*Full Paper***Effects of Dofetilide and EGIS-7229, an Antiarrhythmic Agent Possessing Class III, IV, and IB Activities, on Myocardial Refractoriness in Hyperkalemia, Hypokalemia, and During β -Adrenergic Activation in the Rabbit Papillary Muscle In Vitro**Anikó Kovács¹ and Gábor Szénási^{1,*}¹Pharmacology Laboratory I, EGIS Pharmaceuticals, Ltd., H-1475 Budapest 10, POB 100, Hungary

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Abstract. Lengthening of the effective refractory period (ERP) by EGIS-7229, a class III/IV drug, and by dofetilide, a selective I_{Kr} blocker, was compared in normokalemia (NK), hypokalemia (LK), and hyperkalemia (HK) in right ventricular papillary muscles of rabbits paced at 0.5, 1, and 2 Hz, in vitro, and also during β -adrenergic activation. In NK, EGIS-7229 (3 and 10 μ M) and dofetilide (30 and 100 nM) similarly lengthened ERP in a steeply reverse frequency-dependent manner. The two compounds produced smaller ERP prolongations at 0.5 Hz in HK and LK, so rate-dependence of ERP changes decreased. EGIS-7229 lengthened ERP more at 2 Hz than at 0.5 Hz at 10 μ M in LK, that is, the effect of EGIS-7229 turned into positive frequency-dependence from 3 to 10 μ M. Furthermore, EGIS-7229 lengthened ERP at 10 μ M more than dofetilide at 100 nM at 2 Hz stimulation rate ($P < 0.05$). Isoproterenol (30 nM) eliminated the effect of dofetilide on ERP, while EGIS-7229 prolonged ERP during β -adrenergic activation. In conclusion, efficacy of EGIS-7229 was superior to that of dofetilide in LK and during β -adrenergic stimulation, suggesting improved antiarrhythmic action for EGIS-7229 under certain conditions in the patient.

Keywords: dofetilide, antiarrhythmic agent, hypokalemia, hyperkalemia, effective refractory period

Introduction

Pure class III drugs prolong action potential duration (APD) and effective refractory period (ERP) more at slow than at rapid driving frequencies, that is, their effect is reverse frequency-dependent (1). The consequence of this characteristic is a relatively weak effect during arrhythmia when the heart rate is high. On the other hand, the strong effect during slow heart rate increases the risk of arrhythmogenesis due to extended duration of calcium flow into the myocytes (2). Induction of “torsades de pointes” polymorphic ventricular tachycardia and fatal ventricular fibrillation considerably limit the therapeutic use of class III antiarrhythmic drugs. Furthermore, the QT lengthening side effect of some

marketed drugs (acquired long QT syndrome) creates a serious ethical and regulatory problem (3). Besides bradycardia, hypokalemia (LK) is one of several factors that can augment the arrhythmogenicity of drugs, which prolong QT interval (4). Extracellular potassium concentration ($[K^+]_e$) can frequently decrease in clinical practice, especially in patients treated with diuretics. There are some reports regarding the influence of LK and hyperkalemia (HK) on the frequency-dependent APD and ERP prolongation of pure class III agents in vitro (5–8). However, the potential modifying effect that may arise from further ion channel blockade in addition to I_{Kr} (rapid component of the delayed rectifier potassium current) blockade has not yet been tested at altered $[K^+]_e$.

β -Adrenergic activation attenuates lengthening of APD and QT interval of the ECG caused by class III drugs (9, 10). This phenomenon is undesirable, as

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tachycardia due to increased sympathetic tone and ensuing β -adrenergic activation has been implicated as one of the provokers of malignant ventricular arrhythmias (11). Compared to selective class III drugs, the effect of antiarrhythmic agents with combined mode of action may be resistant to β -adrenergic activation. Amiodarone (class III/Ib/IV/II) was effective during sympathetic activation (10). Bidisomide (class I/III) maintained APD and ERP prolongation in the presence of isoproterenol via an action not related to β -adrenergic receptor blockade (12).

EGIS-7229 is a class III (I_{Kr} blocker) antiarrhythmic compound with Ib and IV components (13–15). The aim of the present work was to compare the effects of EGIS-7229 and dofetilide on ERP in LK, HK, and during β -adrenergic activation in the right ventricular papillary muscle of rabbits *in vitro*. The rabbit seems to be a suitable species for studying the cardiac effects of EGIS-7229, as we have shown previously that the electrophysiological effects of EGIS-7229 were similar in the rabbit and human myocardium (15). The basis of the antiarrhythmic effect of class III drugs is prolongation of refractoriness to electrical stimulation. Therefore, the effects of the two antiarrhythmic drugs on refractoriness were evaluated as done previously (14).

Materials and Methods

Male New Zealand white rabbits (Charles River Hungary) were housed in standard conditions and food and water were available *ad libitum* at all times. Care and use of experimental animals were in accordance with the 86/609/EEC directive and the local Animal Ethics Committee approved all experiments.

Rabbits weighing 2600–3200 g were killed by a blow on the head after light anesthesia (Nembutal; CEVA-Phylaxia, Budapest, Hungary), and then the hearts were rapidly excised and placed in a Petri dish containing carbogenized normal Krebs solution. Thin (<1 mm) papillary muscles were isolated from the right ventricle and they were vertically mounted in 20-ml organ bathes in normokalemic, hypokalemic, or hyperkalemic Krebs solution (Schuler organ bath; Hugo-Sachs, Hugstetten, Germany). The tendinous end of the muscle was connected to a force-displacement transducer coupled to a recorder via a DC-bridge amplifier for measuring isometric tension. Immediately after the application of the preload (500 mg), the preparations were stimulated (field stimulation) with 1-ms square-wave pulses at the rate of 2 Hz and at $1.3\times$ threshold voltage. Resting tension was maintained during the 90-min equilibration period and the preparations were rinsed with fresh bathing solution every 30 min. The

bathing solution was carbogenized (95% O_2 / 5% CO_2) and its temperature was kept at 35°C.

ERP was measured using a standard extrastimulus technique. Extrastimuli (S_2) were interjected between normal paced stimuli (S_1) in such a way that the S_1 - S_2 interval was initially below the refractory period of the tissue. The S_1 - S_2 interval was then widened in increments of 1 ms after each 10th normal stimuli until postextrasystolic potentiation (>120% of the control contraction force) was observed. The effective refractory period was defined as the smallest S_1 - S_2 interval that produced postextrasystolic potentiation.

Frequency-dependent effects of antiarrhythmic drugs on ERP in normokalemia (NK), LK, and HK in the right ventricular papillary muscle of rabbits in vitro

Three groups of tissues ($n=9-14$) were bathed in Krebs solutions containing 4, 2, or 8 mM $[K^+]_e$ (composition of normal Krebs solution: 120 mM NaCl, 3.0 mM KCl, 27.2 mM $NaHCO_3$, 11.1 mM glucose, 1.2 mM $MgSO_4$, 1.0 mM KH_2PO_4 , 2.6 mM $CaCl_2$, pH = 7.4). Each concentration of the antiarrhythmic compounds was tested in NK, LK, and HK in separate groups of tissues. After a 90-min incubation period, the ERP was measured at 2, 1, and 0.5 Hz stimulation frequencies (baseline), and then EGIS-7229 at 3 or 10 μ M or dofetilide at 30 or 100 nM was applied and incubated for 60 min or 30 min at 2 Hz, respectively. ERP was measured in the presence of the test compound at 2-, 1-, and 0.5-Hz stimulation rates (treated). ERP measurements were always started 3 min after changing the stimulation frequency.

Effects of β -adrenergic activation on prolongation of ERP by antiarrhythmic agents in the right ventricular papillary muscle of rabbits in vitro

Baseline ERP value at 2-Hz pacing rate was measured at the end of the incubation period. The effects of the test compounds (EGIS-7229: 3 and 10 μ M, $n=8-10$; dofetilide: 30 and 100 nM, $n=8-10$); dofetilide (100 nM) co-administered with lidocaine (30 μ M, $n=9$) or verapamil (300 nM, $n=8$); and of lidocaine alone (30 μ M, $n=8$) on ERP were determined after incubation for 60 min. Then one test concentration of isoproterenol (3, 10, or 30 nM) was added to the bath in separate groups of tissues, and in the presence of the test compound/s and isoproterenol, ERP was measured once again. An attempt was made to measure ERP at the peak effect of the β -adrenoceptor agonist (3–4 min after its addition).

Drugs

EGIS-7229 and dofetilide were synthesized at the

Division of Chemical Research of EGIS Pharmaceuticals, Ltd. (Budapest, Hungary). Lidocaine HCl (2% injection) was produced by EGIS Pharmaceuticals Ltd. Isoproterenol was obtained from Fluka Chemie AG (Buchs, Switzerland). Verapamil was prepared by Research Biochemicals International (RBI, Natick, MA, USA). Other chemicals were made by Reanal, Ltd. (Budapest, Hungary).

Data analyses

Data given are the means $\pm$ S.E.M. Frequency dependence of the effects of test compounds on ERP was evaluated by one-way ANOVA for repeated measures (Statistica software package; Statsoft, Tulsa, OK, USA). Other statistical comparisons of the results were performed by the Newman-Keuls post hoc test after checking the homogeneity of variances with two-way ANOVA for repeated measures.

Results

The frequency- and concentration-dependent effects of dofetilide and EGIS-7229 on ERP at different potassium concentrations

The effect of stimulation frequency on baseline ERP in LK and HK: Baseline ERP values were similar at all stimulation rates and proved to be reverse frequency-dependent in NK and LK (Table 1). HK reduced ERP at 1 and 0.5 Hz compared to values measured in NK, and ERP was longer at 1 Hz than at 0.5 and 2 Hz in HK.

Effects of LK and HK on ERP changes caused by dofetilide and EGIS-7229: Dofetilide markedly lengthened ERP in a reverse frequency-dependent manner at both test concentrations and at all $[K^+]_e$ (Fig. 1A). Both LK and HK suppressed the dofetilide-induced ERP changes at 1.0 and 0.5 Hz pacing rates at both test concentrations. The ERP lengthening effect of dofetilide remained unchanged at 2.0 Hz except at 30 nM in LK. EGIS-7229 also markedly lengthened ERP at both test concentrations and at all three $[K^+]_e$ (Fig. 1B). The effects of EGIS-7229 on ERP were usually reverse frequency-dependent. However, EGIS-7229 lengthened

Fig. 1. Comparison of the effects of hypokalemia (LK), normokalemia (NK), and hyperkalemia (HK) on the effective refractory period prolongations by dofetilide (A) and EGIS-7229 (B) at 2-, 1-, and 0.5-Hz stimulation frequencies. The data are expressed as % changes in relation to the baseline values. The effects of the lower concentration (left panel) and higher concentration (right panel) of dofetilide and EGIS-7229 on ERP are shown. ** = $P < 0.01$, *** = $P < 0.001$ vs NK; ° = $P < 0.05$, °° = $P < 0.01$, °°° = $P < 0.001$ vs 2 Hz.

Table 1. Baseline ERP values measured in normokalemia (NK) ($[K^+]_e$: 4 mM), hypokalemia (LK) ($[K^+]_e$: 2 mM), and hyperkalemia (HK) ($[K^+]_e$: 8 mM) at 2-, 1-, and 0.5-Hz stimulation frequencies

$[K^+]_e$		ERP (ms)		
		2 Hz	1 Hz	0.5 Hz
LK	2 mM (n = 63)	158.6 $\pm$ 2.4	174.1 $\pm$ 2.9*	166.2 $\pm$ 2.6*
NK	4 mM (n = 62)	158.5 $\pm$ 2.5	174.5 $\pm$ 2.5*	167.0 $\pm$ 2.4*
HK	8 mM (n = 65)	156.0 $\pm$ 2.1	169.7 $\pm$ 2.1*#	158.9 $\pm$ 2.0#

ERP values from all experiments were combined. n = number of experiments. * = $P < 0.001$ vs 2 Hz; # = $P < 0.05$ vs NK.

ERP less at 0.5 Hz than at 2 Hz ($P < 0.05$) at $10 \mu\text{M}$ in LK, that is, the effect of the higher concentration of EGIS-7229 on ERP turned into positive frequency-dependence at LK. Changes in ERP caused by EGIS-7229 were more resistant to LK and HK at higher pacing rates. The EGIS-7229-induced ERP prolongations were shorter at 0.5 Hz both in LK and HK than in NK both at 3 and $10 \mu\text{M}$. Compared to changes measured in NK, EGIS-7229 produced less ERP prolongation at 1.0 Hz at $3 \mu\text{M}$ in HK and at $10 \mu\text{M}$ in LK. The EGIS-7229-induced ERP changes were similar at 2-Hz stimulation rate at all $[\text{K}^+]_e$. Moreover, the EGIS-7229-induced ERP lengthening was larger at the higher test concentration than at the lower test concentration at all stimulation rates in HK (data not shown).

Comparison of ERP changes caused by dofetilide and EGIS-7229 at different potassium concentrations: The effects of dofetilide and EGIS-7229 on ERP were similar at both test concentrations and all stimulation rates in NK and HK (Fig. 2: A and B). EGIS-7229 produced significantly larger ERP prolongations at $3 \mu\text{M}$ than dofetilide at 30 nM at all stimulation rates in LK (Fig. 2A). Furthermore, the effect of EGIS-7229 on ERP was greater than that of dofetilide at 2 Hz in LK at their higher test concentrations (Fig. 2B).

Effects of dofetilide and EGIS-7229 on ERP during β -adrenergic activation

Before β -adrenergic stimulation, EGIS-7229 prolonged ERP by 49, 53, and 52 ms ($P < 0.001$) at $10 \mu\text{M}$, which were changed to 39, 58, and 28 ms in the presence of 3, 10, and 30 nM of isoproterenol, respectively. Thus, β -adrenergic stimulation did not reduce ERP prolongation caused by EGIS-7229 at $10 \mu\text{M}$ (Fig. 3A). On the contrary, isoproterenol significantly reduced the effect of dofetilide on ERP from 53 to 25 ms at 10 nM, and dofetilide failed to lengthen ERP in the presence of 30 nM isoproterenol (Fig. 3B). Combined treatment with lidocaine ($30 \mu\text{M}$) and dofetilide (100 nM) caused larger ERP lengthening than dofetilide alone, and 10 nM isoproterenol did not decrease their effect on ERP compared to that seen before β -adrenergic stimulation (Fig. 4). When dofetilide (100 nM) was combined with verapamil (300 nM), 10 nM isoproterenol reduced the dofetilide-induced ERP prolongation (Fig. 4). Lidocaine prolonged ERP at $30 \mu\text{M}$, and 10 nM isoproterenol did not change the effect of lidocaine (Fig. 5).

Discussion

It is well known that pathophysiological conditions influence basic electrophysiological characteristics of the myocardial tissue by altering ion channel functions

Fig. 2. Comparison of the effects of dofetilide and EGIS-7229 on the effective refractory period at their lower (A) and higher (B) concentrations in hypokalemia (LK), normokalemia (NK), and hyperkalemia (HK) at 2-, 1-, and 0.5-Hz stimulation frequencies. The data are expressed as % changes in relation to the baseline values. The effects of the lower and higher concentrations of dofetilide and EGIS-7229 on the effective refractory period are shown in LK (left panel), NK (middle panel), and HK (right panel). * = $P < 0.05$ between dofetilide and EGIS-7229.

(16–18). Furthermore, efficacy of antiarrhythmic drugs may often worsen under these conditions.

EGIS-7229 is an I_{Kr} blocker, class III drug with I/B and IV antiarrhythmic components (13, 15, 19). EGIS-7229 suppressed I_{Kr} with an IC_{50} of $1 \mu\text{M}$, while it blocked I_{Ca} from $3 \mu\text{M}$ in isolated ventricular myocytes of the dog (19). The effects of EGIS-7229 on V_{max} , which is a measure of I_{Na} blockade, started at 3 or $10 \mu\text{M}$ in different myocardium preparations. Kinetic analysis of V_{max} revealed that sodium channel blockade caused

Fig. 3. Effects of isoproterenol (ISO) on the effective refractory period prolongation caused by EGIS-7229 (A) and dofetilide (B) at 2-Hz stimulation frequency. Open bars show baseline ERP values; gray bars show the effects of EGIS-7229 (10 μ M) and dofetilide (100 nM) on ERP before the administration of isoproterenol; hatched bars show the effects of EGIS-7229 and dofetilide after the administration of isoproterenol at 3, 10, or 30 nM. ^{oo} = $P < 0.01$, ^{ooo} = $P < 0.001$ vs baseline; *** = $P < 0.001$ vs dofetilide alone (gray bar).

by EGIS-7229 falls into the I/B subclass (15). As a consequence of multiple ion channel actions, EGIS-7229 caused peak APD₅₀ and APD₉₀ lengthening at 3 or 10 μ M, depending on the species studied, and APD declined if the concentration of EGIS-7229 was increased further (13, 15).

We tested the frequency-dependent effects of EGIS-7229 on ERP at two concentrations in comparison with those of dofetilide in order to evaluate if blockade of other ion channels besides I_{Kr} improves antiarrhythmic efficacy. We chose two concentrations of dofetilide and EGIS-7229 that produced near-maximal effects on ERP in NK and determined if LK and HK altered the effects

Fig. 4. Effects of isoproterenol (ISO) on the effective refractory period prolongation caused by dofetilide (Dof) alone and by dofetilide co-administered with lidocaine (Lid) or verapamil (Ver) at 2-Hz stimulation frequency. Open bars show baseline ERP values, gray bars show the effects of dofetilide (100 nM) alone, and dofetilide co-administered with lidocaine (30 μ M) or verapamil (300 nM) before the administration of isoproterenol, and hatched bars show the effects of the above test items after the administration of isoproterenol (10 nM). ^{oo} = $P < 0.01$, ^{ooo} = $P < 0.001$ vs baseline, *** = $P < 0.001$ vs the effects of test items before the administration of isoproterenol.

Fig. 5. Effects of isoproterenol (ISO) on the effective refractory period prolongation caused by lidocaine at 2-Hz stimulation frequency. Open bars show baseline ERP values, gray bars show the effects of lidocaine (30 μ M) on ERP before the administration of vehicle or isoproterenol, and hatched bars show the effects of lidocaine after the administration of vehicle or isoproterenol (10 nM). ^{ooo} = $P < 0.001$ vs baseline.

of the two compounds on ERP at those concentrations.

It has been demonstrated that LK prolonged and HK shortened baseline APD₉₀ in the guinea-pig papillary muscle (5, 6) and in the dog Purkinje fiber (7), and ERP changed in parallel with APD₉₀ (6, 7). However, rabbits

seem to respond differently because LK (2 mM $[K^+]_e$) did not change monophasic APD in the left ventricle of isolated rabbits hearts, but shortened epicardial ERP (20). LK did not change monophasic APD in isolated rabbit hearts (21), and no change in left ventricular ERP was found in similar conditions, either (22). Our results are compatible with previous findings in rabbits because baseline ERP was similar in NK and LK at all stimulation rates. In our experiments, HK shortened baseline ERP at 1- and 0.5-Hz pacing rates; therefore, our results are similar to those obtained in guinea-pig and dog myocardial preparations in the above-mentioned studies. However, published data are contradictory regarding the cardiac electrophysiological effects of HK. Cobbe et al. (23) demonstrated that APD_{90} proportionally shortened in rabbit right ventricular papillary muscle when $[K^+]_e$ rose from 4 to 8 and 12 mM but ERP did not change when $[K^+]_e$ rose from 4 to 8 mM, and it even increased when $[K^+]_e$ rose from 4 to 12 mM. Similarly, HK (10 mM) shortened APD_{90} but lengthened ERP in guinea-pig papillary muscle (8) and prolonged ERP in ferret papillary muscle (24). The explanation for these inconsistent results is not apparent.

Most studies have shown that the effects of selective I_{Kr} blockers on APD and especially on ERP declined in both HK and LK, and the rate-dependency of drug-evoked APD and ERP prolongations abated (5, 8, 23, 24). However, the efficacy of dofetilide in prolonging APD_{90} did not change at 2.5, 3.5, or 5.6 mM $[K^+]_e$ in papillary muscle preparations of the guinea pig (6). Dofetilide-induced ERP changes and its frequency-dependency were smaller in LK and HK than in NK in the present study.

The main new finding of this study was that EGIS-7229 had improved efficacy in prolonging ERP in LK in comparison with that of dofetilide, despite the effects of the two compounds being similar in NK in the right ventricular papillary muscle of the rabbit in vitro. EGIS-7229 prolonged ERP at 10 μ M in a direct frequency-dependent manner in LK, and the effect of EGIS-7229 on ERP was significantly larger than that of dofetilide at 2-Hz pacing rate.

The positive frequency-dependence of ERP prolongation caused by EGIS-7229 in LK can be related to two factors. The role of I_{Kr} in repolarization decreased in LK as indicated by smaller actions of dofetilide and EGIS-7229 on ERP at all pacing rates in LK. Therefore, the relative importance of sodium channel blockade can increase in LK. Since the effects of I_{Na} blockers are use-dependent, mixed I_{Na} and I_{Kr} blockade can produce positive frequency-dependent ERP lengthening in LK. This possibility was supported by our previous findings that co-administration of dofetilide and lidocaine at the

same concentrations as in the present experiment lengthened ERP similarly at all pacing rates in NK in the right ventricular papillary muscle of the rabbit (13).

Positive rate-dependence of ERP lengthening by EGIS-7229 in LK is favorable, as LK can multiply the incidence of arrhythmia due to APD lengthening, so smaller ERP and possibly ADP prolongation at slow stimulation rate can lower the incidence of torsades de pointes. Conversely, strong effects on ERP can increase efficacy against arrhythmia. Another advantage of EGIS-7229 was that it prolonged ERP more at 10 μ M than at 3 μ M in HK at all stimulation rates. Although this change did not create a difference between EGIS-7229 and dofetilide, a strong antiarrhythmic action at high potassium concentration can be beneficial in patients suffering from ischemic heart disease.

Thus, EGIS-7229 possessed advantageous effects on myocardial refractoriness under pathophysiological $[K^+]_e$ in comparison to those of dofetilide. Improved efficacy of EGIS-7229 in LK and HK may rely on its complex mode of action.

It is known that β -adrenergic activation can eliminate the antiarrhythmic effects of pure class III drugs (9). Isoproterenol fully reversed the effects of sotalolol, a selective I_{Kr} blocker, on APD and myocardial refractoriness in humans in vivo (10). Consistent with previous observations, dofetilide failed to prolong ERP during β -adrenergic stimulation in our study. It is worth mentioning that isoproterenol reduced the dofetilide-induced ERP lengthening at low concentrations (10 and 30 nM), while much higher concentration of isoproterenol was applied in other studies (9). Isoproterenol did not abolish ERP prolongation caused by EGIS-7229. Since EGIS-7229 had no affinity for β -adrenergic receptors, the role of fast sodium channel and L-type calcium channel blockades was studied in an attempt to reveal the mechanism whereby EGIS-7229 maintained its effect on ERP during β -adrenergic activation. When dofetilide and lidocaine (30 μ M) were administered simultaneously, isoproterenol failed to decrease ERP. Furthermore, ERP prolongation caused by lidocaine was also resistant to isoproterenol. On the other hand, β -adrenergic stimulation decreased the dofetilide-induced ERP lengthening if co-administered with verapamil. Therefore, it seems likely that the resistance of ERP lengthening evoked by EGIS-7229 to β -adrenergic stimulation can be due to the sodium channel blocking action of the compound, but additional mechanisms cannot be excluded.

In summary, our results demonstrated that EGIS-7229, a class III/IV antiarrhythmic compound, lengthened myocardial refractoriness with improved characteristics compared to changes caused by dofetilide,

a pure class III antiarrhythmic drug. EGIS-7229 produced a direct frequency-dependent effect on ERP in LK, resulting in strong action at high pacing rate. Isoproterenol eliminated the effect of high concentration of dofetilide on myocardial refractoriness, while EGIS-7229 lengthened ERP during β -adrenergic stimulation. These results suggest that in certain unfavourable conditions EGIS-7229 may produce an improved antiarrhythmic action that is likely accompanied by low arrhythmogenicity in the patient. The beneficial characteristics of EGIS-7229 may be due to its multichannel antiarrhythmic activity, primarily to combined I_{Kr} and Na^+ channel blockades.

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