pedersen, C., Moller, M., Bloch-Thomsen, P.E. et al. *Dofetilide in patients with congestive heart failure and left ventricular dysfunction. Danish Investigations of Arrhythmia and Mortality on Dofetilide Study Group.* N Engl J Med 1999, 341: 857-65.

18. Hondeghem, L.M., Snyders, D.J. *Class III antiarrhythmic agents have a lot of potential but a long way to go: Reduced effectiveness and dangers of reverse use dependence.* Circulation 1990, 81: 686-90.

19. Hohnloser, S.H. *Proarrhythmia with class III antiarrhythmic drugs: Types, risks, and management.* Am J Cardiol 1997, 80: 82-9G.

20. Haverkamp, W., Shenasa, M., Borggrefe, M., Breithardt, G. *Torsade de pointes*. In: Cardiac Electrophysiology: From Cell to Bedside. Zipes, D.P., Jalife, J. (Eds.). Saunders: Philadelphia 1995, 885-99.

21. Jurkiewicz, N.K., Sanguinetti, M.C. *Rate-dependent prolongation of cardiac action potentials by a methanesulfonanilide class III antiarrhythmic agent. Specific block of rapidly activating delayed rectifier K^+ current by dofetilide.* Circ Res 1993, 72: 75-83.

22. Sanguinetti, M.C., Jurkiewicz, N.K., Scott, A., Siegl, P.K.S. *Isoproterenol antagonizes prolongation of refractory period by the class III antiarrhythmic agent E-4031 in guinea pig myocytes.* Circ Res 1991, 68: 77-84.

23. Schreieck, J., Wang, Y., Gjini, V. et al. *Differential effect of β-adrenergic stimulation on the frequency-dependent electrophysiologic actions of the new class III antiarrhythmics dofetilide, ambasilide, and chromanol 293B.* J Cardiovasc Electrophysiol 1997, 8: 1420-30.

24. Wellens, H.J., Brugada, P., Faber, J. *Ventricular arrhythmias: Mechanisms and actions of antiarrhythmic drugs.* Am Heart J 1984, 107: 1053-7.

25. Yusuf, S., Peto, R., Lewis, J. et al. *β-Blockade during and after myocardial infarction: An overview of the randomized trials.* Prog Cardiovasc Dis 1985, 27: 335-71.

26. Freedman, R.A., Swerdlow, C.D., Echt, D.S. et al. *Facilitation of ventricular tachyarrhythmia induction by isoproterenol.* Am J Cardiol 1984, 54: 765-70.

27. Reddy, C.P., Gettes, L.S. *Use of isoproterenol as an aid to electric induction of chronic recurrent ventricular tachycardia.* Am J Cardiol 1979, 44: 705-13.

28. Jazayeri, M.R., Van-Wyhe, G., Avitall, B. et al. *Isoproterenol reversal of antiarrhythmic effects in patients with inducible sustained ventricular tachyarrhythmias.* J Am Coll Cardiol 1989, 14: 705-11.

29. Vanoli, E., Priori, S.G., Nakagawa, H. et al. *Sympathetic activation, ventricular repolarization and I_{Kr} blockade: Implications*

for the antifibrillatory efficacy of potassium channel blocking agents. J Am Coll Cardiol 1995, 25: 1609-24.

30. Sager, P.T., Follmer, C., Uppal, P. et al. *The effects of β-adrenergic stimulation on the frequency-dependent electrophysiologic actions of amiodarone and sematilide in humans.* Circulation 1994, 90: 1811-9.

31. Takumi, T., Ohkubo, H., Nakanishi, S. *Cloning of a membrane protein that induces a slow voltage-gated potassium current.* Science 1988, 242: 1042-5.

32. Kass, R.S., Freeman, L.C. *Potassium channels in the heart.* Trends Cardiovasc Med 1993, 3: 149-59.

33. Noble, D., Tsien, R.W. *Outward membrane currents activated in the plateau range of potential in cardiac Purkinje fibers*. J Physiol 1969, 200: 205-31.

34. Sanguinetti, M.C., Jurkiewicz, N.K. *Two components of cardiac delayed rectifier K^+ currents: Differential sensitivity to block by class III antiarrhythmic agents.* J Gen Physiol 1990, 96: 195-215.

35. Balser, J.R., Bennet, P.B., Roden, D.M. *Time-dependent outward current in guinea pig ventricular myocytes. Gating kinetics of the delayed rectifier.* J Gen Physiol 1990, 96: 835-63.

36. Sanguinetti, M.C., Jurkiewicz, N.K. *Delayed rectifier outward K^+ current is composed of two currents in guinea pig atrial cells.* Am J Physiol 1991, 260: H393-9.

37. Folander, K., Smith, J.S., Antanavage, J.B.C. et al. *Cloning and expression of the delayed-rectifier I_{sK} channel from neonatal rat heart and diethylstilbestrol-primed rat uterus.* Proc Natl Acad Sci USA 1990, 87: 2975-9.

38. Suessbrich, H., Busch, A.E. *The I_{Ks} channel: Coassembly of I_{sK} (minK) and KvLQT1 proteins.* Rev Physiol Biochem Pharmacol 1999, 137: 191-226.

39. Lesage, F., Attali, B., Lakey, J. et al. *Are Xenopus oocytes unique in displaying functional I_{sK} channel heterologous expression?* Receptors Channels 1993, 1: 143-52.

40. Busch, A.E., Busch, G.L., Ford, E. et al. *The role of the I_{sK} protein in the specific pharmacological properties of the I_{Ks} channel complex*. Br J Pharmacol 1997, 122: 187-9.

41. Attali, B., Giullemare, E., Lesage, F. et al. *The protein I_{sK} is a dual activator of K^+ and Cl- channels.* Nature 1993, 365: 850-2.

42. Wang, Q., Curran, M.E., Splawsi, I. et al. *Positional cloning of a novel potassium channel gene: KvLQT1 mutations cause cardiac arrhythmias.* Nat Genet 1996, 12: 17-23.

43. Barhanin, J., Lesage, F., Guillemare, E. et al. *KvLQT1 and I_{sK} (minK) proteins associate to form the I_{Ks} cardiac potassium current.* Nature 1996, 384: 78-80.

44. Sanguinetti, M.C., Curran, M.E., Zou, A. et al. *Coassembly of KvLQT1 and minK (I_{sK}) proteins to form cardiac I_{Ks} potassium channel.* Nature 1996, 384: 80-3.

45. Romey, G., Attali, B., Chouabe, C. et al. *Molecular mechanism and functional significance of the minK control of the KvLQT1 channel activity.* J Biol Chem 1997, 272: 16713-6.

46. Busch, A.E., Suessbrich, H. *Role of the I_{sK} protein in the I_{minK} channel complex.* Trends Pharmacol Sci 1997, 18: 26-9.

47. Lerche, C., Seebohm, G., Wagner, C.I. et al. *Molecular impact of minK on the enantiospecific block of I_{Ks} by chromanoles.* Br J Pharmacol 2000, 131: 1503-6.

48. Bleich, M., Warth, R. *The very small-conductance K^+ channel KvLQT1 and epithelial function.* Pflügers Arch 2000, 440: 202-6.

49. Suessbrich, H., Busch, A., Ecke, D. et al. *Specific blockade of slowly activating I_{sK} channels by chromanols: Impact on the role of I_{sK} channels in epithelia.* FEBS Lett 1996, 396: 271-5.

50. Lohrmann, E., Burhoff, I., Nitschke, R.B. et al. *A new class of inhibitors of cAMP-mediated Cl- secretion in rabbit colon, acting by the reduction of cAMP-activated K^+ conductance.* Pflügers Arch 1995, 429: 517-30.

51. Busch, A.E., Suessbrich, H., Waldegger et al. *Inhibition of I_{Ks} in guinea pig cardiac myocytes and guinea pig I_{sK} channels by the chromanole 293B.* Pflügers Arch 1996, 432: 1094-6.

52. Salata, J.J., Jurkiewicz, N.K., Sanguinetti, M.C. et al. *The novel class III antiarrhythmic agent L-735,821 is a potent and selective blocker of I_{Ks} in guinea pig ventricular myocytes.* Circulation 1996, 94: I-529.

53. Lloyd, J., Schmidt, J.B., Ahmad, S. et al. *Design and synthesis of potent, selective, and orally bioavailable potassium (I_{Ks}) blockers.* 220th ACS Natl Meet (Aug 20-24, Washington, DC) 2000, MEDI-327.

54. Englert, H.C., Wirth, K., Gehring, D. et al. *Airways pharmacology of the potassium channel opener, HOE 234, in guinea pigs: In vitro and in vivo studies.* Eur J Pharmacol 1992, 210: 69-75.

55. Lerche, C., Brüggemann, A., Seebohm, G. et al. *Block of KvLQT1 related potassium channels by the chromanole 293B.* Biophys J 1999, 76: A89.

56. Loussouarn, G., Charpentier, F., Mohammad-Panah, R. et al. *KvLQT1 potassium channel but not I_{sK} is the molecular target for trans-6-cyano-4-(N-ethylsulfonyl-N-methylamino)-3-hydroxy-2,2-dimethyl-chromane.* Mol Pharmacol 1997, 52: 1131-6.

57. Seebohm, G., Lerche, C., Kelety, B. et al. *Chromanol 293B acts on KvLQT1 and I_{Ks} channels in an enantiomer specific manner.* Arch Pharmacol 1999, 359(Suppl.): 76, 290.

58. Lerche, C., Seebohm, G., Wagner, C.I. et al. *Mapping the binding site of the chromanol 293B on the I_{Ks} channel.* Biophys J 2000, 78: 204A.

59. Bosch, R.F., Gaspo, R., Busch, A.E. et al. *Effects of the chromanole 293B, a selective blocker of the slow component of the delayed rectifier K^+ current, on repolarization in human and guinea pig ventricular myocytes.* Cardiovasc Res 1998, 38: 441.

60. Seebohm, G., Lerche, C., Kelety, B. et al. *State, frequency and enantiomer dependent block of the potassium channel I_{ks} by the chromanol 293B.* Biophys J 1999, 76: A187.

61. Lu, Z., Kamiya, K., Kodama, I. *Effects of chromanol 293B, a selective blocker of the slow component of the delayed rectifier K^+ current (I_{Ks}), on the action potential in rabbit ventricular myocytes.* Environ Med 1999, 43: 114-6.

62. Fujisawa, S., Ono, K., Iijima, T. *Time-dependent block of the slowly activating delayed rectifier K^+ current by chromanol 293B in guinea-pig ventricular cells.* Br J Pharmacol 2000, 129: 1007-13.

63. Lei, M., Varghese, A., Gerlach, U. et al. *Inhibition of I_{Ks} in rabbit sino-atrial node cells by the chromanole 293B: Effect on pacemaker rate.* 33rd Int Cong Physiol 1997, PO36.09.

64. Ono, K., Shibata, S., Iijima, T. *Properties of the delayed rectifier potassium current in porcine sino-atrial node cells.* J Physiol 2000, 524: 151-62.

65. Wirth, K.J., Gerlach, U., Brendel, J., Busch, A. *Wirkung des selektiven I_{Ks}-Kanalblockers HMR1556 auf die Refraktärzeit des Vorhofs und Ventrikels am Schwein.* Z Kardiol 2000, 89(Suppl. 5): 735.

66. Shiimizu, W., Antzelevitch, C. *Cellular basis for the ECG features of the LQT1 form of the long-QT syndrome.* Circulation 1998, 98: 2314-22.

67. Burashnikov, A., Antzelevitch C. *Block of I_{Ks} does not induce early afterdepolarization activity but promotes β-adrenergic agonist-induced delayed after depolarization activity.* J Cardiovasc Electrophysiol 2000, 11: 458-65.

68. Bauer, A., Müller, M., Gerlach, U. et al. *Frequency-dependent effect of the new I_{Ks} blocker chromanol 293B in intact dog myocardium.* Z Kardiol 1998, 87(Suppl. 1): 283.

69. Bauer, A., Becker, R., Freigang, K.D. et al. *Rate- and site-dependent effects of propafenone, dofetilide, and the new I_{Ks}-blocking agent chromanole 293B on individual muscle layers of the intact canine heart.* Circulation 1999, 100: 2184-90.

70. Bauer, A., Becker, R., Lang, H.J. et al. *Are there differential effects of the new I_{Ks} blocking agent chromanole 293B in normal and infarcted hearts?* J Am Coll Cardiol 1999, 33(Suppl. A): 152A.

71. Bauer, A., Becker, Freigang, K.D. et al. *Electrophysiologic effects of the new I_{Ks}-blocking agent chromanol 293B in the postinfarction canine heart. Preserved positive use-dependence and preferential prolongation of refractoriness in the infarct zone.* Basic Res Cardiol 2000, 95: 324-32.

72. Varro, A., Balati, B., Iost, N. et al. *The role of the delayed rectifier component I_{Ks} in dog ventricular muscle and Purkinje fiber repolarization.* J Physiol 2000, 523: 167-81.

73. Merot, J., Probst, V., Debailleul, M. et al. *Electropharmacological characterization of cardiac repolarization in German shepherd dogs with an inherited syndrome of sudden death: Abnormal response to potassium channel blockers.* Am Coll Cardiol 2000, 36: 939-47.

74. Gerlach, U., Brendel, J., Brüggemann, A. et al. *Synthesis and SAR of novel I_{Ks}-channel blockers: From K_{ATP}-channel opener to I_{Ks}-channel blocker.* 219th ACS Natl Meet (March 26-30, San Francisco) 1999, MEDI-159.

75. Gerlach, U., Brendel, J., Brüggemann, A. et al. *Synthesis and SAR of novel I_{Ks}-channel blockers: From KATP-channel opener to I_{Ks}-channel blocker.* J Med Chem, in review.

76. Gögelein, H., Brüggemann, A., Gerlach, U. et al. *Inhibition of I_{Ks} channels by HMR1556.* Naunyn Schmiedebergs Arch Pharmacol 2000, 362: 480-8.

77. Eckardt, L., Kirchhof, P., Gerlach, U. et al. *Regional unterschiedliche elektrophysiologische Effekte einer I_{Ks}-Blockade am isolierten Kaninchenherz.* Z Kardiol 2000, 89(Suppl. 5): 1172.

78. Eckardt, L., Haverkamp, W., Mertens, H. et al. *Drug-related torsades de pointes in the isolated rabbit heart: Comparison of clofilium, d,l-sotalol, and erythromycin.* J Cardiovasc Pharmacol 1998, 32: 425-34.

79. Bock, M.G., DiPardo, R.M., Evans, B.E. et al. *Benzodiazepine gastrin and brain cholecystokinin receptor ligands: L-365,260.* J Med Chem 1989, 32: 13-6.

80. Selnick, H.G., Liverton, N.J., Baldwin, J.J. et al. *Class III antiarrhythmic activity in vivo by selective blockade of the slowly activating cardiac delayed rectifier potassium current I_{Ks}, by (R)-2-(2,4-trifluoromethyl)-N-[2-oxo-5-phenyl-1-(2,2,2-trifluoroethyl)-2,3-dihydro-III-benzo[e][1,4]diazepin-3-yl]acetamide.* J Med Chem 1997, 40: 3865-8.

81. Cordeiro, J.M., Spitzer, K.W., Giles, W.R. *Repolarizing K^+ currents in rabbit heart Purkinje cells.* J Physiol 1998, 508: 811-23.

82. Shi, Y.J., Wells, K.M., Pye, P.J. et al. *Crystallization-induced asymmetric transformation: Stereospecific synthesis of L-768,673.* Tetrahedron 1999, 55: 909-18.

83. Shi, Y.J., Lynch, J.E., Choi, W.B. et al. *Process research and development for an antiarrythmic drug candidate.* 219th ACS Natl Meet (March 26-30, San Francisco) 2000, ORGN-206.

84. Lynch, J.J., Houle, M.S., Stump, G.L. et al. *Antiarrhythmic efficacy of selective blockade of the cardiac slowly activating delayed rectifier current, I_{Ks}, in canine models of malignant ischemic ventricular arrhythmia.* Circulation 1999, 100: 1917-23.

85. Billman, G.E., Houle, M.S., Lynch, J.J. *Selective I_{Ks}, but not I_{Kr}, blockade protects against ventricular fibrillation induced by mycardial ischemia.* Circulation 1998, 98: I-52.

86. Vanoli, E., Hull, S.S., Adamson, P.B. et al. *K^+ channel blockade in the prevention of ventricular fibrillation in dogs with acute ischemia and enhanced sympathetic activity.* J Cardiovasc Pharmacol 1995, 26: 847-54.

87. Robinson, C.P., Robinson, K.A., Castañer, J. *Azimilide hydrochloride.* Drugs Fut 1997, 22: 601-7.

88. Fermini, B., Jurkiewicz, N.K., Jow, B. et al. *Use-dependent effects of the class III antiarrhythmic agent NE-10064 (azimilide) on cardiac repolarization: Block of delayed rectifier potassium and L-type calcium currents.* J Cardiovasc Pharmacol 1995, 26: 259-71.

89. Vanoli, E., Hull, S.S., Jackman, W.M. et al. *Combined blockade of I_{Kr} and I_{Ks} enhances the arrhythmogenic effect of sympathetic stimulation.* Circulation 1996, 94: I-160.

90. Heath, B.M., Terrar, D.A. *Block by propofol and thiopentone of the minK current (I_{sK}) expressed in Xenopus oocytes.* Naunyn Schmiedebergs Arch Pharmacol 1997, 356: 404-9.

91. Bachmann, A., Mueller, S., Kopp, K. et al. *Inhibition of cardiac potassium currents by barbiturates.* Br J Pharmacol 2001, in review.

92. Schwartz, P.J. *Do animal models have clinical value?* Am J Cardiol 1998, 81: 14D.

93. Wang, Z., Fermini, B., Nattel, S. *Rapid and slow components of delayed rectifier current in human atrial myocytes.* Cardiovasc Res 1994, 28: 1540-6.

94. Li, G.R., Feng, J., Yue, L. et al. *Evidence for two components of delayed rectifier K^+ current in human ventricular myocytes.* Circ Res 1996, 78: 689-96.

95. Gintant, G.A. *Two components of delayed rectifier current in canine atrium and ventricle. Does I_{Ks} play a role in the reverse rate dependence of class III agents?* Circ Res 1996, 78: 26-37.

96. Liu, D.W., Antzelevitch, C. *Characteristics of the delayed rectifier current (I_{Kr} and I_{Ks}) in canine ventricular epicardial, midmyocardial, and endocardial myocytes.* Circ Res 1995, 76: 351-65.

97. Salata, J.J., Jurkiewicz, N.K., Jow, B. et al. *I_K of rabbit ventricle is composed of two currents: Evidence for I_{Ks}.* Am J Physiol 1996, 271: H2477-89.

98. Lei, M., Brown, H.F. *Two components of the delayed rectifier potassium current, I_K, in rabbit sino-atrial node cells.* Exp Physiol 1996, 81: 725-41.

99. Felipe, A., Knittle, T.J., Doyle, K.L. et al. *Differential expression of I_{sK} mRNAs in mouse tissue during development and pregnancy.* Am J Physiol 1994, 267: C700-5.

100. Wang, L., Feng, Z.P., Kondo, C.S., Sheldon, R.S., Duff, H.J. *Developmental changes in the delayed rectifier K^+ channels in mouse heart.* Circ Res 1996, 79: 79-85.

101. Lesage, F., Attali, B., Lazdunski, M., Barhanin, J. *I_{sK} a slowly activating voltage-sensitive K^+ channel. Characterization of multiple cDNAs and gene organization in the mouse.* FEBS Lett 1992, 301: 168-72.

102. Hume, J.R., Giles, W., Robinson, K., Shibata, E.F. *A time and voltage-dependent K^+ current in single cardiac cells from bullfrog atrium.* J Gen Physiol 1986, 88: 777-98.

103. Markus, D.C., Shen, Z. *Slowly activating voltage-dependent K^+-conductance is the apical pathway for K^+-secretion in vestibular dark cells.* Am J Physiol 1994, 267: C857-64.

104. Markus, D.C., Shen, Z. *I_{sK} channel is a apical pathway for K^+ secretion by inner ear epithelia.* J Gen Physiol 1994, 104: 16A.

105. Boyle, M.B., Azhderian, E.M., MacLusky, N.J. et al. *Very slowly activating K^+ current from rat uterine smooth muscle expressed in RNA-injected Xenopus oocytes.* Science 1987, 235: 1221-4.

106. Hwang, T.H., Suh, D.J., Bae, H.R., Lee, S.H., Jung, J.S. *Characterization of K^+ channels in the basolateral membrane of rat tracheal epithelia.* J Membr Biol 1996, 154: 777-98.

107. Sugimoto, T., Tanabe, Y, Shigemoto, R. et al. *Immunohistochemical study of a rat membrane protein which induces a selective potassium permeation: Its localization in the apical membrane portion of epithelial cells.* J Membr Biol 1990, 113: 39-47.

108. Kim, S.J., Greger, R. *Voltage-dependent, slowly activating K^+ current (I_{Ks}) and its augmentation by carbachol in rat pancreatic acini.* Pflügers Arch 1999, 438: 604-11.

109. Zhang, Z.H., Follmer, C.H., Sarma, J.S. et al. *Effect of ambasilide, a new class III agent, on plateau currents in isolated guinea pig ventricular myocytes: Block of delayed outward potassium current.* J Pharmacol Exp Ther 1992, 263: 40-8.

110. Salata, J.J., Brooks, R.R. *Pharmacology of azimilide dihydrochloride (NE-10064), a class III antiarrhythmic agent.* Cardiovasc Drug Rev 1997, 15: 137-56.

111. Carmeliet, E., Mubagwa, K. *Antiarrhythmic drugs and cardiac ion channels: Mechanisms of action.* Prog Biophys Mol Biol 1998, 70: 1-72.

112. Delpon, E., Valenzuela, C., Tamargo, J. *Blockade of cardiac potassium and other channels by antihistamines.* Drug Safety 1999, 21(Suppl. 1): 11-8.

113. Suessbrich, H., Schonherr, R., Heinemann, S.H. et al. *Specific block of cloned HERG channels by clofilium and its tertiary analog LY97241.* FEBS Lett 1997, 414: 435-8.

114. Arena, J.P., Kass, R.S. *Block of heart potassium channels by clofilium and its tertiary analogs: Relationship between drug structure and type of channel blocked.* Mol Pharmacol 1988, 34: 60-6.

115. Suessbrich, H., Waldegger, S., Lang, F., Busch, A.E. *Blockade of HERG channels expressed in Xenopus oocytes by the histamine receptor antagonists terfenadine and astemizole.* FEBS Lett 1996, 385: 77-80.

116. Suessbrich, H., Schonherr, R., Heinemann, S.H. et al. *The inhibitory effect of the antipsychotic drug haloperidol on HERG potassium channels expressed in Xenopus oocytes.* Br J Pharmacol 1997, 120: 968-74.

117. Turgeon, J., Daleau, P., Bennett, P.B. et al. *Block of I_{Ks}, the slow component of the delayed rectifier K^+ current, by the diuretic agent indapamide in guinea pig myocytes.* Circ Res 1994, 75: 879-86.

118. Yang, T., Roden, D.M. *Extracellular potassium modulation of drug block of I_{Kr}. Implications for torsade de pointes and reverse use-dependence.* Circulation 1996, 93: 407-11.

119. Po, S., Wang, D., Yang, I. et al. *Modulation of HERG potassium channels by extracellular magnesium and quinidine.* J Cardiovasc Pharmacol 1999, 33: 181-5.

120. Dukes, I.D., Cleemann, L., Morad, M. *Tedisamil blocks the transient and delayed rectifier K^+ currents in mammalian cardiac and glial cells.* J Pharmacol Exp Ther 1990, 254: 560-9.

121. Daleau, P., Turgeon, J. *Triamterene inhibits the delayed rectifier potassium current (I_K) in guinea pig ventricular myocytes.* Circ Res 1994, 74: 1114-20.

122. Young, J.B., Vandermolen, L.A., Pratt, C.M. *Torsade de pointes: An unusual manifestation of chloral hydrate poisoning.* Am Heart J 1986, 112: 181-4.