

**Antiarrhythmic properties of novel
antianginal drugs in dog and human
cardiac preparations**

PhD Thesis

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2012

STUDIES RELATED TO THE THESIS

I. Szél Tamás, Koncz István, Jost Norbert, Baczkó István, Husti Zoltán, Virág László, Bussek Alexandra, Wettwer Erich, Ravens Ursula, Papp Gyula, Varró András

Class I/B antiarrhythmic property of ranolazine, a novel antianginal agent, in dog and human cardiac preparations

European Journal of Pharmacology, 2011, 662(1-3):31-39.

IF.: 2,516

II. Koncz István, Szél Tamás, Bitay Miklós, Cerbai Elisabetta, Jaeger Kristian, Fülöp Ferenc, Jost Norbert, Virág László, Orvos Péter, Tálosi László, Kristóf Attila, Baczkó István, Papp J Gyula, Varró András

Electrophysiological effects of ivabradine in dog and human cardiac preparations: Potential antiarrhythmic actions

European Journal of Pharmacology, 2011, 668(3):419-426.

IF.: 2,516

III. Koncz István, Szél Tamás, Jaeger Kristian, Baczkó István, Cerbai Elisabetta, Romanelli M Novella, Papp J Gyula, Varró András

Selective pharmacological inhibition of the pacemaker channel isoforms (HCN1-4) as new possible therapeutical targets

Current Medicinal Chemistry, 2011, 18(24):3662-74.

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OTHER STUDIES

I. Esther Pueyo, Alberto Corrias, László Virág, Norbert Jost, Tamás Szél, András Varró, Norbert Szentandrassy, Péter P. Nánási, Kevin Burrage, Blanca Rodríguez

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Novel blockers of hyperpolarization-activated current with isoform selectivity in recombinant cells and native tissue

British Journal of Pharmacology, 2011, 166(2):602-16.

IF.: 4,409

INTRODUCTION

Cardiac action potential

The electrophysiologic behavior of the heart is determined by ordered propagation of excitatory stimuli that result in rapid depolarization and slow repolarization, thereby generating action potentials (AP) in myocytes, which reflect the sequential activation and inactivation of inward (Na^+ and Ca^{2+}) and outward (K^+) currents carrying ion channels. The electrical cycle of the myocytes has been divided in five phases. When the excitation threshold is exceeded, cardiomyocytes are depolarized (phase 0) by a rapid inflow of Na^+ ions generating a large and fast inward Na^+ current (I_{Na}). This current determines also the velocity of impulse propagation through the His-Purkinje system, working atrial and ventricular myocytes ('fast-channel tissues'). In sino-atrial and atrioventricular node mainly the Ca^{2+} current (I_{Ca}) is responsible for depolarization ('slow-channel tissues'). Phase 1 is the initial part of repolarization, mainly governed by the transient outward current (I_{to}). In phase 2, also named plateau, the late components of inward currents (late I_{Na} , late I_{Ca}) oppose the outward repolarizing currents i.e. the rapid and slow components (I_{Kr} and I_{Ks}) of the delayed rectifier K^+ current and the inward rectifier K^+ current (I_{K1}). Phase 3 is the terminal part of repolarization, which differs from phase 2 for its faster repolarization rate, the outward gradually overcome the inward currents, enabling fast repolarization of the action potential. The loss of one repolarizing current may not lead to excessive AP lengthening, since other unimpaired K^+ channels may provide sufficient repolarizing capacity, i.e. there is a redundancy of the repolarization process ('*repolarization reserve*'). The key players of the reserve are I_{Kr} , I_{Ks} , I_{K1} , and presumably I_{to} [2-5]. Phase 4 describes membrane potential during diastole. Time-independent (or background) currents may also contribute to the whole action potential course i.e. the Na^+/K^+ pump current (I_{NaK}), the $\text{Na}^+/\text{Ca}^{2+}$ exchanger current (I_{NCX}) and the ATP-sensitive potassium current ($I_{\text{K(ATP)}}$). In the sino-atrial node heart rate is regulated by spontaneous electrical pacemaker activity mainly controlled by the I_{f} current. This current determines the slope of diastolic depolarization and cardiac frequency, and its inhibition causes heart rate reduction. The 'funny' (I_{f}) current is so termed because of its unusual characteristics, including that of being an inward current that is activated on hyperpolarization and not on depolarization like other known currents.

Antiarrhythmic drugs

Most antiarrhythmic agents are traditionally grouped according to Vaughan Williams classification based on their dominant electrophysiological action. Class I antiarrhythmic agents have a blockade of the fast-inward sodium channel on myocardium. Class II agents are antisympathetic drugs, particularly the β -adrenoceptor blockers. Class III drugs act through delaying repolarization of cardiac myocytes and thus cause a lengthening of action potential duration (potassium-channel blockers). Class IV antiarrhythmic drugs are the calcium channel blockers and class V drugs are specific bradycardic agents.

Class I antiarrhythmic drugs

These compounds share the ability to block the fast Na^+ channels responsible for the upstroke and rapid conduction of the cardiac action potential. In the ECG, this effect may be reflected as widening of the P wave, widening of the QRS complex, prolongation of the PR interval, or a combination. They are capable not only of slowing but blocking intracardiac conduction and induce arrhythmias. This phenomenon has become widely recognized, and has led to reduction in the usage of these agents. Class I antiarrhythmic

drugs usually exert post-repolarization refractoriness, when the refractory period extends well beyond phase 3, even after complete repolarization. Class I drugs are subdivided based on the kinetics of the Na⁺ channel effects, that determine the heart rates at which their electrophysiologic effects become manifest.

i. Class I/A drugs have intermediate kinetics, their fast-channel tissue conduction slowing effects may or may not be evident on an ECG obtained during normal rhythm at normal rates. Class I/A drugs block repolarizing K⁺ channels also, prolonging the repolarization and refractory periods (the interval from the beginning of the AP until the fiber is able to conduct another AP) of fast-channel tissues. On the ECG, this effect is reflected as QT-interval prolongation even at normal rates.

ii. Class I/B drugs have fast kinetics, they express their electrophysiologic effects only at fast heart rates. Thus, an ECG obtained during normal rhythm at normal rates usually shows no evidence of fast-channel tissue conduction slowing.

iii. Class I/C drugs have slow kinetics, they express their electrophysiologic effects at all heart rates. Thus, an ECG obtained during normal rhythm at normal heart rates usually shows fast-channel tissue conduction slowing. Class I/B drugs and class I/C drugs do not block K⁺ channels directly.

Class I drugs can be useful in supraventricular and ventricular tachyarrhythmias also, but they are not generally recommended for patients with structural heart disorder.

The Cardiac Arrhythmia Suppression Trial (CAST) showed that flecainide and encainide, two Class I/C sodium channel blocker antiarrhythmic drugs, increased mortality rates compared with placebo due to proarrhythmic effects. Consequently, the interest of drug development for treatment of ventricular tachycardia (VT) and atrial fibrillation (AF) has been shifted toward those agents that prevent and terminate re-entrant arrhythmias by prolonging the action potential duration.

Class II antiarrhythmic drugs

Class II drugs are β -blockers. These agents have been given many names in the literature (beta-adrenoceptor blockers, b-adrenergic-blocking agents, b-adrenergic antagonists, b-antagonists, b-adrenergic receptor antagonists). They are particularly used not only for the management of cardiac arrhythmias, but hypertension, angina pectoris, congestive heart failure, essential tremor, glaucoma, migraine prophylaxis and myocardial infarction. As antiarrhythmic agents they suppress beta-adrenergic signaling in the heart by competitively inhibiting agonist binding to beta-adrenergic receptors. They are useful in preventing sudden death due to ventricular tachyarrhythmias associated with acute myocardial ischemia, congenital long QT syndrome, and congestive heart failure. They are also quite valuable in controlling the ventricular rate in patients with atrial fibrillation. β -blockers affect predominantly slow-channel tissues (SA and AV nodes), where they decrease rate of automaticity and slow conduction velocity. Thus, heart rate is slowed and the PR interval is lengthened on the ECG.

Class II drugs are used primarily to treat supraventricular tachycardias (SVT), including sinus tachycardia, AV nodal reentry, atrial fibrillation, and atrial flutter. These drugs are also used to treat VTs to raise the threshold for ventricular fibrillation (VF) and reduce the ventricular proarrhythmic effects of β -adrenoceptor stimulation.

Class III antiarrhythmic drugs

Class III antiarrhythmic action, *i.e.* lengthening of cardiac action potential duration (APD), is usually caused by blockade of one or more potassium channels. A great number of non-cardiac drugs cause lengthening of APD in both ventricular muscle cells and Purkinje fibres by using a similar mode of action. The drugs act also in slow- and fast-

channel tissues. Because repolarization and refractoriness are prolonged, rate of automaticity is reduced. QT-interval prolongation is the predominant effect on the ECG. These drugs are used to treat supraventricular and ventricular arrhythmias. Class III drugs have a risk of proarrhythmia, particularly torsades de pointes ventricular tachycardia.

Class IV antiarrhythmic drugs

Class IV drugs are the nondihydropyridine Ca channel blockers, which depress Ca-dependent action potentials in slow-channel tissues and thus decrease the rate of automaticity, slow conduction velocity, and prolong refractoriness. Heart rate is slowed, the PR interval is lengthened, and the AV node transmits rapid atrial depolarizations at a lower frequency. These drugs are used primarily to treat supraventricular tachycardias or tachyarrhythmias.

Class V antiarrhythmic drugs

Class V drugs are specific bradycardic agents with sino-atrial pacemaker current (I_f) inhibitory property. I_f plays an important role in spontaneous diastolic depolarization. The current flows through hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. Block of the pacemaker current reduces the steepness of diastolic depolarization. Thus, heart rate is slowed and the RR interval is lengthened on the ECG.

Medical treatment of angina pectoris

Stable angina is a form of coronary artery disease. A multifaceted approach is required in the management of angina. It includes lifestyle intervention, medical arsenal and revascularization therapy. The current pharmacological treatment of stable angina pectoris is based on beta adrenoceptor blockers, calcium channel antagonists, nitrates (and related derivatives). Number of patients have contraindications or remain unrelieved from anginal discomfort and have relative intolerances to maximum doses of traditional antianginal agents. β -blockers and many calcium channel blockers have similar depressive hemodynamic and significant electrophysiological effects. Therefore antianginal drugs without serious limitations are needed. New classes of treatments (ivabradine, ranolazine) with entirely different mechanisms of action have now been added.

Beta blockers

Heart rate has a prominent role in the development and pathophysiology of myocardial ischaemia. In patients with coronary artery disease, ischaemic episodes could be generated by an elevation in heart rate that elicits imbalance between myocardial oxygen supply and demand. The antiischemic effect of beta blockers is attributable, at least in part, to heart rate reduction and that is regarded as an important therapeutic target in preventing ischaemia by reducing myocardial oxygen consumption. Beta blockers apart from the numerous, proven, valuable effects in the treatment of cardiovascular diseases (angina pectoris, arrhythmias, myocardial infarction, heart failure) have several side effects including bronchospasm, atrioventricular block, lassitude, sleep disturbance, depression, change in lipid status, sexual dysfunction, possible increase in blood sugar level, rebound phenomena. The hemodynamic effects might be also limiting factors at application e.g. the negative inotropic effect. Furthermore beta blockers (except carvedilol and nebivolol) may be contraindicated in patients with peripheral vascular disease. In ST-segment elevation myocardial infarction (STEMI) early i.v. use of beta blockers is clearly contraindicated in patients with clinical signs of hypotension or congestive heart failure. In the absence of

contraindications, beta-blockers remain the reference treatment to prevent angina attacks, particularly after myocardial infarction.

Calcium channel blockers

Calcium channel blockers reduce calcium influx into the cell, producing dual vasodilating and negative inotropic effects, which respectively vary depending on the different compounds. Dihydropyridines (amlodipine, felodipine, isradipine, nicardipine and nifedipine) mostly have a peripheral vasodilating action and could increase heart rate. Usually they must be associated with a beta-blocker or, failing this, with an alternative cardiac slowing agent (e.g. ivabradine). The non-dihydropyridines (diltiazem and verapamil) both have a peripheral vasodilating action and probradycardic and myocardial depressant effects. The hemodynamic effects might be also limiting factors at application (the considerable blood pressure lowering property or the negative inotropic effect). In patients with angina pectoris the most common coexisting cardiovascular risk factor is hypertension and, therefore, dihydropyridines or other calcium channel blockers (diltiazem or verapamil) have a role in combined treatment with or as a substitute for beta blockers.

Nitrates

Nitrates are indirect nitric oxide donors, which block calcium entry in smooth muscle cells and promote their relaxation through production of cyclic guanosine monophosphate (cGMP). Their main haemodynamic effect is to reduce preload by venodilatation, although at high doses they have an arterial vasodilating effect, causing dilatation of the epicardial arteries. Nitrates are usually well tolerated, although they may cause headaches, restricting their use. Because of their vasodilating action, they increase heart rate and it is recommended to combine them with preventative treatments such as beta-blockers, ivabradine or calcium channel blockers, which have a heart rate-lowering effect.

Ranolazine

Ranolazine (Ranexa[®]) is a novel antianginal agent (a piperazine derivative) shown to exert anti-ischemic effects without causing significant bradycardia or hypotension. The drug reduces ischemia via inhibition of the late phase of the inward sodium current (late I_{Na}) during cardiac repolarization, with a consequent reduction in intracellular sodium and calcium overload which could contribute to its therapeutic effect. The drug was approved in 2006 in the US for use in management of chronic angina pectoris. Ranolazine is useful in patients who have not achieved an adequate response with other antianginal medications, and is used in combination with amlodipine, β -blockers, or nitrates.

In addition, ranolazine has been proposed to possess antiarrhythmic potential which was principally related to the inhibition of sodium current (I_{Na}), rapid delayed rectifier potassium current (I_{Kr}) and calcium current (I_{Ca}). A suppression of ventricular tachycardias by ranolazine after non-ST-segment elevation myocardial infarction has been proven in the MERLIN-TIMI 36 trial. Experimental models and clinical reports have revealed an antiarrhythmic potential of ranolazine also in long QT syndrome. Ranolazine has been associated with good safety profile during clinical usage. The side-effects are mild and tolerable (e.g. dizziness, nausea, constipation, headache).

Ivabradine

Ivabradine (Procoralan[®]) is the only clinically available selective heart rate reducing agent and it exerts anti-ischaemic effects in patients with chronic stable angina. Ivabradine inhibits the pacemaker (I_f) current in the heart with reasonable selectivity and with minimal effect on haemodynamic parameters. According to the BEAUTIFUL (morBidity-mortality

Evaluation of the I_f inhibitor ivabradine in patients with coronary disease and left ventricular dysfunction) study, ivabradine can be used to reduce the incidence of coronary artery disease outcomes in a subgroup of patients who have heart rates of 70 bpm or greater. Ivabradine has been associated with a good safety profile during its clinical development. In a trial, mild, tolerable visual symptoms (e.g. abrupt changes in light intensity) were reported as side effects. Ivabradine is beneficial in patients with chronic stable angina pectoris equally to beta receptor blocker and calcium channel antagonist drugs. Contrary to several heart rate-reducing drugs, ivabradine lowers heart rate both at rest and during exercise without producing any negative inotropic or vasoconstrictor effect. The results of the recent SHIFT study support the significance of heart-rate reduction with ivabradine for improvement of clinical outcomes in heart failure and confirm the important role of heart rate in the pathophysiology of this disorder. Improvement of regional myocardial blood flow and function and reduction of infarct size by ivabradine have also been described.

This selective heart rate reducing agent, lacking the negative inotropic effect, may offer a new therapeutic perspective in the treatment of ischaemic heart diseases e.g. in stable angina pectoris, particularly in patients with left ventricular dysfunction. Based on the recent clinical trials and experiments, widening the indication of ivabradine for the treatment of heart failure or myocardial infarction is expected.

Aims of the study

The primary goal of the present study was to investigate in detail the cellular electrophysiological effects of ranolazine and ivabradine in dog and human heart preparations using conventional microelectrode technique. The effects of the drugs were mainly investigated in dog, a species resembling human in heart size, spontaneous frequency and repolarization.

RESULTS

Effects of ranolazine on transmembrane action potentials

Dog Purkinje fibre

Ranolazine (dose- and rate-dependently) decreased the maximum rate of rise of the action potential upstroke (V_{max}) in isolated dog cardiac Purkinje fibres. Action potential duration measured at 50% and 90% of repolarization (APD_{50} , APD_{90}) was shortened in a concentration-dependent manner at pacing with a constant cycle length of 500 ms. The reduction of APD_{50} was more pronounced than that of APD_{90} which resulted in an action potential with a triangular shape. The depression of V_{max} evoked by ranolazine was strongly dependent upon stimulation frequency ("use-dependency"); i.e., as pacing cycle length was decreased, the depression of V_{max} was increased. The block was statistically significant only at stimulation rates faster or equal of 2 Hz.

Dog papillary muscle

In dog right ventricular papillary muscle at a stimulation cycle length of 1000 ms ranolazine exerted no statistically significant effect on action potential duration. Ranolazine decreased the V_{max} and increased the impulse conduction time (CT) dose- and rate-dependently. This effect was significant also at the concentration of 5 μ M at basic cycle length of 300-500 ms. The decrease in V_{max} was more pronounced in case of elevating extracellular potassium concentration from 4 to 6 mM and produced a shift in the normalized V_{max} -membrane potential curve to more negative potentials.

Human papillary muscle

In human right ventricular papillary muscle ranolazine moderately shortened the APD in 2 out of 4 experiments at a stimulation cycle length of 1000 ms. On average ranolazine exerted no statistically significant effect on APD. Ranolazine dose- and rate-dependently decreased the V_{\max} . In human preparations pronounced biphasic effect was evident during the incubation, i.e. first shortening (faster development of late sodium channel block) and later prolongation of APD (formation of I_{Kr} block) was detected suggesting multiple ion channel block with different drug binding kinetics.

Onset and offset kinetics of V_{\max} block

In dog papillary muscles, after 1 minute of rest, train of stimuli driven at the cycle length of 400 ms, the onset kinetics of 20 μ M ranolazine induced V_{\max} block was fitted by a single exponential, resulting in the onset rate kinetic constant of $\tau = 3.5 \pm 0.8 \text{ beat}^{-1}$. At the same stimulation cycle length of 400 ms, the recovery of V_{\max} (offset kinetics) during control was best fit to a single exponential relation. The time constant for recovery of V_{\max} during control was fast ($\tau_{\text{fast}} = 35.7 \pm 5.4 \text{ ms}$) and before final repolarization of the basic action potential, it was almost complete. In the presence of 20 μ M ranolazine the recovery kinetics of V_{\max} was best fitted with a two exponential relation. In addition to a fast component ($\tau_{\text{fast}} = 29.1 \pm 2.9 \text{ ms}$) which reflects recovery of the drug-free sodium channels, a slow component ($\tau_{\text{slow}} = 1.58 \pm 0.25 \text{ s}$) of recovery of V_{\max} was revealed following exposure to ranolazine. This second slow component for recovery of V_{\max} may reflect effects on drug-affected sodium channels.

Dog midmyocardial preparations

In dog midmyocardial tissue (M-cells) 10 μ M ranolazine exerted no significant effect on APD at basic cycle length of 1000 ms, however, at higher concentration (20 μ M) it produced a slight prolongation of APD. In these preparations ranolazine decreased the maximum rate of rise of the action potential upstroke (V_{\max}) which was significant only in higher concentration (20 μ M) at basic cycle length of 1000 ms.

Dispersion of repolarization

In dog heart ranolazine decreased the dispersion of repolarization i.e. the difference in APD₉₀ values between Purkinje fibres and papillary muscles. The drug produced abbreviation in APD of Purkinje fibre and at the same time exerted no significant effect on the subendocardial layers (papillary muscle).

Dog atria

In atria from dog in sinus rhythm (SR) a statistically significant prolongation of the APD was observed in the presence of 10 μ M ranolazine and this lengthening further increased at 20 μ M. In addition, the drug exerted a marked and significant use-dependent depression of V_{\max} at basic cycle length of 300-700 ms. This block was significantly more expressed in atria than in ventricle at fast stimulation frequencies (BCL= 300-400 ms). In tachypacing induced remodelled dog atrial preparations ranolazine also produced statistically significant prolongation of APD₉₀ with a concomitant shortening of APD₅₀. The repolarization (APD₉₀) lengthening and V_{\max} blocking effects were more pronounced in normal (healthy) atria (SR), than in remodelled atria (AF).

Effects of ivabradine on transmembrane action potentials

Dog Purkinje fibre

In dog Purkinje strands, stimulation was terminated to allow development of spontaneous activity. The developed spontaneous frequency in the control preparations was 0.47 ± 0.06 Hz. Ivabradine concentration-dependently decreased the steepness of spontaneous diastolic depolarization and slowed spontaneous rate of firing of the Purkinje fibres. Spontaneous activity was completely abolished by 10 μ M ivabradine in all preparations. In isolated dog Purkinje fibres ivabradine concentration- and rate-dependently decreased the maximum rate of rise of the action potential upstroke (V_{\max}), and action potential amplitude while action potential duration measured at 50% of repolarization was shortened in a concentration-dependent manner at pacing with a constant cycle length of 500 ms. The depression of V_{\max} evoked by 1 and 10 μ M ivabradine was strongly dependent upon stimulation frequency (“use-dependent”); i.e., as pacing cycle length was decreased, the depression of V_{\max} was increased.

Dog papillary muscle

In dog right ventricular papillary muscle at a stimulation cycle length of 1000 ms ivabradine lengthened the action potential repolarization in a concentration dependent manner. Ivabradine decreased the V_{\max} and increased the impulse conduction time (CT) dose- and rate-dependently.

The drug moderately prolonged the APD in case of normal repolarization reserve, while after attenuation of the repolarization reserve by inhibition of the inward rectifier potassium current by adding 30 μ M BaCl₂ it further lengthened the ventricular repolarization.

Human papillary muscle

In human ventricular muscle preparations at a stimulation cycle length of 1000 ms 1 μ M ivabradine did not change ventricular repolarization and a small, but significant prolongation of the action potential repolarization was observed only in the presence of high (10 μ M) concentration of ivabradine. Ivabradine dose- and rate-dependently decreased the V_{\max} and increased the impulse conduction time.

Onset and offset kinetics of V_{\max} block

In dog ventricular muscles driven at the cycle length of 400 ms the onset kinetics of V_{\max} block induced by 10 μ M ivabradine was fitted to a single exponential, resulting in the onset rate kinetic constant of $\tau = 13.9 \pm 3.2$ beat⁻¹. In dog ventricular muscles at the stimulation cycle length of 400 ms, the recovery of V_{\max} during control was best fit to a single exponential relation. The time constant for recovery of V_{\max} during control was fast ($\tau_{\text{fast}} = 46.2 \pm 4.3$ ms) and before final repolarization of the basic action potential, it was almost complete. In the presence of 10 μ M ivabradine the recovery kinetics of V_{\max} was best fit with a twoexponential relation. In addition to a fast component ($\tau_{\text{fast}} = 41.2 \pm 8.2$ ms) which reflects recovery of the drug-free sodium channels, a slow component ($\tau_{\text{slow}} = 8.76 \pm 1.34$ s) of recovery of V_{\max} was revealed following exposure to ivabradine. This second slow component for recovery of V_{\max} may reflect effects on drug-affected sodium channels.

Dispersion of repolarization

In dog heart ivabradine preserved the dispersion of repolarization i.e. the difference in APD₉₀ values between Purkinje fibres and papillary muscles at basic cycle length of

1000 ms. The drug produced prolongation in APD of Purkinje fibre and exerted the same effect on the subendocardial layers (papillary muscle).

DISCUSSION

Major findings

In this thesis several effects of novel antianginal drugs on transmembrane action potentials are summarized. The effects of ranolazine and ivabradine were mainly investigated in dog, a species resembling human in heart size, spontaneous frequency and repolarization. The results led us to the conclusion that ranolazine and ivabradine at relatively high concentrations in dog and human cardiac preparations produce a concentration- and frequency-dependent depression of V_{\max} and able to prolong action potential duration i.e. exerts Class I and III antiarrhythmic actions. Ranolazine produces depression of V_{\max} with rather fast onset and offset kinetics, i.e. exerts Class I/B antiarrhythmic action (similar to that of mexiletine) not only in normal and remodelled atria, but also in the ventricle. Other important finding is that due to its multiple ion channel blocking property, ranolazine alters the repolarization in a complex manner in remodelled atria. Ivabradine (at high concentrations) can be considered as a Na^+ channel blocker antiarrhythmic drug with slow kinetic onset and recovery i.e. exerts Class I/C antiarrhythmic action (similar to that of flecainide or propafenone). Ranolazine and ivabradine are devoid from the effect to increase the dispersion of repolarization between Purkinje fibres and the subendocardial muscle layers.

Class I antiarrhythmic action

The dose- and frequency dependent V_{\max} block (induced by ranolazine and ivabradine) could be attributed to the inhibition of the fast/peak Na^+ current. V_{\max} measurements are indicative for I_{Na} function, but they cannot be used for quantitative estimation of sodium channel availability, since it could be underestimated. It was demonstrated that V_{\max} could be regarded as a nonlinear indicator of the fast inward sodium current.

V_{\max} block in the ventricle

The V_{\max} block of ranolazine was similar in frequency-dependent characteristic than that of class I/B antiarrhythmic drugs (e.g. mexiletine). In previous studies the drug was reported to produce atrial-predominant sodium channel block and postrepolarization refractoriness which was postulated in the mechanism of suppressing atrial fibrillation. The investigators did not apply properly wide range of stimulation frequencies (BCL= 300-5000 ms) in the presence of ranolazine at therapeutically meaningful concentration using the conventional microelectrode technique. Therefore the effect of ranolazine on peak I_{Na} and conduction in the ventricle might have been underestimated and neglected. In a previous study the investigators found that ranolazine blocked peak I_{Na} with high IC_{50} values (at 1, 2 and 5 Hz were 260, 157 and 154 μM , respectively) in HEK293 cells using whole-cell patch-clamp technique at room temperature which is about one order of magnitude higher than our results based on V_{\max} measurements and should be extrapolated with caution to intact heart including humans. Fredj et al. found preferential ranolazine block of sustained vs peak Na^+ channel current for LQT-3 mutant channels (IC_{50} = 15 vs 135 μM) also in HEK293 cells using patch-clamp technique at room temperature which ratio in our experiments - in 'healthy' cardiac preparations at 37°C - is almost 1:1. The effects of ranolazine on onset and offset kinetics of V_{\max} indicate that ranolazine kinetically resembles fast/intermediate (Class I/B) antiarrhythmic agents. Similar results were

described by others earlier at high concentration using different protocol to determine unbinding kinetics.

The effects of ivabradine on recovery of V_{\max} observed in dog ventricular muscle indicate that ivabradine resembles kinetically slow antiarrhythmic agents. Frequency-dependent V_{\max} block has been demonstrated previously only in small animals at high concentration (50 μM) of ivabradine. The possible decrease of I_{Na} by ivabradine may contribute to the inhibition of the pacemaker function.

The sodium current determines greatly the velocity of impulse propagation through the His-Purkinje system, working atrial and ventricular cardiac tissue. Since ranolazine and ivabradine produced significant depression of V_{\max} at fast stimulation frequencies, it can therefore be expected that the drugs may suppress the impulse conduction at fast heart rate i.e. during tachycardia or that extrasystoles with short coupling interval during cardiac arrhythmias.

V_{\max} block in atria vs. ventricle

The less negative resting potential, the greater density of sodium channels and the more negative half-inactivation voltage in atrial myocytes could make the sodium channel block more vigorous. We investigated the possible difference in V_{\max} block between atria and ventricle in the example of ranolazine, since this drug was reported to produce atrial-predominant use dependent block of sodium channels and postrepolarization refractoriness which was postulated in the mechanism of suppressing atrial fibrillation. Similarly to that described by others earlier the decrease in V_{\max} was more pronounced in the atria than in the ventricle. However, in our experiments the V_{\max} block and consequently the CT were also substantial (at fast stimulation frequencies) in ventricular preparations.

V_{\max} block in normal vs. remodelled atria

Since it has been suggested that ranolazine application could be effective in atrial fibrillation, we investigated the possible difference in V_{\max} block between normal and remodelled atria in the example of ranolazine. Remodelled atrial preparations were obtained from tachypacing induced remodelled dog hearts (used as an established model for mimicking the pathological tissue in atrial fibrillation). The frequency dependent V_{\max} block was less pronounced in remodelled than in normal atria. This is in accordance with a previous study in which reduced peak I_{Na} density was detected in AF. The less pronounced effect on V_{\max} by ranolazine in remodelled atrial tissue can be explained by shorter repolarization and, as a consequence, longer diastolic intervals allowing more recovery at each cycle. In AF the use-dependent Na^+ channel block by ranolazine most likely enhances postrepolarization refractoriness in addition to the repolarization lengthening in the remodelled atria. Despite the prolongation in the APD_{90} , ranolazine decreased the APD_{50} value in remodelled atria, which may reflect inhibitory effect on late I_{Na} . The latter current was recently reported to be upregulated in isolated human atrial myocytes obtained from AF patients in experiments with patch-clamp technique.

V_{\max} block in case of elevated extracellular potassium concentration

Block of sodium current has been demonstrated to be more pronounced when the resting membrane potential is partly depolarized e.g. in ischemic tissues. We investigated this phenomenon in the example of ranolazine induced V_{\max} block in case of normal (4 mM) and elevated (6 mM) extracellular potassium concentration. Depression of V_{\max} and conduction were more pronounced in case of elevated potassium concentration, e.g. when the resting membrane potential is partly depolarized, even as in the presence of an ischaemic cardiac substrate.

It can therefore be expected that the drugs may suppress the impulse conduction at fast heart rate i.e. during tachycardia or that extrasystoles with short coupling interval during cardiac arrhythmias in patients with angina pectoris.

Class III antiarrhythmic action

The repolarization lengthening effect of ranolazine and ivabradine could be best explained by the drug evoked I_{Kr} block. In various cardiac preparations, the effect of ranolazine and ivabradine on APD depends on the different density of various ion channels (late I_{Na} , late I_{Ca} , I_{Kr} , I_{Ks}) and the extent of blocking effects of the compounds on these channels. The antiarrhythmic efficacy of most pure class III drugs is compromised by their inherent property to induce excessive lengthening of the action potential (reverse frequency dependence) and their inability to prolong the action potential when most needed, namely during tachycardia. Overall, an ideal antiarrhythmic agent does not exist, and drug selection should be highly individualized. In case of ranolazine and ivabradine the additional sodium channel block would limit action potential prolongation at slow rate due to the I_{Kr} inhibition.

Ranolazine has been shown to cause a slight prolongation of the QT interval on the ECG. In dog midmyocardial preparations ranolazine (20 μ M) produced prolongation of repolarization in accordance with the previous (clinical) observation. Ivabradine exerted significant prolongation of repolarization in human preparations only at higher concentration (10 μ M).

Attenuated repolarization reserve

Remodelling, pharmacological modulation or genetic channelopathies of certain potassium channels, which normally contribute to repolarization, can attenuate the capability of the heart to repolarize. In these situations inhibition of other potassium channels may lead to unexpectedly augmented APD prolongation, resulting in proarrhythmic reactions. In previous studies the investigators found that ivabradine at 3 and 10 μ M prolongs repolarization in guinea-pig papillary muscles. Our results support the action potential lengthening effect of ivabradine also in dog and human cardiac preparations especially in case of attenuated repolarization reserve.

Class V antiarrhythmic action

Ivabradine is originally a class V antiarrhythmic agent with antianginal property, since the drug exerts well-known I_f blocking effect. Ivabradine induces (a marked exponential use-dependent) blockade of the hyperpolarization activated I_f current with relatively high (2.8 μ M) IC_{50} in rabbit sinus node cells. Ivabradine concentration-dependently decreased the steepness of spontaneous diastolic depolarization and slowed spontaneous rate of firing of the Purkinje fibres which effect could be best explained by the drug evoked I_f block. This ability on Purkinje fibres might be considered as an antiarrhythmic property and might suppress the initiation of arrhythmias. Ranolazine does not exert class V antiarrhythmic action.

Dispersion of ventricular repolarization

In Purkinje fibres, where the late I_{Na} is robust, ranolazine and ivabradine produced shortening of APD_{50} . This impact on the plateau slope might be related to the inhibitory effect of the drugs on the persistent, 'window' (late I_{Na}) current. Block of this Na^+ current might also have an additional therapeutic value and would also limit action potential prolongation at slow rate due to the I_{Kr} inhibition of the drugs at higher concentration.

Owing to this 'dirty' (mixed) characteristic, ranolazine and ivabradine are devoid from the effect to increase the dispersion of repolarization between Purkinje fibres and the subendocardial muscle layers, oppositely in case of 'pure' class III drugs. This ability can be considered as an advantageous property. Excessive shortening of the Purkinje fibre APD may increase the risk of reentry, but in the case of ranolazine and ivabradine the mentioned I_{Kr} blocking effect and the postrepolarization refractoriness (due to the sodium channel blockade) attenuate this potential risk factor also.

Human cardiac tissue

To our knowledge our results were the first in which the effects of ranolazine and ivabradine were investigated in human ventricular muscle preparations with conventional microelectrode technique. We observed depression of the V_{max} to similar extent as in dog papillary muscles. Ivabradine at 10 μ M exerted significant effect on action potential duration also in human ventricular preparations. Our results suggest that late I_{Na} has less contribution to repolarization in dog than in human ventricular preparations. In case of ranolazine APD₅₀ values were shortened in human but not in dog ventricular muscle. In this context it should be noted that biphasic time dependent changes were observed (especially in human preparations) with ranolazine, i.e. the APD shortening effect always preceded the drug evoked tendency to repolarization lengthening. The initial shortening of APD was much greater in human than in dog, which may also indicate, that late I_{Na} has less contribution to repolarization in dog.

Therapeutically relevant concentrations of the drugs

The therapeutically relevant concentration of ranolazine and ivabradine should be interpreted with great caution. For example in previous studies the authors interpreted 2-10 μ M ranolazine as therapeutically relevant concentration, but they did not provide a cross reference to a factual effective plasma level. Authors report steady state trough levels at a therapeutic dose in man to be 464 ng/ml. This equates to a plasma concentration of 1 micromolar. Thus the doses used in our study may 5- to 20-fold higher than therapeutic concentration according to this article.

In case of ivabradine though therapeutic plasma concentrations are about 0.04–0.07 μ M, the drug has been tested at higher (0.17 μ M) concentration in healthy volunteers. Other investigators interpreted 3 μ M ivabradine as „clinically relevant” concentration. Therefore the (0.1–1–10 μ M) concentrations applied in our experiments may be relevant, since the tissue concentration can be expected to be higher than that of the plasma, in accordance with the high volume of distribution (close to 100 L) value of the drug. In this context, the relatively low therapeutic plasma concentration might be the result of possibly meaningful tissue appearance of ivabradine. We did not find significant effect of 0.1 μ M ivabradine on V_{max} and APD measurements.

Clinical implications

It has to be emphasized that the drugs so far have proved to be safe and free from any proarrhythmic events in clinical trials. The sodium channel blocking ability on Purkinje fibres might be considered as an antiarrhythmic property and might suppress the initiation of an extrasystole, limit any repolarization lengthening and most importantly, it could decrease dispersion of repolarization. Since ranolazine and ivabradine produced significant depression of V_{max} at fast stimulation frequencies, it can therefore be expected that the drugs **may** suppress the impulse conduction at fast heart rate i.e. during tachycardia or that extrasystoles with short coupling interval during cardiac arrhythmias. However the

possible contribution of this effect to the clinical benefit of the drug is still unclear. The properties of ranolazine resemble that of chronically administered amiodarone and **may** represent antiarrhythmic property. Amiodarone has minimal proarrhythmic risk, but has numerous noncardiac toxicities that require frequent monitoring. Clinical findings support the suggestion that ranolazine might be efficacious not only in supraventricular but also in ventricular arrhythmias. Although proarrhythmic side effects do not seem to be a major concern during ranolazine and ivabradine application, they can not be completely ruled out in some pathological conditions, e.g. in case of drug accumulation or intoxication, or in case of attenuated repolarization reserve like in heart failure, diabetes or in long QT syndromes. Therefore, further studies with ranolazine and ivabradine are needed to determine its safety and efficacy in future clinical use.

CONCLUSIONS

Based on the cellular cardiac electrophysiological properties of ranolazine and ivabradine it can be concluded that these antianginal drugs also exert Class I and III antiarrhythmic properties (at higher concentrations) which can be advantageous in the treatment of patients with ischemic heart disease, heart failure liable to disturbances of cardiac rhythm. Ranolazine decreases (and ivabradine does not increase) the dispersion of ventricular repolarization (the difference in APD₉₀ values between Purkinje fibres and papillary muscles), which can contribute to the antiarrhythmic property of the drugs. Ranolazine exerts amiodaron-like antiarrhythmic properties, without serious side-effects. Ivabradine is beneficial in patients with angina pectoris equally to beta receptor blockers without producing any negative inotropic or vasoconstrictor effect. Based on the recent clinical trials and experiments, widening the indication of the drugs is expected (heart failure and atrial fibrillation for ranolazine; myocardial infarction and heart failure for ivabradine).

These ‘dirty’ drugs combine several modes of antiarrhythmic actions which might be the key to find a drug that has powerful antiarrhythmic potential with lack of proarrhythmic side effects.

ACKNOWLEDGEMENT

I am especially thankful to my supervisors **László Virág PhD** for introducing me to cardiac cellular electrophysiology, and for providing me the opportunity for research in the laboratory, and to **Professor András Varró MD, DSc** for his continuous support and personal guidance at the Department of Pharmacology & Pharmacotherapy.

I am very grateful to **Professor Julius Gy. Papp MD, DSc, academician** and to **István Koncz MD** for their continuous support, criticism and suggestions.

I wish to thank my colleagues, **Norbert Jost PhD; István Baczkó MD, PhD; Zoltán Husi MD** for their continuous support and help in my work.

I am also very thankful to **Zsuzsanna Molnár, Gyula Horváth, and Gábor Girst** for their helpful technical assistance.

Finally, I wish to thank, and dedicate this thesis to my whole family and to my friends for their love, help and encouragement.

The publication is supported by the European Union and co-funded by the European Social Fund. Project title: “Broadening the knowledge base and supporting the long term professional sustainability of the Research University Centre of Excellence at the University of Szeged by ensuring the rising generation of excellent scientists.” Project number: TÁMOP-4.2.2/B-10/1-2010-0012.