

β -adrenoceptor activation plays a role in the reverse rate-dependency of effective refractory period lengthening by dofetilide in the guinea-pig atrium, *in vitro*

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1 Blockers of the rapid component of the delayed rectifier potassium current (I_{Kr}) prolong cardiac action potential duration (APD) and effective refractory period (ERP) in a reverse rate-dependent manner. Since activation of β -adrenoceptors attenuates prolongation of APD evoked by I_{Kr} blockers, rate-dependent neuronal noradrenaline liberation in the myocardium may contribute to the reverse rate-dependent nature of the effects of I_{Kr} blockers. In order to test this hypothesis, we studied the effects of dofetilide, a pure I_{Kr} blocker, on ERP after activation or blockade of β -adrenoceptors and after catecholamine depletion in guinea-pig left atrial myocardium paced at 3, 2 and 1 Hz, *in vitro*.

2 Dofetilide (100 nM) lengthened ERP in a reverse rate-dependent manner in the left atrial myocardium of guinea-pigs. Strong activation of β -adrenoceptors using 10 nM isoproterenol abolished the dofetilide-induced lengthening of ERP at all pacing rates.

3 Blockade of the β -adrenoceptors with metoprolol (1 μ M), atenolol (3 μ M) or propranolol (300 nM) increased the dofetilide-evoked prolongation of ERP at 3 and 2 Hz, but not at 1 Hz. As a consequence, metoprolol attenuated while propranolol and atenolol fully eliminated the reverse rate-dependent nature of the dofetilide-induced ERP lengthening. In catecholamine-depleted atrial preparations of the guinea-pig (24 h pretreatment with 5 mg kg⁻¹ reserpine i.p.), the effect of dofetilide on ERP was not frequency dependent, and propranolol did not alter the effects of dofetilide.

4 In contrast to results obtained in guinea-pig atrial preparations, propranolol failed to change the reverse rate-dependent effect of dofetilide on ERP in the right ventricular papillary muscles of rabbits and guinea-pigs.

5 As an indication of the functional consequences of rate-dependent noradrenaline liberation, propranolol decreased twitch tension at 3 and 2 Hz but not at 1 Hz in the atrial myocardium of control guinea-pigs, whereas no such effect was detected in catecholamine-depleted atrial preparations. Propranolol failed to change contractility of ventricular myocardium in guinea-pigs and rabbits.

6 It is concluded that rate-dependent noradrenaline release and the ensuing β -adrenoceptor activation contributed to the reverse rate-dependent nature of ERP prolongation caused by I_{Kr} blockers in isolated guinea-pig atrial myocardium.

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Abbreviations: APD, action potential duration; ERP, effective refractory period; I_{Kr} , rapid component of the delayed rectifier potassium current; I_{Ks} , slow component of the delayed rectifier potassium current; I_{Kur} , ultrarapid component of the delayed rectifier potassium current; I_{to} , transient outward potassium current; I_{K1} , inward rectifier potassium current; I_f , hyperpolarization-activated current

Introduction

The rapid and slow components of the delayed rectifier potassium currents (I_{Kr} and I_{Ks} , respectively) play an important role in the initiation of repolarisation and in determining the duration of action potential (APD), and effective refractory period (ERP) in mammalian myocardial cells (Noble & Tsien, 1969; Sanguinetti & Jurkiewicz, 1990; Varró *et al.*, 1993; Iost *et al.*, 1998). The majority of class III antiarrhythmic drugs inhibit I_{Kr} . It has been shown that the lengthening effect of I_{Kr} blockers on APD and ERP is more

pronounced at slow than at rapid driving rates (Hondeghem *et al.*, 1990). This phenomenon, known as reverse frequency-dependence, is undesirable since antiarrhythmic effect is most needed during tachyarrhythmias. According to the classic hypothesis, reverse rate-dependency is due to differences in the kinetics of I_{Kr} and I_{Ks} channels (Jurkiewicz & Sanguinetti, 1993). However, some recent experimental findings contradicted this theory (Groh *et al.*, 1997; Gintant, 1998; Lengyel *et al.*, 2001; Rocchetti *et al.*, 2001). Thus, the precise mechanism of the reverse rate-dependent effect of I_{Kr} blockers has not yet been explained despite the great practical significance of understanding this phenomenon.

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β -Adrenoceptor activation can counteract the electrophysiological effects of various antiarrhythmic drugs (Jazayeri *et al.*, 1989; Sanguinetti *et al.*, 1991). High doses of isoproterenol, a nonselective β -adrenoceptor agonist, fully reverted the effects of I_{Kr} blockers on APD *in vitro* (Newman *et al.*, 1993; Martin *et al.*, 1996; Marschang *et al.*, 2000), and abolished the sematilide-induced prolongation of APD and myocardial refractoriness in humans *in vivo* (Sager *et al.*, 1994). Thus, gradual increases in the stimulation frequency and the concomitant β -adrenoceptor activation due to neuronal noradrenaline release in the myocardium can proportionally shorten the prolongation of APD and ERP caused by I_{Kr} blockers. However, D,L-sotalolol, a mixed β -adrenoceptor antagonist and I_{Kr} blocker caused a reverse frequency-dependent prolongation of ERP in right ventricular papillary muscle of rabbits and guinea-pigs *in vitro* (Berman & Dorian, 1993; Kovács *et al.*, 2001). These results exclude the application of this hypothesis to the ventricular myocardium, but there are no data available on such effects of β -adrenoceptors in the atrial myocardium. Our aim was to study the role of β -adrenoceptor activation in the reverse frequency-dependency of ERP prolongation elicited by I_{Kr} blockers. Therefore, the effects of dofetilide on ERP were studied either in the absence and presence of β -adrenoceptor blockade or after catecholamine depletion in guinea-pig left atrial tissues stimulated at 3, 2 and 1 Hz, *in vitro*.

Methods

The study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996) and was approved by the local Animal Ethics Committee.

Preparations

Male Dunkin–Hurtley guinea-pigs (400–600 g) and male New Zealand white rabbits (2.5–3.0 kg) were killed by cervical dislocation in pentobarbitone sodium anaesthesia and the heart was rapidly excised. The left atrium of guinea-pigs was dissected and mounted vertically in a 20 ml organ chamber filled with Krebs solution, containing (in mM) NaCl 118, KCl 4.6, NaHCO₃ 27.2, glucose 11.1, MgSO₄ 1.2, KH₂PO₄ 1.0, CaCl₂ 2.6, pH 7.4 (Schuler organ bath, Hugo-Sachs, Hugstetten, Germany). The tissue was connected to a force transducer coupled to a recorder through a DC-bridge amplifier allowing the measurement of isometric tension. Immediately after the application of preload (500 mg), the preparations were stimulated (field stimulation) using rectangular current pulses of 1 ms duration and 1.3 times of diastolic threshold amplitude at the rate of 2 Hz. Resting tension was maintained during the 90 min equilibration period and the preparations were rinsed with fresh bathing solution every 20 min. The bathing solution was bubbled with carbogene (95% O₂ plus 5% CO₂) and the temperature was set to 35°C.

ERP was measured using a standard extrastimulus technique. Extrastimuli (S₂) were interposed between normally paced stimuli (S₁) in such a way that the S₁–S₂ interval was initially below the refractory period of the tissue. The S₁–S₂ interval was then lengthened gradually by 1 ms increment after every 10th normal stimulus until postextrasystolic potentiation

(120% of the control twitch tension) was observed. ERP was defined as the smallest S₁–S₂ interval, which produced postextrasystolic potentiation.

The concentration- and frequency-dependent effects of dofetilide were studied at concentrations of 10, 30 and 100 nM. As the effect of dofetilide on ERP only slightly increased from 30 to 100 nM, the latter concentration was selected for further experiments. The concentration-dependent effects of β -adrenoceptor antagonists on the increase in twitch tension induced by isoproterenol (30 nM) were tested in separate experiments. The concentration of β -adrenoceptor antagonists selected for further studies inhibited the positive inotropic effect of isoproterenol by 84–92% in the atrial myocardium of guinea-pigs.

Experimental protocol

Control (baseline) ERP values were measured at 3, 2 and 1 Hz stimulation frequencies. The test compounds were applied and incubated for 30 min at 2 Hz. At the end of the incubation period, ERP values were measured at 3, 2 and 1 Hz stimulation rates. Measurement of ERP always commenced 3 min after changing stimulation frequency. When the effect of β -adrenoceptor blockade was examined, four groups of atrial tissues were studied (control, β -adrenoceptor antagonist, dofetilide and β -adrenoceptor antagonist + dofetilide). The effects of isoproterenol (10 nM) on the dofetilide-induced ERP prolongation were studied at three pacing rates. Isoproterenol was applied 3 min after changing stimulation frequency. ERP was measured close to the time of peak effect of isoproterenol (3 min) and then a 15-min interval was allowed for recovery. Catecholamine depletion was performed by pretreatment of the guinea-pigs with reserpine (5 mg kg⁻¹ i.p.) 24 h before the experiment. The compound was dissolved in 10% ascorbic acid solution and injected in a volume of 1 ml kg⁻¹ b.w.

Similar experimental techniques were applied when measuring ERP in the right ventricular papillary muscles of guinea-pigs or rabbits. The rabbit was also chosen for measuring the frequency-dependent effect of dofetilide on ERP in the ventricular tissue, since both I_{Kr} density and the ERP lengthening effect of I_{Kr} blockade were shown to be small in guinea-pig ventricular myocardium (Ohmoto-Sekine *et al.*, 1999). The right ventricle was opened and the papillary muscles were dissected and mounted in the experimental chamber. Following a 120-min period of incubation, the experiments were performed in the same way as described for the guinea-pig atrial myocardium.

Drugs

Reserpine, metoprolol and dofetilide were synthesised at the Division of Chemical Research of EGIS Pharmaceuticals Ltd (Budapest, Hungary). Propranolol and atenolol were purchased from Research Biochemicals International (Natick, MA, U.S.A.) and Sigma Chemical Co. (St Louis, MO, U.S.A.), respectively, and isoproterenol was obtained from Fluka Chemie AG. (Buchs, Switzerland).

Statistics

Data are presented as mean \pm s.e.m. values. Statistical analysis of the results involved Newman–Keuls *post hoc* test after

checking the homogeneity of variances with two-way ANOVA for repeated measures (Statistica Software Package, Statsoft, Tulsa, U.S.A.).

Results

All baseline ERP values obtained in the guinea-pig atrial myocardium in the control, propranolol-, dofetilide- and dofetilide+propranolol-treated groups were pooled for untreated and reserpinised animals. In untreated tissues, ERP was short and showed reverse frequency-dependence (Table 1). The frequency-dependent changes in ERP were small but statistically significant. In contrast to these untreated preparations, ERP values were not frequency-dependent in atrial tissues obtained from reserpine-treated animals. Thus, reverse frequency-dependence of the baseline ERP was eliminated by catecholamine depletion.

The effects of dofetilide on ERP showed reverse frequency-dependent properties (Figure 1a–c). Metoprolol, atenolol and propranolol did not influence ERP at any driving rate (even though ERP values were different between 1 and 3 Hz in the group treated with atenolol). Metoprolol (1 μ M) increased the dofetilide-induced prolongation of ERP at high (3 and 2 Hz), but not at low (1 Hz) stimulation frequency (Figure 1a), that is, the reverse frequency-dependent character of the dofetilide-induced ERP prolongation was attenuated by metoprolol. Similar results were obtained when other β -receptor blockers, like propranolol (300 nM, Figure 1b) or atenolol (3 μ M, Figure 1c), were applied. In these experiments, pretreatment with either propranolol or atenolol completely abolished the reverse frequency-dependent nature of the dofetilide-induced lengthening of ERP, while this effect was only partial with metoprolol.

The frequency-dependent effects of dofetilide, with or without pretreatment with propranolol, were also studied in guinea-pig atrial preparations obtained from animals, having their noradrenaline pools depleted by pretreatment with reserpine. In these reserpinised tissues, dofetilide (100 nM) caused 24.4, 27.9 and 22.7% increases in ERP at 3, 2 and 1 Hz, respectively, which were statistically not different from each other (Figure 2). Thus, the dofetilide-induced ERP prolongation was independent of stimulation frequency, and propranolol (300 nM) failed to alter the dofetilide-induced

Table 1 Effect of stimulation frequency on the effective refractory period in left atrial myocardium of untreated and reserpinised guinea-pigs

Treatment	ERP (ms)		
	3 Hz	2 Hz	1 Hz
Untreated (n = 39)	67.4 \pm 0.7	70.7 \pm 0.8***	72.0 \pm 1.0***
Reserpinised (n = 35)	73.2 \pm 1.5 ^{oo}	72.9 \pm 1.6 ^o	72.9 \pm 1.7

All ERP values, measured before drug treatment in the control-, propranolol-, dofetilide- and dofetilide+propranolol-treated groups, were combined for both untreated and reserpinised guinea-pigs. Values are means \pm s.e.m., *n* indicates the number of experiments. Data were evaluated using two-way ANOVA for repeated measures (pacing rate), followed by Newman–Keuls *post hoc* test (***) $P < 0.001$ vs 3 Hz in the same group, ^o $P < 0.05$, ^{oo} $P < 0.001$ vs untreated tissues at the same frequency).

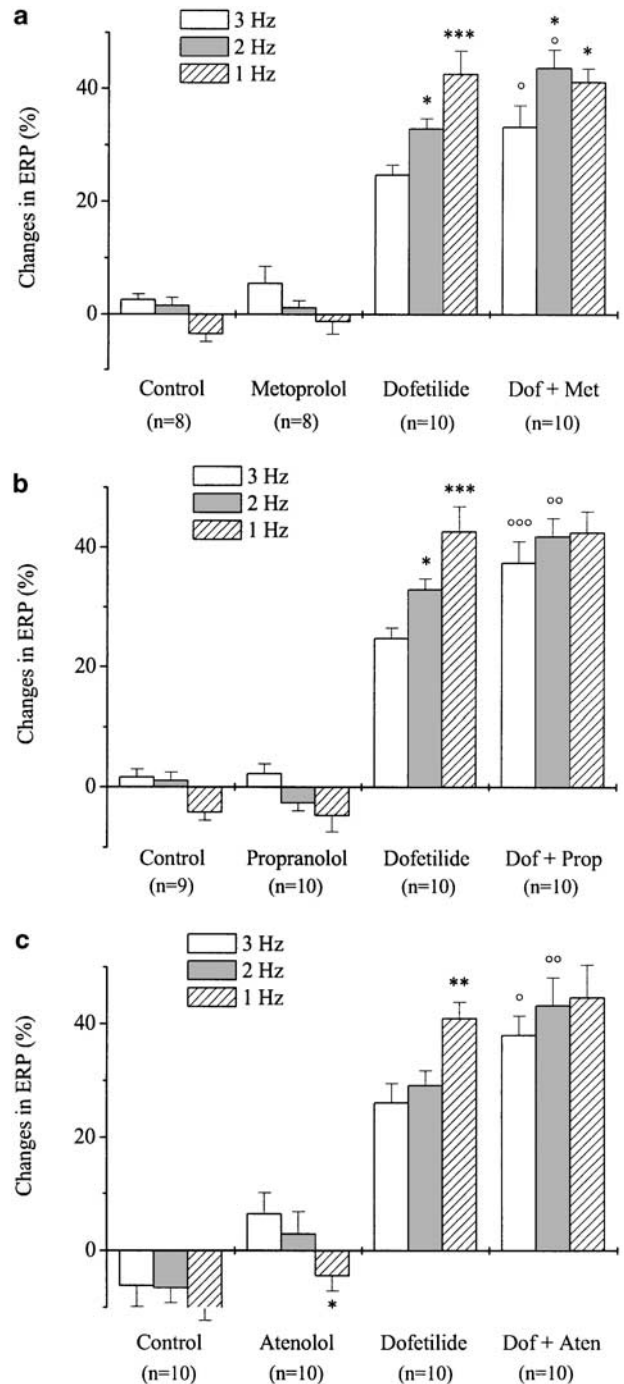


Figure 1 Frequency-dependent effects of dofetilide in the presence of metoprolol (a), propranolol (b) or atenolol (c) on the effective refractory period in guinea-pig left atrial myocardium. After measuring baseline values, separate groups of preparations were treated with either metoprolol (1 μ M), dofetilide (100 nM), dofetilide+metoprolol (Dof+Met) or were used as control in (a). In panels (b and c) propranolol (300 nM) or atenolol (3 μ M) was used, respectively. The incubation period lasted for 30 min. Values are means \pm s.e.m., *n* indicates the number of experiments. Data are expressed as percentage changes in comparison with the baseline values (* $P < 0.05$, *** $P < 0.001$ vs 3 Hz in the same group; ^o $P < 0.05$, ^{oo} $P < 0.01$, ^{ooo} $P < 0.001$ vs dofetilide at the same frequency).

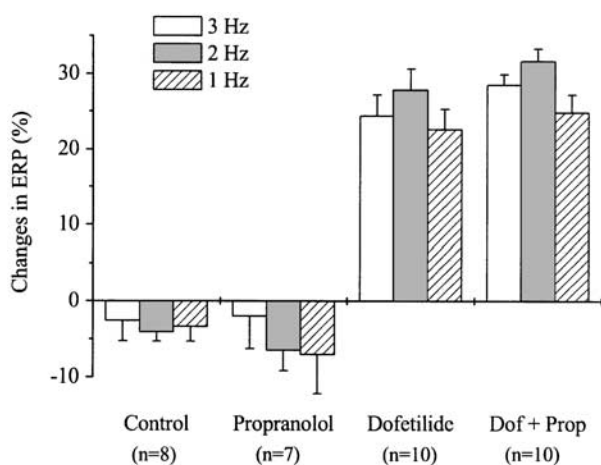


Figure 2 Frequency-dependent effects of dofetilide and propranolol on the effective refractory period in atria obtained from reserpinised guinea-pigs. Reserpine (5 mg kg^{-1} i.p.) was administered 24 h before the experiments. After measuring baseline values, separate groups of preparations were treated with propranolol (300 nM), dofetilide (100 nM), dofetilide + propranolol (Dof + Prop) or were used as control. Each drug was applied for 30 min. Values are means \pm s.e.m., n indicates the number of experiments. Data are expressed as percentage changes in comparison with the baseline values. No significant changes were observed between the dofetilide- and the dofetilide + propranolol-treated groups.

changes in ERP in reserpinised atria. The effects of dofetilide on ERP were directly compared in untreated and reserpinised tissues in Table 2. The ERP lengthening effect of dofetilide was not different in the two groups of preparations at 3 Hz, it was moderately reduced at 2 Hz in the reserpine-treated hearts, while at 1 Hz the prolongation of ERP was smaller in atria of reserpinised animals than in untreated ones.

Application of isoproterenol (10 nM), a full activator of β -adrenoceptors, eliminated the dofetilide-evoked prolongation of ERP at all pacing rates (Table 2), demonstrating that the effect of an I_{K_r} blocker on ERP is critically dependent on the intensity of β -adrenoceptor activation.

In order to study the β -adrenoceptor-mediated functional effects of rate-dependent catecholamine liberation in the atrial myocardium, the effects of propranolol on contractility in untreated and reserpinised tissues were also measured (Figure 3). In untreated preparations, propranolol induced a marked reduction in twitch tension at 3 Hz, it produced a moderate decrease at 2 Hz, but failed to alter contractility at 1 Hz pacing rate compared with values measured in the control group at the same frequencies (Figure 3a). On the other hand, in atria with depleted noradrenaline pools, propranolol did not change twitch tension at either stimulation frequency (Figure 3b). Compared with values measured in control tissues, dofetilide increased twitch tension at all three pacing rates in both untreated and reserpinised atria. Interestingly, this effect was not much affected by the presence of propranolol.

All experiments presented above were performed in the left atrial myocardium of the guinea-pig. For studying the role of β -adrenoceptor activation in the reverse rate dependency of ERP prolongation elicited by dofetilide in ventricular myocardium, the frequency-dependent effects of dofetilide on ERP were also evaluated in the right ventricular papillary muscles of guinea-pigs and rabbits. Dofetilide (100 nM) prolonged ERP in a

Table 2 Effects of catecholamine depletion and isoproterenol on the dofetilide-induced lengthening of ERP in guinea-pig left atrial tissues

Treatment	ERP prolongation (%)		
	3 Hz	2 Hz	1 Hz
Untreated ($n=10$)	24.7 ± 1.8	$32.9 \pm 1.8^*$	$42.6 \pm 4.2^{***}$
Reserpinised ($n=10$)	24.4 ± 2.8	27.9 ± 2.8	$22.7 \pm 2.6^{***}$
Isoproterenol ($n=10$)	$3.3 \pm 4.3^{***}$	$3.8 \pm 5.9^{***}$	$3.8 \pm 6.6^{***}$

Reserpine (5 mg kg^{-1} i.p.) was administered 24 h before the experiments. Isoproterenol (10 nM) was repeatedly applied with a 15-min interval between subsequent measurements in left atrial myocardium of untreated guinea-pigs. Values are means \pm s.e.m., n indicates the number of experiments. Data were evaluated with two-way ANOVA for repeated measures (pacing rate), followed by Newman-Keuls *post hoc* test ($*P < 0.05$, $***P < 0.001$ vs 3 Hz in the same group, $^{***}P < 0.001$ vs untreated tissues at the same frequency).

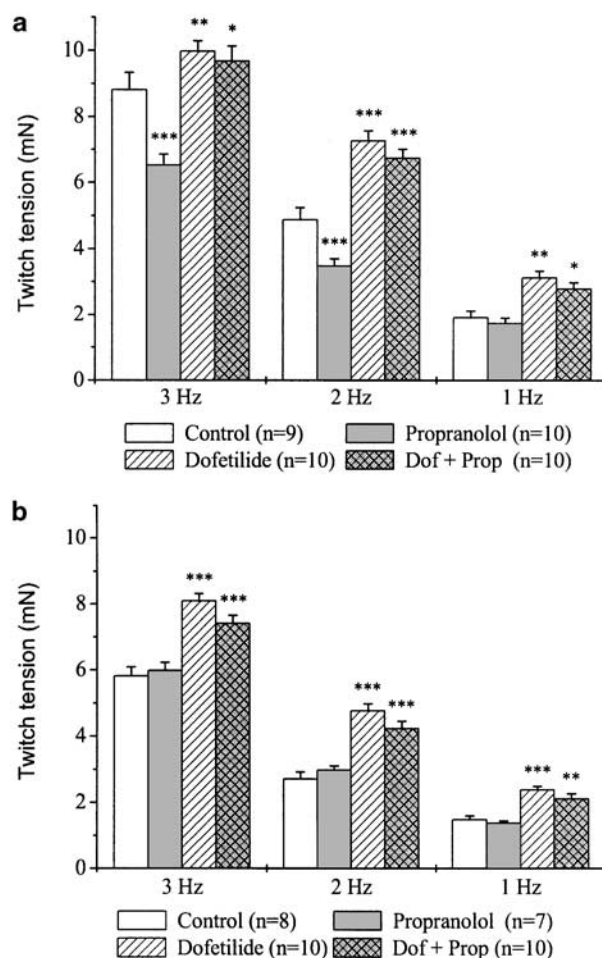


Figure 3 Frequency-dependent effects of dofetilide and propranolol on twitch tension in left atria obtained from untreated (a) and reserpinised (b) guinea-pigs. Reserpine (5 mg kg^{-1} i.p.) was administered 24 h before the experiments. After measuring baseline values, separate groups of preparations were treated with propranolol (300 nM), dofetilide (100 nM), dofetilide + propranolol (Dof + Prop) or were used as control. Each incubation period lasted for 30 min. Values are means \pm s.e.m., n indicates the number of experiments. ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs control at the same frequency).

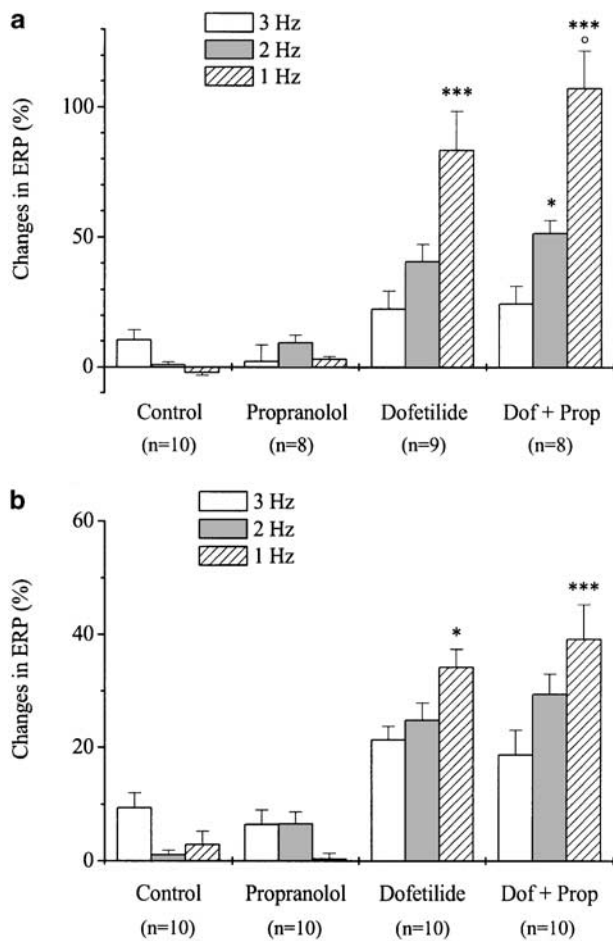


Figure 4 Frequency-dependent effects of dofetilide and propranolol on the effective refractory period in the right ventricular papillary muscles of rabbits (a) and guinea-pigs (b). After measuring baseline values, separate groups of preparations were treated with propranolol (300 nM), dofetilide (100 nM), dofetilide + propranolol (Dof + Prop) or were used as control. Each incubation period lasted for 30 min. Values are means \pm s.e.m., *n* indicates the number of experiments. Data are expressed as percentage changes in comparison with the baseline values (* $P < 0.05$, *** $P < 0.001$ vs 3 Hz in the same group. ° $P < 0.05$ vs dofetilide at the same frequency).

reverse rate-dependent manner in papillary muscle preparations obtained from both rabbits and guinea-pigs; however, its effect on ERP was much larger in the former than in the latter species (Figure 4a–b). The frequency-dependence of the dofetilide-induced ERP response was not influenced by propranolol in either species. In contrast to results obtained in guinea-pig atrial myocardium, propranolol increased the effect of dofetilide on ERP at 1 Hz but not at other stimulation rates in rabbit papillary muscles, while it did not change the effect of dofetilide in guinea-pig papillary muscle preparations.

The level of the rate-dependent β -adrenoceptor activation was smaller in ventricular myocardium than in atrial tissues, as propranolol failed to decrease twitch tension in papillary muscles of either species at any stimulation rate (Figure 5a–b). Dofetilide and dofetilide + propranolol augmented twitch tension in the rabbit ventricle, at 1 Hz only. The inotropic effect of dofetilide was much larger in the ventricular myocardium of rabbits than in guinea-pigs, similarly to its effects on ERP between the two species.

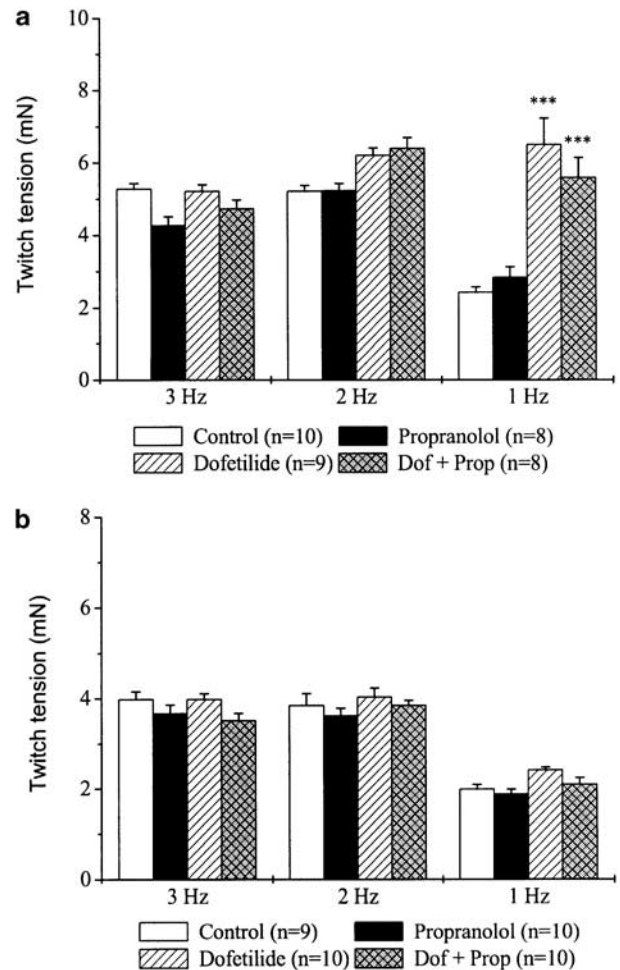


Figure 5 Frequency-dependent effects of dofetilide and propranolol on twitch tension in right ventricular papillary muscles of rabbits (a) and guinea-pigs (b). After measuring baseline values, separate groups of tissues were treated with propranolol (300 nM), dofetilide (100 nM), dofetilide + propranolol (Dof + Prop) or were used as control. Each incubation period lasted for 30 min. Values are means \pm s.e.m., *n* indicates the number of experiments. Asterisks (***) denote significant differences vs control at the same frequency.

Discussion

In this study, dofetilide, one of the most selective I_{K_r} blockers known, was chosen to examine a feasible mechanism of the phenomenon called reverse frequency-dependence. Dofetilide evoked a reverse frequency-dependent prolongation of ERP in the guinea-pig left atrial myocardium. These results are in accordance with earlier results obtained in atrial and ventricular preparations of various species (Martin *et al.*, 1996; Lee *et al.*, 1998; Marschang *et al.*, 1998; Li *et al.*, 2001). The dofetilide-induced lengthening of ERP was augmented by β -adrenoceptor blockade (metoprolol, atenolol and propranolol) in case of high-frequency stimulation, whereas application of β -receptor blockers alone failed to modify ERP substantially. The modulatory effects of β -adrenoceptor blockers on the dofetilide-induced lengthening of ERP were not observed in catecholamine-depleted animals.

The most important finding in this study was that full activation of the β -adrenergic pathway (by isoproterenol)

and its suppression (by β -adrenoceptor blockers or reserpine pretreatment) abolished the dofetilide-induced ERP prolongation or eliminated its reverse rate-dependent character in the guinea-pig atrial myocardium. Therefore, our observations suggest that the rate-dependent release of endogenous catecholamines and the concomitant β -adrenoceptor activation play an important role in the development of reverse rate-dependency of the dofetilide-induced prolongation of ERP in this preparation. In contrast, the modulatory action of β -adrenoceptor stimulation was not observed in the ventricular myocardium of rabbits and guinea-pigs.

It is obvious and well documented that noradrenaline release increased proportionally with the rate of electrical stimulation in various cardiac preparations (Junstad & Wennmalm, 1973; Johnston & Majewski, 1986; Foucart & Majewski, 1989; Lamontagne *et al.*, 1990; Murphy *et al.*, 1992; Molderings *et al.*, 2000). Under the conditions of the present experiments, a functional consequence of rate-dependent noradrenaline liberation was that propranolol decreased twitch tension at 3 and 2 Hz but not at 1 Hz in atrial myocardium of untreated animals. As it was expected, propranolol did not alter contractility in catecholamine-depleted preparations. These observations clearly show that the level of β -adrenoceptor activation was high enough to increase contractility and to reduce the dofetilide-induced ERP lengthening at high rates of electrical stimulation in atrial myocardium. On the other hand, propranolol did not change contractility in the papillary muscles of either species indicating that the level of noradrenaline release was insufficient to induce detectable functional changes up to 3 Hz in ventricular myocardium. Thus, in various parts of the heart reverse rate-dependency of APD and ERP prolongation by I_{Kr} blockers may be the consequence of diverse mechanisms. The reason for this inconsistency is unclear at present; however, it can likely be attributed to differences in the composition, kinetics and regulation of ionic currents governing repolarisation in atrial and ventricular myocardium (Hume & Uehara, 1985; Hafner *et al.*, 1988; Baskin & Lynch, 1998).

β -Adrenoceptor stimulation may enhance a great variety of ion currents and pumps in cardiac cells, including the inward sodium current (Matsuda *et al.*, 1992), the delayed rectifier potassium current (Hoffmann & Singer, 1967), the Na–K pump current (Boyett & Fedida, 1984), the transient outward potassium current (Nakayama & Fozzard, 1988), the chloride current (Harvey & Hume, 1989), the slow calcium current (Reuter, 1983) and the pacemaker current (Di Francesco, 1985). It has been clearly demonstrated that I_{Kr} current was also increased by β -adrenoceptor stimulation in guinea-pig ventricular myocytes, an effect mediated both by protein kinase A and protein kinase C and involved calcium entry into the cells (Heath & Terrar, 2000). I_{Kr} current was less sensitive to blockade by E-4031, a pure I_{Kr} blocker, in myocytes treated with isoproterenol than in control myocytes. It was also demonstrated that I_{Kr} currents in both the humans and guinea-pigs were suppressed (having their activation curve shifted towards positive voltages) by a cAMP- and protein kinase A-dependent pathway that was due to phosphorylation of the channel protein (Kiehn *et al.*, 1998; Thomas *et al.*, 1999). The reduced I_{Kr} current and decreased sensitivity of I_{Kr} current to blockade by drugs after increasing intracellular cAMP levels, demonstrated in these

studies, are compatible with our findings. Since all previous results on this issue were obtained in ventricular myocytes and the dofetilide-induced reverse frequency-dependent ERP prolongation was diminished by β -adrenoceptor blockade only in atrial but not ventricular myocardium in our study, to explain the precise mechanisms by which β -adrenoceptor activation attenuated the effects of dofetilide on ERP at high pacing rate in the atrium remains to be elucidated.

Dofetilide induced smaller changes in ERP at all stimulation rates in reserpinised preparations than in untreated ones exposed or not to β -adrenoceptor blockers. The effects of noradrenaline on myocardial α -adrenoceptors may be an important factor in these seemingly contradictory findings. Activation of α -adrenoceptors prolonged APD in rabbit and human atrium by inhibiting I_{to} (Fedida *et al.*, 1989; 2000), reducing I_{to} and I_{K1} in canine ventricular myocytes (Wang *et al.*, 2001), decreasing I_{Kur} , in canine and human atrial myocardium (Li *et al.*, 1996; Yue *et al.*, 1999) and also by suppressing a repolarising chloride current (Oleksa *et al.*, 1996; Hool & Harvey, 1997). When any repolarising current is reduced, the relative contribution of other currents to repolarisation may increase (Biliczki *et al.*, 2002). Therefore, the effect of dofetilide on ERP can be more pronounced after β -adrenoceptor blockade than after catecholamine depletion. However, it is not known which currents were involved in such an effect in the guinea-pig atrial myocardium in our study. The release of acetylcholine from the cholinergic nerve terminals can be inhibited by noradrenaline *via* activation of prejunctional α_2 -adrenoceptors in guinea-pig atria (Loiacono *et al.*, 1986). Acetylcholine can reduce the effects of selective I_{Kr} blockers on APD and ERP, and this action is more pronounced in noradrenaline-depleted preparations than in control ones (Zaza *et al.*, 1995). In addition, α_1 -adrenoceptor activation is known to desensitise acetylcholine-activated potassium currents ($I_{K,Ach}$) that cannot occur in preparations obtained from reserpine-pretreated guinea-pigs (Cho *et al.*, 2001). Another important factor involved could be cardiac remodelling due to exposition of the heart to high catecholamine concentrations after reserpine treatment (Ramirez *et al.*, 2000; Wichter *et al.*, 2000). The combination of some or all of the above effects may have brought about adjustments in several ion currents that resulted in a diminished lengthening of ERP by dofetilide in catecholamine-depleted atrial myocardium of the guinea-pig.

ERP is a more reliable parameter for the assessment of therapeutic utility of antiarrhythmic agents than APD, because it reflects functional alterations of electrical excitability of the myocardium. Although ERP and APD can change disproportionately (Pankucsi *et al.*, 1997; Watanabe *et al.*, 2001), changes in ERP and APD are strictly proportional for atrial and ventricular myocardium in the case of selective I_{Kr} blockers (Yang *et al.*, 1992a, b; Ishii *et al.*, 1995; Marschang *et al.*, 1998). Our results were based on measurements of ERP detected as changes in myocardial contractility, which is a proper method that has been used previously by several investigators (Gwilt *et al.*, 1991; Lynch *et al.*, 1995; Baskin & Lynch, 1998; Kovács *et al.*, 2001; Li *et al.*, 2001; 2002). Baseline ERP values were within the range obtained in electrophysiological measurements in the guinea-pig atrial myocardium (Ishii *et al.*, 1995; Raatikainen *et al.*, 2000; Li *et al.*, 2001).

We have shown that in the presence of propranolol and atenolol or after catecholamine depletion, the dofetilide-evoked ERP prolongation turned to be frequency independent in guinea-pig left atrial preparations. Our results are in agreement with the findings of some previous studies in which the authors did not aim to reveal the role of β -adrenoceptors in the development of reverse frequency-dependent effects of I_{Kr} blockers. In anaesthetised and vagotomised newborn dogs pretreated with propranolol, both dofetilide and ibutilide evoked reverse rate-dependent monophasic APD prolongation in the ventricle, but their effect was independent of the stimulation rate in the atrium (Pickoff & Stolfi, 2001). Ishii *et al.* (1995) found that in isolated left atrial preparations obtained from reserpinised guinea-pigs, the effect of sematilide on APD₉₀ was independent of the pacing frequency. Nifecantal, a pure I_{Kr} blocker, prolonged atrial monophasic APD and ERP in a reverse rate-dependent manner, whereas absolute changes in ERP did not depend on the pacing rate after treatment with D,L-sotalol in a human electrophysiological study *in vivo* (Watanabe *et al.*, 2001). These results together strongly suggest that the interaction between

β -adrenoceptor stimulation and I_{Kr} blockade is likely a general regulatory mechanism in the mammalian atrium, and it is not a specific feature of the guinea-pig. However, according to our knowledge, we were the first to demonstrate that both β -adrenoceptor antagonists and catecholamine depletion attenuated the reverse frequency-dependent character of the dofetilide-evoked ERP prolongation in a mammalian atrial tissue.

In summary, β -adrenoceptor inhibition or catecholamine depletion attenuated or fully eliminated the reverse frequency-dependency of the dofetilide-induced prolongation of the effective refractory period in the left atrial myocardium of guinea-pigs. These results suggest that rate-dependent noradrenaline release and ensuing β -adrenoceptor activation can explain, at least partly, the development of the reverse frequency-dependent nature of the effects of I_{Kr} blockers in the mammalian atrium. However, no such mechanism was detected in the ventricular tissue of guinea-pigs and rabbits.

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