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"Use-dependent" effects of cisapride on postrest action potentials in rabbit ventricular myocardium

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Abstract

Repercussions of cisapride-induced blocking effects on repolarisation of K^+ channels in open and inactivated states investigated in rabbit ventricular myocardium during rest and under stimulation were compared with effects of K^+-blocking drugs (4-aminopyridine, dofetilide, terikalant). Major lengthening in the first postrest action potential indicates affinity for closed channels. Gradual lengthening during stimulation implies affinity for open channels. Four (control, add-in, steady-state, washout) 20-min rest periods were alternated with regular stimulation (0.5 Hz). Each drug was added during add-in and steady-state periods. Similarly to dofetilide (10 nM) and terikalant (0.3 μM), cisapride (1 μM) increasingly lengthened action potentials during stimulation, whereas 4-aminopyridine (1 mM) prolonged mostly the first postrest action potential. Our results indicate that cisapride induced use-dependent lengthening of repolarisation, compatible with an affinity for open K^+ channels. We also found that in isolated rabbit ventricular myocytes, cisapride (1–10 μM) decreased the inward rectifier K^+ current, an effect contributing to the proarrhythmic potential.

Keywords: Cisapride; Action potential duration; Inward rectifier current; Ventricular myocardium, rabbit; Myocyte; K^+ channel blocker

1. Introduction

The gastrointestinal prokinetic agent, cisapride (Propulsid®), is a benzamide derivative that is widely prescribed for the treatment of dyspepsia, gastroparesis and gastrooesophageal reflux diseases (Wiseman and Faulds, 1994). In recent years, a number of reports have appeared associating cisapride use with QT interval prolongation in the electrocardiogram and development of polymorphic ventricular tachycardia, the so-called torsades de pointes (Ahmad and Wolfe, 1995; Bran et al., 1995; Lewin et al., 1996; Wysowski and Bacsanyi, 1996). Our laboratory was the first to demonstrate experimentally that cisapride induces prolongation of the action potential duration in isolated rabbit Purkinje fibres and causes the development of early afterdepolarisations (Puisieux et al., 1996) in a range of clinically relevant concentrations (0.1–3 μM). The maximal mean plasma concentration of unchanged cisapride averaged 0.15–0.3 μM when the clinically effective doses were given to healthy volunteers or to patients with gastrointestinal discomfort (Wiseman and Faulds, 1994), but it can reach 10- or 20-fold higher values in patients after overdosing or inhibition of its metabolism by other drugs (Ahmad and Wolfe, 1995; Wysowski and Bacsanyi, 1996). More recently, cisapride has been found to block concentration dependently the rapid component of the delayed rectifier K^+ current (I_{Kr}) measured in single ventricular myocytes from rabbit (Carlsson et al., 1997) or from guinea-pig (Drolet et al., 1998). Furthermore, cisapride was shown to display specific, high affinity block of the human *ether-a-gogo*-related gene (HERG) K^+ channel that expresses the delayed rectifier current, I_{Kr}, in human atria and ventricle (Mohammad et al., 1997; Rampe et al., 1997). The potency of cisapride effects on the HERG channel was comparable to that observed for the class III antiarrhythmic agent dofetilide. As with dofetilide, HERG inhibition by cisapride required channel activation, which implies a predominant affinity for open and inactivated states of the HERG channel (Walker et al., 1999). However, our previous results showed that cisapride caused a reverse rate-dependent prolongation of action potential du-

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ration (Puisieux et al., 1996). Whether cisapride affinity for open and inactivated HERG channels may result in use-dependent lengthening of action potential duration remains unclear.

The voltage-dependent transient outward I_{to} and delayed rectifier I_K K^+ channels open during depolarisation and close at diastolic potential. If a drug binds preferentially to K^+ channels in the closed state, the subsequent block of the channel may be expected to be reverse use-dependent, leading to more potent prolonging effects on action potential duration at low than at high stimulation rates (Hondeghem and Snyders, 1990). After prolonged quiescence, the full prolonging effect on action potential duration will be obtained with the first action potential elicited when regular stimulation is resumed (Ohler and Ravens, 1994; Ohler et al., 1994). In contrast, if drug binding occurs on the K^+ channel in the open state, the prolonging effect on action potential duration will be expected to develop increasingly during repetitive stimulation, as was demonstrated for dofetilide on I_{Kr}, the rapid component of I_K (Carmeliet, 1992; Ohler et al., 1994).

Whether or not a use-dependent block of K^+ channels could influence a drug-induced prolonging effect and its subsequent proarrhythmic potential remains unclear. Thus, aiming to improve our understanding about the repercussions of cisapride interaction with K^+ channels on action potential duration, we used a standard microelectrode to study its time-course effects on action potential duration after rest and during regular stimulation in rabbit ventricular muscle. Cisapride effects were compared with those of 4-aminopyridine which blocks predominantly the Ca^{2+}-independent component of I_{to} (Campbell et al., 1993) and to a lesser extent I_{Kr} (Mitcheson and Hancox, 1999), dofetilide, a selective blocker of I_{Kr} (Carmeliet, 1992) and terikalant, which has been demonstrated to block I_{Kr} (Jurkiewicz et al., 1996), but also the inward rectifier K^+ current, I_{K1} (Escande et al., 1992). In addition, we used the patch-clamp technique to evaluate the effects of cisapride on I_{K1} in rabbit ventricular myocytes.

2. Materials and methods

2.1. Action potentials in multicellular preparations

Experiments were performed with tissues dissected from male New Zealand white rabbits (1.5–2.0 kg) obtained from Charles River (Le Plessis-Robinson, France) in accordance with the official recommendations of the European Community guidelines. Rabbits were killed by cervical dislocation and exsanguinated. The thorax was opened and the heart was removed rapidly and placed in a Tyrode's solution of the following composition (mM): NaCl 108.2; KCl 4; $CaCl_2$ 1.8; $MgCl_2$ 1; NaH_2PO_4 1.8; $NaHCO_3$ 25; glucose 11; pH 7.35 ± 0.05, gassed with carbogen (95% O_2, 5% CO_2). Ventricular strips (about 3 mm long, 2 mm wide and 0.5 mm thick) were dissected out from the left ventricular free wall and mounted, endocardium upward, in an organ bath maintained at 36.5 ± 0.5°C. They were superfused at a constant rate (2.5 ml min^{-1}) with the Tyrode's solution and paced at 1 Hz using a UHS 20 Biotronik stimulator which delivered square wave pulses of 1-ms duration and 1.5 times threshold voltage through a bipolar Teflon-insulated (except at the tip) stainless-steel electrode. Preparations were allowed to equilibrate for 2 h at 1 Hz, then the stimulation frequency was reduced from 1 to 0.5 Hz.

Transmembrane action potentials were recorded using conventional glass microelectrodes filled with 3 M KCl and with a tip resistance of 10–20 MΩ, coupled with an Ag–AgCl bath electrode and connected to an impedance amplifier (VF 102 Bio-Logic). Action potentials were viewed on an oscilloscope (Gould DSO 1602), analysed by an external computer system (Datapac, Bio-Logic) and stored on a magnetic digital tape recorder (DTR 1202, Bio-Logic) which allowed the display on paper recordings (Gould TA 240) of the action potential profiles and electrical abnormalities (e.g. early afterdepolarisations). The following parameters of action potential were measured: resting membrane potential, action potential amplitude, maximal rate of depolarisation (V_{max}) and action potential duration at 30% and 90% repolarisation.

Under control conditions (so-called baseline action potentials), the action potentials recorded from the rabbit left ventricular free wall differed as to plateau height, leading us to divide the action potentials into two groups, the "low-plateau" and the "high-plateau" action potentials. The action potential duration at 30% repolarisation was shorter than 100 ms in the low-plateau action potentials and longer in the high-plateau action potentials, and/or the ratio of action potential duration at 30% repolarisation to action potential duration at 90% repolarisation was less than 60% in the low-plateau and higher than 60% in the high-plateau action potential group.

A diagram of the experimental protocol is given in Fig. 1: after 30 min of stimulation at 0.5 Hz, a baseline action potential was recorded, then four rest periods of 20 min were alternated with intercalated in 0.5-Hz regular stimulation. The first rest period was followed by 10 min of stimulation, this giving the predrug (so-called "control") postrest adaptation of action potential duration after quiescence. Stimulation was then interrupted for the second rest period concomitantly with the start of exposure ("add-in") to the drug tested. Stimulation was resumed for 40 min during drug exposure, this constituting the add-in postrest period. A third period of rest ("steady state") was introduced, the drug being kept in the superfusate, and was followed by 10 min of regular stimulation at 0.5 Hz; this was named steady-state postrest period. Finally the drug was withdrawn from the superfusate ("washout") immediately at the start of the fourth rest period, followed by 10 min of stimulation, this being the washout postrest period.

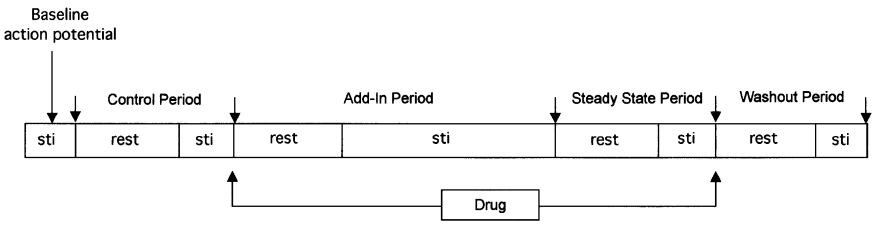

Fig. 1. Diagram of the protocol used for rabbit ventricular myocardium. Four 20-min rest periods were alternated with regular stimulation (sti) at 0.5 Hz. Each period (rest + stimulation) lasted 30 min except the add-in period, which was 60 min. The baseline action potential was recorded before the control rest period. In each experiment, drug-induced effects on the 300 action potentials recorded during the 10-min stimulation periods were time-matched with control values.

The time course of changes in action potential duration after stimulation was resumed was evaluated by analysing the 15 first action potentials that followed each postrest period, then the action potentials were recorded at regular time intervals and identified by their running number. Thus, the values for action potential duration measured during the add-in, the steady-state and the washout postrest periods could be time-matched with the control one. The concentrations were chosen on the basis of the dose–response curves for action potentials recorded from rabbit Purkinje fibers (Puisieux et al., 1996; Dumotier et al., 1999).

2.2. Voltage clamp studies on isolated rabbit ventricular myocytes

Single ventricular myocytes were dissociated enzymatically according to the method described by Mitra and Morad (1985). All solutions used during the cell isolation procedure were oxygenated and kept at 37°C. Briefly, rabbit heart was rapidly excised and cannulated on a Langendorff perfusion apparatus. The heart was first perfused retrogradely through the aorta with K^+-enriched Tyrode's solution (containing in mM: NaCl 135; KCl 27; $CaCl_2$ 1.8; $MgSO_4$ 1; NaH_2PO_4 0.33; glucose 20; taurine 20; HEPES 10; pH 7.15) then with a Ca^{2+}-free Tyrode's solution for 10 min. An enzymatic Ca^{2+}-free Tyrode's solution containing 0.7 mg/ml collagenase B (Boehringer Mannheim, Mannheim, Germany) and 0.045 mg/ml protease XIV (Sigma, St. Quentin Fallavier, France) was perfused for 17–20 min. Then the heart was rinsed with Kraftbrühe (KB) solution (in mM: KCl 85; K_2HPO_4 30; $MgSO_4$ 5, Na_2ATP 5; taurine 20, glucose 20, ethylene glycol-bis(β aminethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) 0.2; phosphocreatine 5; pyruvic acid 5, HEPES 10, pH 7.30). The ventricles were then chopped finely. After gentle agitation, the resulting cell suspension was filtered and the cells allowed to settle for 10 min at 37°C. The supernatant was discarded and the cells were resuspended in 1 mM Ca^{2+}-containing Tyrode's solution. The cell preparation was stored at room temperature and used between 2 and 8 h after isolation.

The whole-cell configuration of the patch-clamp technique was used to record the inward rectifier K^+ currents, I_{K1} (Hamill et al., 1981). Currents were recorded using a RK 400 amplifier (Bio-Logic) and pClamp software (Axon Instruments). The standard voltage clamp protocol was a series of 240-ms voltage steps from −140 to 0 mV (10-mV increments) from a holding potential of −40 mV, delivered every 5 s. Currents were sampled at 6.65 kHz using a 12-bit analog-to-digital converter (Labmaster TL-1, Scientific Solutions), low-pass filtered at 3 kHz. Borosilicate glass pipettes (Clark Electromedical Instruments) had a resistance of 1.5–1.7 MΩ when filled with the pipette solution (in mM: KCl 130; $MgCl_2$ 2; K_2-ATP 3; EGTA 10; phosphocreatine 5; HEPES 10; pH 7.3). The superfusion solution had the following composition (in mM): NaCl 135; KCl 4; $MgCl_2$ 1; $CaCl_2$ 1.8; NaH_2PO_4 0.8; glucose 10; HEPES 10; nifedipine 0.005; pH 7.4). Control and drug-containing solutions were applied to the exterior of the cell by placing the cell at the opening of 300 μm inner diameter catheters fixed on the rotating head of a RSC 200 (Rapid solution changer, Bio-Logic). Cell capacitance was measured by integrating the area of the capacitive transient elicited by 5-mV hyperpolarising steps. Currents were normalised to the membrane capacity to allow for differences in cell size. All experiments were done at room temperature (20–22°C).

2.3. Drugs / reagents

Stock solutions (10 mM) of cisapride, 4-aminopyridine, dofetilide or terikalant were prepared daily and later diluted in Tyrode's solution to the desired concentration. Cisapride, dofetilide and terikalant were dissolved in dimethylsulfoxide (DMSO) and 4-aminopyridine in Tyrode's solution. In three time-matched experiments, it was verified that the highest concentration of DMSO used in the Tyrode's solution (0.1 per 1000 expressed as v/v) was without any effect on action potential parameters. Cisapride was kindly supplied by Hoechst Marion Roussel (Romainville, France), dofetilide was a gift from Pfizer (Orsay, France) and terikalant was donated by Rhone Poulenc Rorer (Vitry-sur-Seine, France); 4-aminopyridine and other reagents were purchased from Sigma.

2.4. Statistics

Where appropriate, data are expressed as means ± S.E.M. for n experiments. In studies on multicellular preparations, significance tests were performed using Student's t-test for paired or unpaired observations. In whole-cell patch-clamp experiments, the differences in values for the four conditions (control, 1, 3 and 10 μM cisapride) were compared by analysis of variance (ANOVA) for repeated measures. Differences between means were taken as statistically significant at $P < 0.05$.

3. Results

3.1. Drug-induced changes in action potential duration after prolonged quiescence

As illustrated in Fig. 2, the baseline action potentials showed a prominent spike and flattened plateau in the low-plateau action potentials ($n = 24$) and a rather small spike and well-developed dome in the high-plateau action potentials ($n = 30$). Table 1 indicates that action potential duration was significantly shorter for low-plateau than for high-plateau action potentials at any level of repolarisation, but low-plateau action potentials showed significantly less negative resting membrane potential and smaller amplitude than high-plateau action potentials. The maximal rate of

Table 1
Action potential parameters in low-plateau and in high-plateau action potentials recorded in rabbit ventricular muscle stimulated at 0.5 Hz

	Low-plateau ($n = 24$)	High-plateau ($n = 30$)
RMP (mV)	-80.4 ± 0.4[a]	-81.6 ± 0.6
APA (mV)	118.9 ± 0.8[a]	121.1 ± 0.7
V_{max} (V/s)	172.1 ± 8.6	183.6 ± 7.8
APD_{30} (ms)	90.4 ± 4.0[a]	126.0 ± 4.3
APD_{90} (ms)	174.8 ± 4.8[a]	201.2 ± 4.8

RMP = Resting Membrane Potential; APA = Action Potential Amplitude; V_{max} = Maximal rate of phase 0 depolarisation; APD_{30}, APD_{90}: Action Potential Duration at 30% and 90% repolarisation, respectively.

[a] $P < 0.05$ as compared with high-plateau action potentials using Student's unpaired t-test.

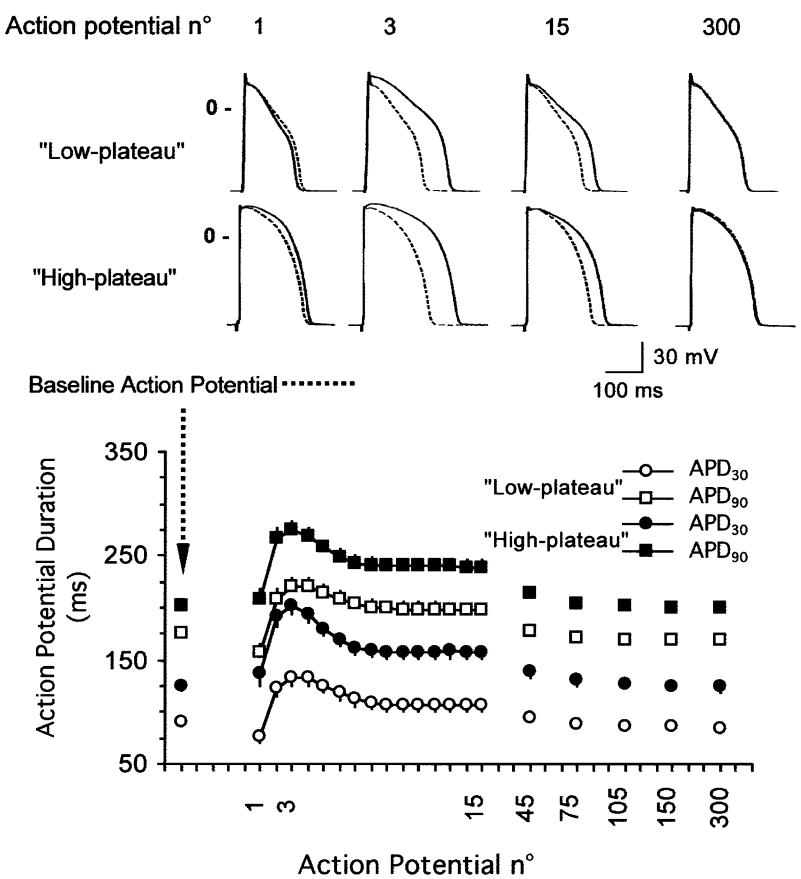

Fig. 2. Time course of changes in action potential duration during the postrest control period, in rabbit ventricular muscle stimulated at 0.5 Hz. Upper panels show typical examples of traces recorded from low-plateau and high-plateau action potentials numbered from 1 to 300. Baseline action potentials are indicated as dashed line in traces and with an arrow in the graph. Note the shape of the initial repolarisation in the low-plateau action potentials that showed prominent spike and flattened plateau. In this group ($n = 24$), the first postrest action potential duration was shorter than the baseline one, whereas it was longer in the high-plateau group ($n = 30$). The third postrest action potential was the longest in both groups. Return to baseline values was between the 150th and 300th action potential. APD_{30} and APD_{90}: action potential duration at 30% and 90% repolarisation, respectively.

rise of phase 0 depolarisation (V_{max}) did not differ between the two groups. The time course of postrest changes in action potential duration at 30% and 90% repolarisation after 20 min of quiescence under drug-free (control) conditions is shown in Fig. 2. Compared with the baseline action potentials recorded in each group, the first postrest action potential was slightly and not significantly longer in the high-plateau group, but was significantly shorter in the low-plateau group. Similarly, in both groups, the second and the third postrest action potentials had increasingly prolonged action potentials, which returned to their control values within a few min.

Fig. 3 compares the effects of 1 μM cisapride, 1 mM 4-aminopyridine, 10 nM dofetilide and 0.3 μM terikalant on the changes in action potential duration in the 15 first postrest action potentials of the low- and high-plateau groups during the add-in period and in the subsequent numbered action potentials. Thus, the time course of drug effects during 40 min of stimulation is expressed as a function of successive depolarisations. The action potential changes obtained with cisapride showed a time course quite similar to that observed with dofetilide and terikalant, but noticeably different from 4-aminopyridine effects.

In the presence of cisapride, dofetilide and terikalant, the first postrest action potential was quite similar to the first postrest control action potential. This is clear from Fig. 4, which magnifies the changes (expressed as percentages of the time-matched control ones) in action potential duration at 90% repolarisation of the six first postrest action potentials. A prolonging effect developed progressively with successive depolarisations and became significant with cisapride and terikalant on the 15th action potential (Table 2) as compared with the 15th (time-matched) control action potential, whereas, with dofetilide, significance was only reached on the 150th action potential (data not shown in Table 2). Table 2 indicates that the prolonging effect with these three drugs was well developed and highly significant on the 300th action potential, i.e., after 10 min of stimulation. The intensity of their prolonging effect continued to increase through the 40 min of stimulation, reaching (vs. baseline values) with cisapride $+32.5 \pm 4.6\%$ and $+27.8 \pm 5.3\%$ in low- and in high-plateau action potentials, respectively, with dofetilide $+40.4 \pm 7.5\%$ and $+32.5 \pm 5.2\%$ in low- and in high-plateau action potentials, respectively, and with terikalant $+80.1 \pm 10.7\%$ and $+45.4 \pm 7.8\%$ in low- and in high-plateau action potentials, respectively. Thus, drug effects were somewhat more marked in the low-plateau and the high-plateau groups.

In contrast, the prolongation induced by 4-aminopyridine during the add-in period showed the highest intensity on the first postrest action potential (Fig. 4) then decreased

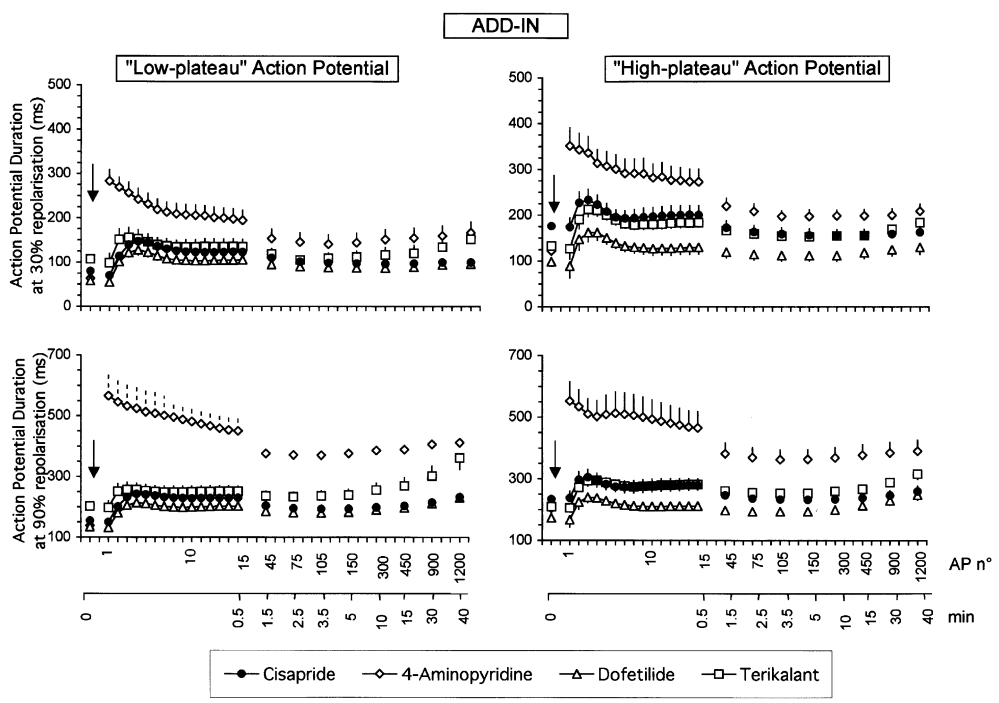

Fig. 3. Time course of changes in action potential duration during the postrest add-in period, in rabbit ventricular muscle stimulated at 0.5 Hz in the presence of 1 μM cisapride ($n = 6$ in low-plateau group and $n = 8$ in high-plateau group), 1 mM 4-aminopyridine ($n = 6$ in low-plateau group and $n = 7$ in high-plateau group), 10 nM dofetilide ($n = 6$ in low-plateau group and $n = 6$ in high-plateau group) and 0.3 μM terikalant ($n = 6$ in low-plateau group and $n = 9$ in high-plateau group). Arrows show baseline action potentials. Dashed bars for S.E.M. in the low-plateau group indicate the occurrence of early afterdepolarisations that resulted in large S.E.M. for action potential duration at 90% repolarisation. Note the major lengthening of the first postrest action potential in the presence of 4-aminopyridine, whereas with other drugs, the prolonging effect developed gradually during repetitive stimulation. AP: action potential.

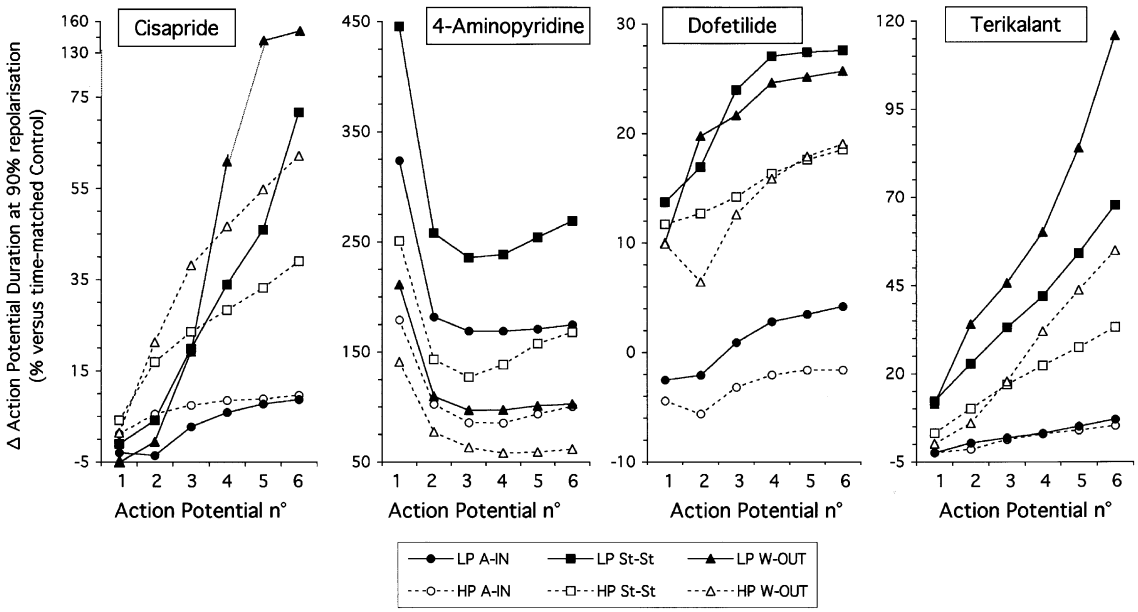

Fig. 4. Time course of changes in action potential duration at 90% repolarisation of the six first postrest action potentials recorded in low-plateau (LP) and high-plateau (HP) groups in the presence of 1 μM cisapride, 1 mM 4-aminopyridine, 10 nM dofetilide and 0.3 μM terikalant during add-in (A-IN), steady-state (St-St) and washout (W-OUT) periods. Drug effects are expressed as percentages of time-matched postrest control values. Note that in the presence of cisapride and terikalant, the first postrest action potential of the steady-state and washout periods was quite similar to the first add-in postrest action potential, this indicating recovery from drug effects during rest. With dofetilide, the first postrest steady-state and washout action potentials were longer than the first postrest add-in one, suggesting that during rest, the preparation did not fully recover from drug-induced block of K^+ current. With 4-aminopyridine, the first postrest steady-state action potential was longer and the first postrest washout one was shorter than the first postrest add-in one, indicating that full effect was not reached within 20-min of add-in rest period and that full recovery from drug effects was not achieved within 20 min of washout rest period.

rapidly during subsequent stimulations (Fig. 3) and after 10 min of stimulation reached a rather stable level (Table 2). Expressed as percentage of the control time-matched values, the intensity of 4-aminopyridine effects appeared greater in the low-plateau than in the high-plateau group. Furthermore, in 3/6 fibres of the low-plateau group, early afterdepolarisations developed in the first 15 postrest action potentials, leading to large standard errors of the mean for action potential duration at 90% repolarisation (Fig. 3, dashed error bars, lower left panel). At the concentration used, 4-aminopyridine effects were always significant as compared to the time-matched control values.

Table 2
Time courses of changes in action potential duration at 90% repolarisation induced by cisapride, 4-aminopyridine, dofetilide and terikalant in rabbit ventricular muscle, expressed as percentages of time-matched control action potentials

Action potential	No.	Cisapride (1 μM)		4-Aminopyridine (1 mM)		Dofetilide (0.01 μM)		Terikalant (0.3 μM)	
		Low-plateau ($n = 6$)	High-plateau ($n = 8$)	Low-plateau ($n = 6$)	High-plateau ($n = 7$)	Low-plateau ($n = 6$)	High-plateau ($n = 6$)	Low-plateau ($n = 6$)	High-plateau ($n = 9$)
Wash-in	1	-2.9 ± 2.1	1.2 ± 1.9	323.9 ± 58.6^a	179.1 ± 30.9^a	-2.5 ± 3.0	-4.4 ± 1.3	-2.4 ± 3.0	-2.3 ± 1.0
	3	2.7 ± 1.8	7.05 ± 2.4^a	168.8 ± 28.9^a	85.8 ± 8.7^a	0.9 ± 4.7	-3.2 ± 1.1	1.8 ± 2.1	1.4 ± 1.7
	15	11.2 ± 1.2^a	12.60 ± 2.6^a	160.0 ± 19.9^a	98.8 ± 10.3^a	6.2 ± 4.5	-0.6 ± 0.8	13.1 ± 3.0^a	9.7 ± 1.7^a
	300	15.7 ± 1.0^a	14.3 ± 2.5^a	149.8 ± 15.5^a	94.6 ± 11.0^a	18.3 ± 4.3^a	10.4 ± 2.6^a	31.3 ± 6.2^a	20.7 ± 2.9^a
Steady-state	1	-1.0 ± 3.3	4.2 ± 4.0	455.6 ± 82.1^a	250.7 ± 49.9^a	13.7 ± 1.7^a	11.7 ± 3.6^a	12.1 ± 4.9	3.1 ± 2.4
	3	19.9 ± 5.0^a	23.6 ± 7.2^a	235.8 ± 57.0^a	127.1 ± 16.7^a	24.0 ± 3.3^a	14.2 ± 3.0^a	33.1 ± 9.7^a	16.9 ± 5.0^a
	15	81.1 ± 25.2^a	56.0 ± 21.0^a	244.9 ± 41.6^a	150.4 ± 16.3^a	31.7 ± 3.5^a	20.6 ± 3.8^a	153.9 ± 36.7^a	56.5 ± 9.7^a
	300	53.4 ± 10.0^a	39.6 ± 7.6^a	165.1 ± 20.9^a	118.8 ± 132.8^a	50.3 ± 8.7^a	39.1 ± 8.1^a	168.2 ± 38.8^a	76.6 ± 10.4^a
Wash-out	1	-5.1 ± 3.9	1.5 ± 5.1	211.3 ± 23.9^a	141.0 ± 30.7^a	10.0 ± 1.6^a	9.9 ± 5.9	11.4 ± 6.2	0.1 ± 2.8
	3	19.2 ± 7.0^a	38.1 ± 17.7^a	96.9 ± 11.1^a	63.1 ± 9.5^a	21.6 ± 1.9^a	12.6 ± 4.0^a	45.7 ± 16.6^a	17.8 ± 7.5^a
	15	153.2 ± 51.9^a	71.5 ± 24.4^a	90.4 ± 13.5^a	62.3 ± 8.7^a	28.4 ± 2.3^a	20.8 ± 4.6^a	358.1 ± 101.4^a	98.2 ± 23.4^a
	300	89.0 ± 29.5^a	47.7 ± 8.8^a	55.7 ± 9.1^a	40.8 ± 6.3^a	33.4 ± 4.1^a	26.0 ± 5.6^a	251.5 ± 80.7^a	110.9 ± 20.5^a

[a] $P < 0.05$ as compared with respective time-matched control action potentials, using Student's paired t-test.

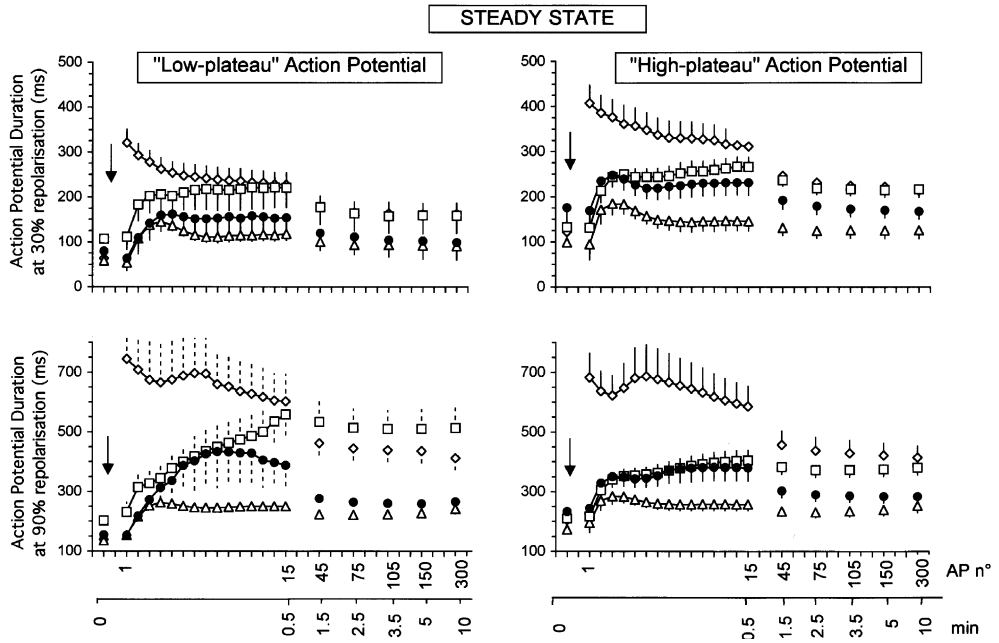

Fig. 5. Time course of changes in action potential duration during the postrest steady-state period, in rabbit ventricular muscle stimulated at 0.5 Hz in the presence of 1 μM cisapride ($n = 6$ in low-plateau group and $n = 8$ in high-plateau group), 1 mM 4-aminopyridine ($n = 6$ in low-plateau group and $n = 7$ in high-plateau group), 10 nM dofetilide ($n = 6$ in low-plateau group and $n = 6$ in high-plateau group) and 0.3 μM terikalant ($n = 6$ in low-plateau group and $n = 9$ in high-plateau group). Arrows show baseline action potentials. Dashed bars for S.E.M in the low-plateau group indicate the occurrence of early afterdepolarisations that resulted in large S.E.M. for action potential duration at 90% repolarisation. Note the major lengthening of the first postrest action potential in the presence of 4-aminopyridine and the abrupt resumption of prolonging effects on the 15 first postrest action potentials in the presence of terikalant and cisapride. AP: action potential.

Fig. 5 illustrates the postrest action potentials during the steady-state stimulation period. With cisapride and terikalant, the 20-min rest period allowed reversal of the prolongation obtained after the 40 min of add-in stimulation. Actually, the first postrest action potential in the low-plateau and high-plateau groups exposed to cisapride and terikalant did not differ from the time-matched control ones (Fig. 4), whereas in the two groups exposed to dofetilide, it was still slightly, but significantly prolonged (Fig. 4, Table 2). Afterwards, the lengthening effect induced by cisapride, dofetilide or terikalant reappeared rapidly, reaching significance level from the third postrest action potentials (Table 2). Early afterdepolarisations developed in the low-plateau groups in 2/6 fibres exposed to cisapride and in 3/6 fibres exposed to terikalant (Fig. 6). After 10 min of stimulation, the prolonging effect induced by the three drugs had returned to the level attained after the 40 min of add-in stimulation period, respectively. Although statistical significance could not be evaluated, due to exaggerated action potential prolongation observed in the case of early afterdepolarisaton appearance, it should be noted that the prolonging effects expressed as percentages of control time-matched values (Table 2) appeared somewhat greater in the low-plateau than in the high-plateau groups.

The 4-aminopyridine-induced prolonging effect on the first postrest action potential of the steady-state period was still more potent than that of the add-in period. Early afterdepolarisations appeared on the 15 first action potentials in 2/6 fibres in the low-plateau group.

In both low-plateau and high-plateau action potentials, during the washout rest period (Fig. 7), there was a partial

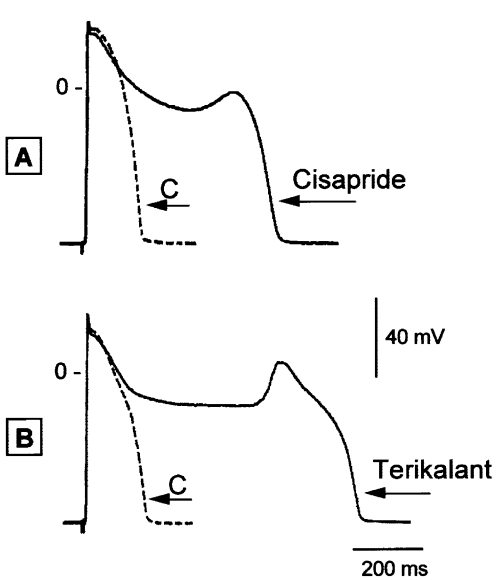

Fig. 6. Examples of early afterdepolarisations occurring on the first 15 action potentials elicited in the low-plateau groups during the steady-state postrest period with exposure to 1 μM cisapride (A) and 0.3 μM terikalant (B). C = time-matched control action potential.

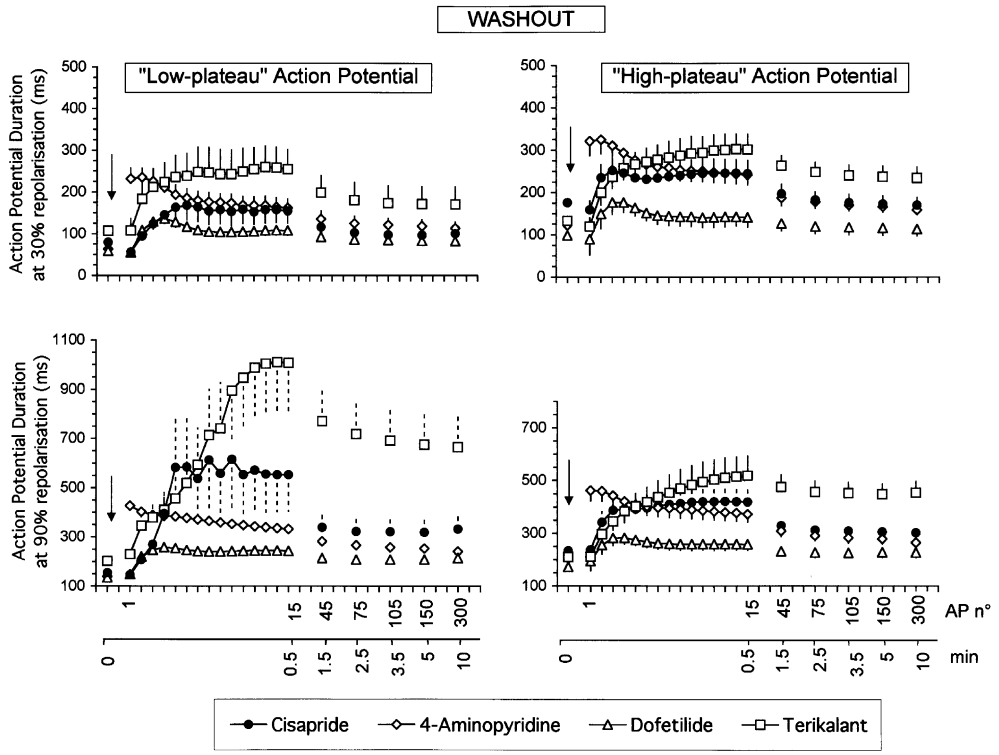

Fig. 7. Time course of changes in action potential duration during the postrest washout period, in rabbit ventricular muscle stimulated at 0.5 Hz and previously exposed to 1 μM cisapride ($n = 6$ in low-plateau group and $n = 8$ in high-plateau group), 1 mM 4-aminopyridine ($n = 6$ in low-plateau group and $n = 7$ in high-plateau group), 10 nM dofetilide ($n = 6$ in low-plateau group and $n = 6$ in high-plateau group) and 0.3 μM terikalant ($n = 6$ in low-plateau group and $n = 9$ in high-plateau group). Arrows show baseline action potentials. Note, in the 4-aminopyridine-treated groups, the partial reversal of the lengthening effect (as compared to Fig. 5) in the first postrest action potentials, whereas in the dofetilide-treated groups, repetitive stimulation was needed for a gradual decrease in action potential duration. In the presence of terikalant and cisapride, prolonging effects resumed abruptly, with occurrence of early afterdepolarisations from the third and the fifth postrest action potential, respectively, and during the 10 min of stimulation, as indicated by the dashed lines of S.E.M. bars. AP: action potential.

reversal of the 4-aminopyridine effects on action potentials as shown by less lengthening of the first postrest action potential than after the add-in and the steady-state rest periods (Fig. 4). Moreover, action potential duration decreased gradually during stimulation, but did not completely return to predrug values after 10 min of regular stimulation, yet remaining significantly prolonged (Table 2). In contrast, in the presence of cisapride, dofetilide or terikalant, the first postrest action potentials of the washout period did not differ from the postrest steady-state ones. In the cisapride and terikalant groups, the subsequent action potentials were greatly prolonged, so that early afterdepolarisations occurred in 2/6 fibres from the low-plateau group and 1/6 fibres from the high-plateau group previously exposed to cisapride and in 2/6 fibres from the low-plateau group exposed to terikalant. In the dofetilide groups, no reversal of prolonging effect was seen during the first 30 s of stimulation (Fig. 4 and Table 2), but partial recovery appeared slowly in the subsequent 10 min. Surprisingly, after the same 10 min of stimulation, action potential prolongation by cisapride and terikalant increased further as compared with that observed during the steady-state postrest period. This was particularly obvious in the low-plateau groups (Table 2).

3.2. Voltage-clamp experiments

The effects of cisapride at three concentrations were tested on the inward rectifier K^+ current, I_{K1}. From a holding potential of −40 mV, pulses increased in 10-mV increments from −140 to −90 mV elicited an early activating and inactivating component followed by a steady-state one. Pulses ranging from −90 to −30 mV resulted in an early activating and a steady-state component. Test potentials ranging above −85 mV produced an outward current much smaller than the inward current, which was typical of an inward rectification. Representative current traces obtained at −140 mV are shown in Fig. 8A after 9 min of 1-, 3- and 10-μM cisapride superfusion. Cisapride decreased the initial peak as well as the steady-state I_{K1} amplitudes as shown in Fig. 8B. Averaged peak (in a potential range of −140 to −90 mV) and steady-state current densities (in a potential range of −140 to −30 mV) are plotted in Fig. 8C and D, respectively, for the control and after 9 min of drug superfusion at each concentration. The blocking effect of cisapride was significant ($P < 0.05$ vs. control, $n = 6$) for 3 and 10 μM for the inward peak and steady-state components. Concerning the outward component, despite a decrease of the current

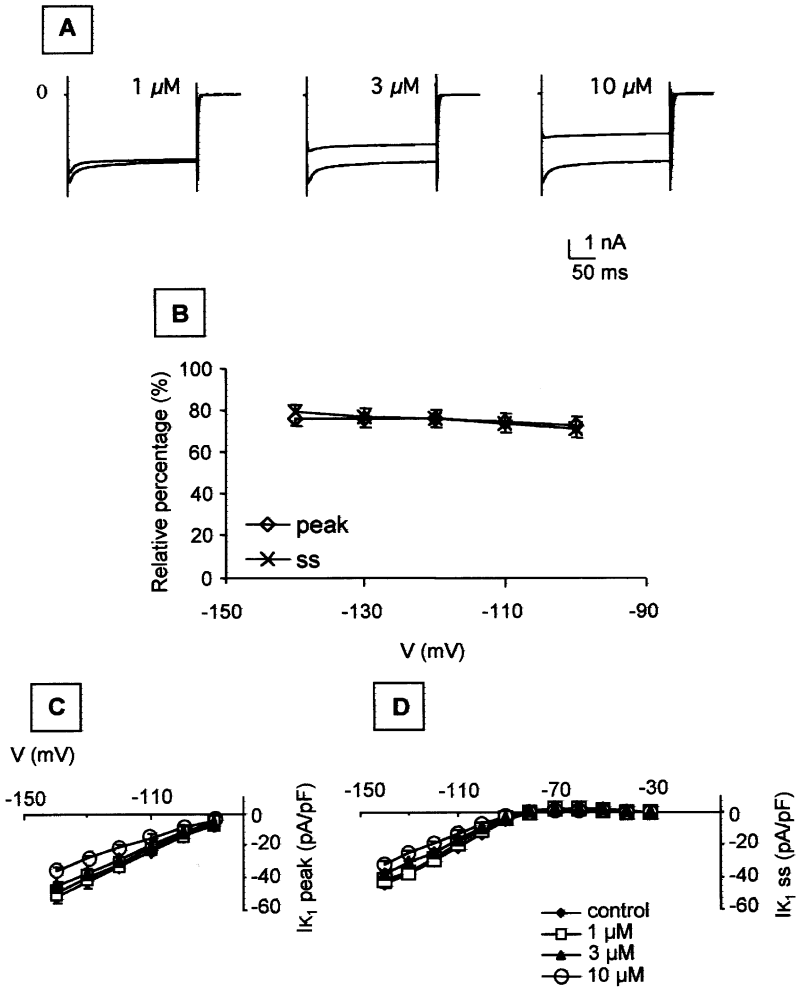

Fig. 8. Concentration-dependent effects of cisapride on I_{K1} in rabbit ventricular myocytes. (A) Representative traces of maximal inward current (obtained at −140 mV) are shown before and after 9-min superfusion of 1, 3 and 10 μM cisapride. (B) Effects of 3 μM superfusion of cisapride on the early activating component (◇) and steady-state component (×) expressed as relative percentage of control I_{K1} vs. potential. (C, D) Averaged current density–voltage relationships of early activating component (I_{K1} peak in C) and steady-state component (I_{K1} ss in D) in control and after 9-min exposure to 1, 3, 10 μM cisapride ($n = 6$, mean ± S.E.M., $^{*}P < 0.05$ vs. control).

densities, no significant block could be reached. Moreover, even after the 20-min washout of the drug, the blocking effect of cisapride was either poorly or not reversed ($n = 3$, data not shown).

4. Discussion

Our results provide evidence that in rabbit ventricular muscle, cisapride prolonged potently the action potential duration. These effects developed increasingly during repetitive stimulation with a time course quite similar to that found with terikalant and to a lesser degree with dofetilide. In addition, we found that cisapride could block the inward rectifier I_{K1}.

The prolongation of action potential duration produced by the drugs is consistent with their previously reported capacity to reduce K^+ currents. Thus, blocking effects on native I_{Kr} channels have been demonstrated with cisapride (Carlsson et al., 1997; Drolet et al., 1998), dofetilide (Carmeliet, 1992) and terikalant (Jurkiewicz et al., 1996), this latter drug also exerting blocking effects on the inwardly rectifying current, I_{K1} (Escande et al., 1994). On the other hand, 4-aminopyridine has been shown to block predominantly the Ca^{2+}-independent component of the transient outward K^+ current, I_{to} (Campbell et al., 1993) and to a lesser degree, I_{Kr} (Mitcheson and Hancox, 1999).

The prolonging of repolarisation caused by the K^+-blocking drugs was shown to be inversely rate-dependent, i.e., smaller prolongation at higher frequencies (Hondeghem and Snyders, 1990). One of the likely mechanisms involved a closed-channel block leading to a more prominent block at long diastolic intervals, i.e., a reverse use-dependent block. Actually, the 4-aminopyridine-induced block of I_{to} has been demonstrated as predominant on the closed state of the I_{to} channels (Castle and Slawsky, 1992; Campbell et al., 1993). Conversely, dofetilide was reported to affect I_{Kr} channels in activated and/or inacti-

vated states (Carmeliet, 1992) and, recently, cisapride was found to exert blocking effects on the HERG channels, with predominant affinity for open and inactivated states (Mohammad et al., 1997; Rampe et al., 1997; Walker et al., 1999), such effects being characteristic of a use-dependent block. However, the dofetilide and cisapride prolonging effect on repolarisation showed an inverse rate dependence (Puisieux et al., 1996; Dumotier et al., 1999). Therefore, it appears difficult to reconcile drug-induced effects on action potential duration and those on K^+ channels because the normal balance between currents responsible for the plateau phase of the action potential is also rate-dependent. The extent of the influence of a use-dependent block of K^+ channels on a drug-induced prolonging effect could be important with respect to the proarrhythmogenic effects of the drug.

The protocol we now used was based on the assumption that drug affinity for K^+ channels in the closed (during rest) or in the open and/or inactivated (during stimulation period) state can be indirectly, but likely correctly, estimated from the subsequent prolongation of action potentials (Ohler and Ravens, 1994; Ohler et al., 1994). A high affinity to closed K^+ channels is expected to result in full prolonging of the first action potential elicited after extended quiescence, this effect declining during regular stimulation until steady state is reached. Conversely, drug interaction with open or inactivated channels cannot occur during rest, but will result in a progressive development of the prolonging effect on action potentials during repetitive stimulation, i.e., repetitive activation of the channel. During drug exposure, complete reversal of the prolonging effect on the first postrest action potential elicited may plausibly indicate that the drug dissociates from the channel in the closed state.

In our study, we included four rest (control, add-in, steady-state and washout) periods alternated with regular stimulation to compare the effects of cisapride with those of K^+ channel blockers. The fact that the full prolonging effect of 4-aminopyridine on repolarisation was obtained on the first postrest action potential and declined rapidly during regular stimulation until a stable level was reached is consistent with the reverse use-dependent block exerted by 4-aminopyridine on I_{to} channels (Castle and Slawsky, 1992; Campbell et al., 1993) although 4-aminopyridine may interfere with other K^+ currents at the concentration used in the present study (Mitcheson and Hancox, 1999). Moreover, the reversal of 4-aminopyridine prolonging effect during the washout rest period is consistent with the dissociation of 4-aminopyridine from the K^+ channel during rest (Castle and Slawsky, 1992; Campbell et al., 1993). It may be assumed that more than 20 min of rest is necessary for complete dissociation and subsequent recovery to the baseline, predrug, action potential duration.

The time course of the cisapride, dofetilide and terikalant effects on action potentials was in strong contrast with that of 4-aminopyridine since with the former compounds, the duration of the first action potential after the rest add-in periods did not differ from the control duration and action potential prolongation developed increasingly during repetitive stimulation. Similar features have been described for dofetilide, which exerts a use-dependent block of I_{Kr} (Carmeliet, 1992) and induces a progressive increase in action potentials (Carmeliet, 1993; Ohler et al., 1994). The progressive development of the cisapride-induced prolonging of action potentials is consistent with its blocking of native I_{Kr} channels (Carlsson et al., 1997; Drolet et al., 1998) and HERG channels, with predominant affinity for open and inactivated states (Mohammad et al., 1997, Rampe et al., 1997, Walker et al., 1999). Similarly, terikalant at a concentration (0.3 μM) found by others to block I_{Kr} channels potently (Jurkiewicz et al., 1996) and to affect the inward rectifier I_{K1} current (Escande et al., 1992) to a lesser degree (Jurkiewicz et al., 1996) lengthened action potentials increasingly with repetitive stimulations during the add-in period, this suggesting higher affinity for I_{Kr} channels in the open or inactivated state or both.

The first action potential after a steady-state rest period did not differ from the control one in the presence of both cisapride and terikalant, whereas it was significantly prolonged with dofetilide. These findings support the hypothesis that cisapride and terikalant dissociate completely and dofetilide only partially, from channels in the closed state during rest. The latter observation is consistent with drug-trapping within the I_{Kr} channel, as proposed for dofetilide by Carmeliet (1992) and Jurkiewicz and Sanguinetti (1993) and recently confirmed by Mitcheson et al. (2000).

Surprisingly, the action potential prolongation induced by both cisapride and terikalant during stimulation was reinforced in the washout period. It may be that despite reversal of the prolonging effect during rest, possibly due to dissociation of cisapride and terikalant from the K^+ channel, and because of their high lipophilicity, these compounds remain within the tissue in the immediate vicinity of the K^+ channels and are, therefore, available for subsequent activation.

The strong similarities between the effects of cisapride and terikalant incited us to investigate the effects of cisapride on the inward rectifier I_{K1} current using the whole-cell patch-clamp technique. At relatively high cisapride concentrations (1–10 μM), we found a significant inhibition of the early activating and inactivating and steady-state inward components, which were poorly reversed by washout. However, at potentials positive to −80 mV, the decrease in outward I_{K1} amplitude by 10 μM cisapride was not significant. Therefore, cisapride proved to be a less potent I_{K1} blocker than terikalant, whereas both drugs block I_{Kr} with similar IC_{50} (Jurkiewicz et al., 1996; Drolet et al., 1998).

Interestingly, it appears from our results that the drug-induced prolonging effects were more potent on the low- than on the high-plateau action potential, resulting in the development of early afterdepolarisations in low-plateau

action potentials. The existence of two types of action potential profiles has been reported for rabbit endocardial myocytes and related to an higher density of I_{to} in the low- than in high-plateau action potentials (Varro et al., 1991; Verkerk et al., 1996). Because a prominent I_{to1} may delay the activation of $I_{Ca(L)}$ (Zygmunt et al., 1997) and reduce plateau height, a decrease in I_{to1} can be expected to keep the membrane potential at a plateau level close to that required for reactivation of $I_{Ca(L)}$ and, therefore, contribute to the occurrence of early afterdepolarisations.

Our study showed that relatively low concentrations of cisapride, dofetilide and terikalant can exert potent lengthening effects on ventricular repolarisation and induce early afterdepolarisations. These concentrations were within the range of effective concentrations previously reported by other authors, 4–10 nM for dofetilide (Segwick et al., 1992; Demolis et al., 1996), 0.1–10 μM for terikalant (Escande et al., 1994; McLarnon and Xu, 1995; Jurkiewicz et al., 1996). The maximal mean plasma concentration of unchanged cisapride can average 0.15–0.3 μM when the clinically effective doses are given to healthy volunteers or to patients with gastrointestinal discomfort (Wiseman and Faulds, 1994), but it can reach 10- or 20-fold higher values in patients after overdosing or inhibition of its metabolism by other drugs (Ahmad and Wolfe, 1995; Wysowski and Bacsanyi, 1996). Furthermore, a recent study with in situ canine heart indicated that the ratio of the tissue/plasma concentrations of cisapride was in a range of 5–6 (Sugiyama and Hashimoto, 1998). Thus, if these results can be carefully extrapolated to rabbit cardiac tissues, the concentrations of cisapride used in our study with both ventricular strips (1 μM) and isolated myocytes (3 and 10 μM) can be considered clinically relevant.

In conclusion, our results showed that in rabbit ventricular myocardium, cisapride induced use-dependent prolonging effects on repolarisation quite consistent with the blockade of K^+ channels in open and/or inactivated states as demonstrated using patch-clamp methods (Drolet et al. 1998). In addition, cisapride decreased the amplitude of the inward rectifier K^+ current, I_{K1}, which may contribute to its proarrhythmic potential. This study confirmed that alternation of rest and stimulation periods can help with understanding the extent of drug-induced prolonging effects and subsequent proarrhythmic potential.

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