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Risperidone prolongs cardiac action potential through reduction of K^+ currents in rabbit myocytes

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Abstract

Prolongation of QT interval by antipsychotic drugs is an unwanted side effect that may lead to ventricular arrhythmias. The antipsychotic agent risperidone has been shown to cause QT prolongation, especially in case of overdosage. We investigated risperidone effects on action potentials recorded from rabbit Purkinje fibers and ventricular myocardium and on potassium currents recorded from atrial and ventricular rabbit isolated myocytes. The results showed that (1) risperidone (0.1–3 μM) exerted potent lengthening effects on action potential duration in both tissues with higher potency in Purkinje fibers and caused the development of early afterdepolarizations at low stimulation rate; (2) risperidone (0.03–0.3 μM) reduced significantly the current density of the delayed rectifier current and at 30 μM decreased the transient outward and the inward rectifier currents. This study might explain QT prolongation observed in some patients treated with risperidone and gives enlightenment on the risk of cardiac adverse events.

Keywords: Risperidone; Action potential; Transient outward current; Delayed rectifier current; Inward rectifier current; Heart, rabbit

1. Introduction

Among the side effects that can be associated with noncardiac drugs, prolongation of cardiac repolarization may be responsible for the development of polymorphic ventricular tachycardia, the so-called torsades de pointes, particularly if predisposing factors such as congenital long QT syndrome, bradycardia, hypokalemia and/or hypomagnesemia are present (for review, see Volders et al., 2000). This proarrhythmic activity is characterized by a prolongation of the QT interval on the electrocardiogram consequently to a reduction of the repolarizing K^+ currents, in particular the rapid component of the delayed rectifier current I_{Kr}. At cellular level, this results in a prolongation of the cardiac action potential duration with development of early afterdepolarizations (for review, see Haverkamp et al., 2000).

Acquired long QT syndrome has been reported to develop under treatment with several antipsychotics such as haloperidol, pimozide, thioridazine, sultopride (Haverkamp et al., 2000; Cavero et al., 2000). Risperidone is a benzisoxazole antipsychotic agent reported to exert antagonistic effects on serotonin (5-HT_2), dopamine (D2), α_1- and α_2-adrenoceptors and histamine (H_1) receptors. Its therapeutic indications are the treatment of schizophrenia and other psychoses. Risperidone is considered as an "atypical" antipsychotic drug compared to the "traditional" antipsychotic treatments (haloperidol, chlorpromazine) because it allowed a clear improvement of the quality of life of the patients by a reduction in specific extrapyramidal effects.

Shortly after its introduction into clinical practice, cases of QT interval lengthening have been reported (Brown et al., 1993; Lo Vecchio et al., 1996; Gesell and Stephen, 1997; Laroussinie et al., 1997; Ravin and Levenson, 1997; Kopala et al., 1998), the majority of them occurring with risperidone overdosage (Brown et al., 1993; Lo Vecchio et al., 1996; Laroussinie et al., 1997; Kopala et al., 1998) in patients with electrolyte disorders such as hypokalemia. However, to our knowledge no case of torsades de pointes was reported with risperidone.

The electrophysiological mechanisms of the QT interval lengthening induced by risperidone have been poorly investigated. In isolated feline heart, Drici et al. (1998) showed

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that risperidone caused concentration-dependent lengthening of QT interval. Frederiksen and Adamantidis (2000) reported briefly the blocking effects of risperidone on the *Human Ether-a-go-go-Related-Gene* (HERG) that expresses the delayed rectifier current I_{Kr} in human ventricle and the prolonging effects on repolarization in action potentials recorded from rabbit Purkinje fibers. The IC_{50} for HERG current was 1.6 μM, a concentration which dramatically prolonged action potential duration.

Nevertheless, risperidone effects on ventricular myocardium and native currents were not yet examined. Therefore, the present study was aimed (i) to compare risperidone effects on action potentials recorded from rabbit Purkinje fibers and rabbit ventricular myocardium and (ii) to examine risperidone effects on the main native voltage-dependent K^+ currents involved in the repolarization of ventricular and atrial isolated rabbit cells.

2. Materials and methods

These studies were carried out on cardiac preparations from male New Zealand white rabbits (1.5 to 2 kg) in agreement with the official recommendations of the European Community guidelines. For the patch clamp studies, animals were anaesthetized by injection of ethylcarbamate (1 g/kg) in a marginal vein of the ear while they were killed by cervical dislocation and exsanguinated for the multicellular preparations.

2.1. Multicellular preparations

The thorax was opened and the heart was removed quickly and placed in a modified Tyrode's solution (in mM: NaCl 118.2; KCl 30; $CaCl_2$ 1.8; $MgCl_2$ 1; NaH_2PO_4 1.8; $NaHCO_3$ 25; glucose 55; pH 7.40 ± 0.05, gassed by carbogen (95% O_2–5% CO_2) at a temperature of about 33 °C. Either Purkinje fibers still attached to the ventricular muscle or strips of contractile myocardium were dissected from the left ventricle free wall. They were pinned carefully, endocardium upward, to the silicone base of the experimental chamber. The preparations were superfused with the modified Tyrode's solution at a flow rate of 2.5 ml/min, gassed with carbogen and thermostated at 36.0 ± 0.5 °C. After 30 min, the superfusate was switched to the normal Tyrode's solution which differed from the previous one by a lower KCl (from 30 to 4 mM) and a lower glucose (from 55 to 11 mM) concentrations. The preparations were stimulated at the frequency of 2 Hz by rectangular pulses of 1-ms duration, with an intensity 1.5 times higher than the diastolic threshold of stimulation. Pulses were delivered by a stimulator (JSI 0198 Beerse, Belgium) through a bipolar Teflon-insulated (except at the tip) stainless-steel electrode. After 30 min at 2 Hz, the stimulation frequency was reduced from 2 to 1 Hz and allowed to equilibrate during at least 2 h.

2.1.1. Action potential recordings

Transmembrane action potentials were recorded using conventional glass microelectrodes filled with 3 M KCl and with a tip resistance of 10 to 25 MΩ, coupled with an Ag–AgCl bath electrode and connected to an impedance amplifier (VF 102 Bio-Logic, Claix, France). Action potentials were displayed on an oscilloscope (Gould DSO 1602, Valley View, OH, USA), analyzed by an external computer system (Datapac, Bio-Logic) and stored on magnetic digital tape recorder (DTR 1205, Bio-Logic) which allowed to display on paper recordings (Gould, Easy Graf TA 240) the action potential profiles and electrical abnormalities (e.g. early afterdepolarisations). The following parameters of the action potential were measured: resting membrane potential (RMP) in mV, action potential amplitude (APA) in mV, maximal rate of depolarization (V_{max}) in V/s and action potential duration at 50% and 90% repolarization (respectively APD_{50} and APD_{90}) in ms.

2.1.2. Experimental protocol: concentration-dependent effects

After at least 2 h stabilization and before application of risperidone, the stimulation frequency was reduced from 1 Hz to 0.2 Hz for 2 min, then returned to 1 Hz. This maneuver was aimed to examine the influence of a low rate of stimulation on action potential characteristics and to verify the absence of abnormality in repolarization, such as early afterdepolarization. Then, increasing concentrations (0.03, 0.1, 0.3, 1, 3 and 10 μM) of risperidone were applied in a cumulative manner, each concentration being maintained during 30 min. The preparation was stimulated at the frequency of 1 Hz except during 2 min (between the 20th and the 22nd min of superfusion of each concentration) where the frequency was lowered to 0.2 Hz then switched back to 1 Hz. Action potential parameters were measured at the end of the 30-min perfusion of each concentration and at the end of each period of 2-min stimulation at 0.2 Hz. Only the results obtained when the impalement was maintained in the same cell throughout the experiment have been considered for quantitative evaluation.

2.2. Voltage-clamp studies on isolated atrial and ventricular myocytes

Single atrial and 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 quickly excised and cannulated by the aorta on a Langendorff perfusion apparatus. The heart was 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) for 4 min 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 collage-

nase B (Boehringer Mannheim, Mannheim, Germany) and 0.06 mg/ml of protease XIV (Sigma, St Quentin Fallavier, France) was perfused for 10–15 min. Then the heart was rinsed with Kraftbrühe (KB) solution (in mM: KCl 85; KH_2PO_4 30; $MgSO_4$ 5; Na_2ATP 5; taurine 20; glucose 20; ethylene glycol-(bis) (β aminethyl ether)–*N*,*N*,*N*′,*N*′,-tetra-acetic acid (EGTA) 0.2; phosphocreatine 5; pyruvic acid 5; HEPES 10; pH 7.35) for 5 min. Atria and ventricles were then taken, cut out in small pieces and gently agitated to dissociate the cells. The atrial cell suspension was filtered and centrifuged at 1000 rpm for 1 min. The pellet was resuspended in 1 mM Ca^{2+} containing Tyrode's solution. The ventricle 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 transient outward current I_{to}, the delayed rectifier current I_K and the inward rectifier current I_{K1}. Currents were recorded using an RK 400 amplifier (Bio-Logic) and pClamp software (Axon instrument, Foster City, CA, USA). The voltage clamp protocol was a series of (i) 750-ms voltage steps from −50 to +60 mV (10-mV increments) from a holding potential of −80 mV for I_{to}, (ii) 3000-ms voltage steps from −30 to +50 mV from a holding potential of −40 mV for I_K and (iii) 240-ms voltage steps from −140 to +10 mV from a holding potential of −40 mV for I_{K1}. Currents were sampled at 3.33 kHz, 333.33 Hz, 6.25 kHz, respectively, for I_{to}, I_K, I_{K1} using a 12-bit analog-to-digital converter (Labmaster TL-1, Scientific Solutions, Solon, OH, USA), low-pass filtered at 3 kHz. Borosilicate glass pipettes (Clark Electromedical Instruments, Reading, UK) had a resistance of 1.7 to 2 MΩ when filled with the pipette solution (in mM: KCl 130; $MgCl_2$ 2; HEPES 10; K_2ATP 3; phosphocreatine 5; EGTA 10; pH 7.4). The superfusion solution had the following composition (in mM): NaCl 54, *N*-methyl-D-glucamine 86; KCl 4; $MgCl_2$ 1; HEPES 10; $CaCl_2$ 1; glucose 10; tetrodotoxin 0.02; $CoCl_2$ 3; pH 7.4). Control and drug-containing solutions were applied to the exterior of the cell by placing this one at the opening of 300-μm inner diameter catheters fixed on the rotating head of a rapid solution changer (RSC, Bio-Logic). Cell capacitance was measured by integrating the area of the capacitive transient elicited by 5-mV hyperpolarizing steps. Currents were normalized to the membrane capacity to eliminate variation of the cell size. All experiments were carried out at room temperature (19–22 °C).

2.3. Drugs/reagents

Stock solution (10 mM) of risperidone was prepared daily and later diluted in Tyrode's solution to the desired concentration. Risperidone was dissolved as followed: 4.1 mg was dissolved in 1 ml of dimethylsulfoxide. The influence of this vehicle has been tested on action potentials recorded from Purkinje fibers by using the same protocol as that used for the drug. The concentration-dependent effects of vehicle dilutions equivalent to a drug concentration range of 1–100 μM are given in Table 1, which indicates that no significant effects were seen. Also on the different currents, vehicle was found without significant effect. Risperidone was firstly given by H. Lündbeck A/S then obtained from Sigma. Tetrodotoxin (Latoxan, Valence, France) was prepared as a 1 mM stock solution in tampon solution. All other chemicals were obtained from Sigma.

Table 1
Concentration-dependent effects of dilutions of dimethylsulfoxide on action potentials recorded from rabbit Purkinje fibers stimulated at 1 Hz

	DMSO (% v/v)					
	0	0.01	0.03	0.1	0.3	1
RMP (mV)	−92 ± 1	−92 ± 1	−93 ± 1	−93 ± 1	−92 ± 1	−92 ± 1
APA (mV)	126 ± 1	126 ± 1	126 ± 1	127 ± 1	126 ± 1	125 ± 2
V_{max} (V/s)	534 ± 38	529 ± 41	518 ± 37	497 ± 29	478 ± 32	472 ± 34
APD_{50} (ms)	251 ± 42	255 ± 43	251 ± 44	249 ± 42	244 ± 42	232 ± 39
APD_{90} (ms)	304 ± 41	309 ± 41	308 ± 40	301 ± 39	303 ± 38	291 ± 35

DMSO = dimethylsulfoxide; RMP = resting membrane potential; APA = action potential amplitude: V_{max} = maximal rate of rise of phase 0 depolarization; APD_{50} and APD_{90}: action potential duration at 50% and 90% repolarization, respectively; values are expressed as mean ± standard error of the mean; $n = 5$ fibers.

2.4. Data analysis and statistical evaluation

The results have been expressed as means ± standard error of the mean (S.E.M). Comparisons vs. control were performed statistically using an analysis of variance (ANOVA) for repeated measures completed by the corrected Dunnett's *t*-test. The threshold of significance retained was $P < 0.05$. The statistical software used was Instat 2 (Graph Pad, San Diego, USA).

3. Results

3.1. Risperidone and action potential

3.1.1. Risperidone effects on action potential recorded from Purkinje fibers

A representative example of risperidone effects (0.1–3 μM) on the action potential profile in rabbit Purkinje fibers is illustrated in Fig. 1. It clearly shows that risperidone induced a concentration-dependent lengthening in action potential duration and that this effect was strongly reinforced by reducing the stimulation frequency from 1 to 0.2 Hz. The height of the plateau was slightly decreased at the concentration of 3 μM under stimulation at 1 Hz, this being

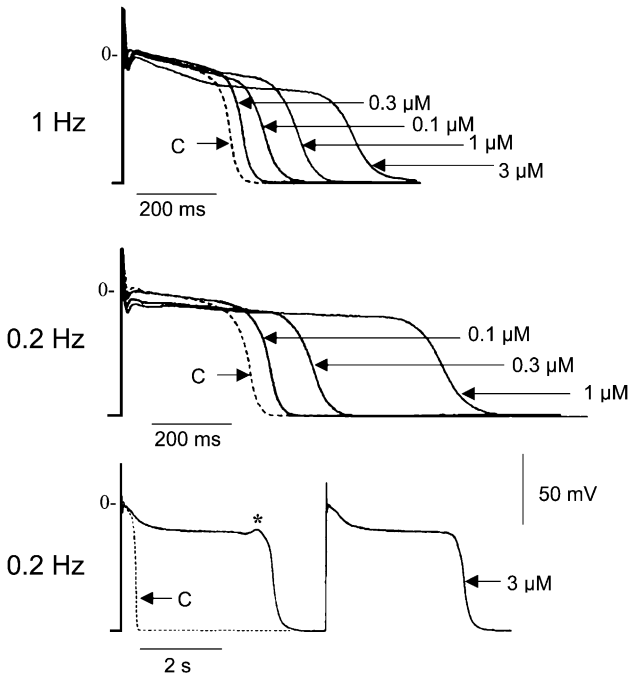

Fig. 1. Representative example of the prolonging effects exerted by risperidone on repolarization in action potentials recorded from a rabbit Purkinje fiber stimulated at 1 and 0.2 Hz. Microelectrode impalement was maintained in the same cell during the whole experiment. Control drug-free action potentials are superimposed in dotted line. Control action potential duration at 90% repolarization was 319 ms at 1 Hz and 374 ms at 0.2 Hz. See how the low stimulation rate of 0.2 Hz magnifies the prolonging effect recorded at 1 Hz. The asterisk indicates a small early afterdepolarization which developed at the end of the plateau.

still more pronounced at 0.2 Hz since it is already visible in the presence of 0.3 μM risperidone. Moreover, in this fiber stimulated at 0.2 Hz and in the presence of 3 μM risperidone, single early afterdepolarizations developed at the end of the plateau.

Table 2
Concentration-dependent effects induced by risperidone on action potential parameters recorded in Purkinje fibers stimulated at the frequency of 1 Hz

	Control	Risperidone (μM)				
		0.03	0.1	0.3	1	3
RMP (mV)	−92 ± 0	−92 ± 1	−92 ± 1	−92 ± 1	−91 ± 1	−86[a] ± 2
APA (mV)	125 ± 2	126 ± 2	126 ± 2	126 ± 2	124 ± 2	118[a] ± 2
OS (mV)	33 ± 2	33 ± 2	34 ± 2	34 ± 1	33 ± 1	32 ± 1
V_{max} (V/s)	510 ± 29	499 ± 31	476 ± 28	425[a] ± 30	412[a] ± 32	350[a] ± 36
APD_{50} (ms)	274 ± 13	285 ± 13	308[a] ± 13	359[a] ± 15	531[a] ± 27	769[a] ± 31
APD_{90} (ms)	329 ± 12	343 ± 12	368[a] ± 13	438[a] ± 19	647[a] ± 34	956[a] ± 44

RMP: resting membrane potential; APA: action potential amplitude; OS: overshoot; V_{max}: maximal rate of depolarization; APD_{50} and APD_{90}: action potential duration at 50% and 90% repolarization respectively. $n = 7$.
[a] $P < 0.05$ vs. control.

Table 2 summarizes the effects obtained on the action potential parameters recorded in seven experiments. The lengthening in repolarization phase was already significant (vs. the control values) at the concentration of 0.1 μM (308 ± 13 vs. 274 ± 13 ms for action potential duration at 50% repolarization and 368 ± 13 vs. 329 ± 12 ms for action potential duration at 90% repolarization). It was observed with the same intensity on the plateau (as expressed by the action potential duration at 50% repolarization) and on the final repolarization (represented by the action potential duration at 90% repolarization). At 3 μM risperidone, this lengthening effect reached 184 ± 18% for the plateau and 193 ± 17% for the final repolarization. Furthermore, in six of the seven Purkinje fibers exposed to 3 μM risperidone, the prolonging effect was so marked that 2/1 responses to stimulation were obtained within the first 20 min of drug exposure. Under stimulation at 0.2 Hz, early afterdepolarizations were observed in the seven fibers (Fig. 2A), and in one of the seven fibers, multiple early afterdepolarizations developed, thus inducing in this fiber a sustained repetitive activity (Fig. 2B).

Resting membrane potential and action potential amplitude were not significantly modified by risperidone except at the highest concentration tested (3 μM) which depressed significantly all parameters. A slight but significant reduc-

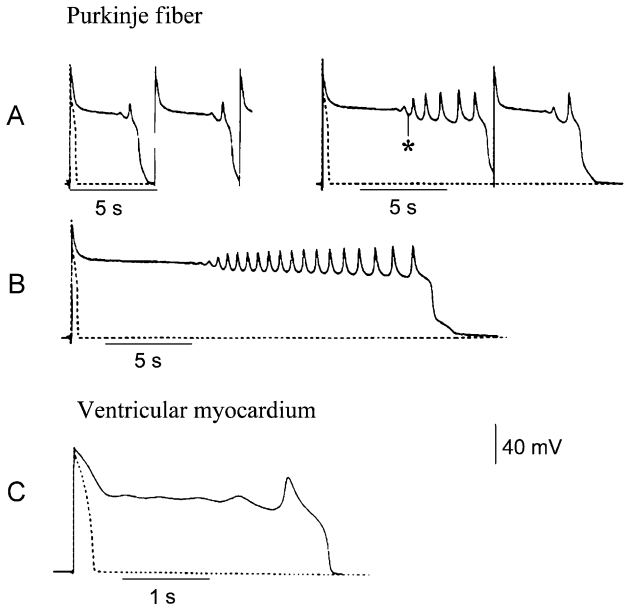

Fig. 2. Development of early afterdepolarizations (EADs) on action potentials recorded from rabbit Purkinje fiber and rabbit ventricular myocardium. Control drug-free action potentials are superimposed in dotted line. Control action potential durations at 90% repolarization were 307 ms in (A), 311 ms in (B), 212 ms in (C). (A) Single or multiple EADs occurring in Purkinje fiber stimulated at 0.2 Hz in the presence of 3 μM risperidone. EADs similar as that on the left side occurred in six out of the seven fibers. On the right side, the prolonging effect exerted by risperidone was so strong that action potential duration exceeded the 5-s period of stimulation (* stimulation artefact). (B) Sustained rhythmical activity in one of the seven Purkinje fibers. (C) EADs observed in ventricular myocardium stimulated at 0.2 Hz in the presence of 10 μM risperidone.

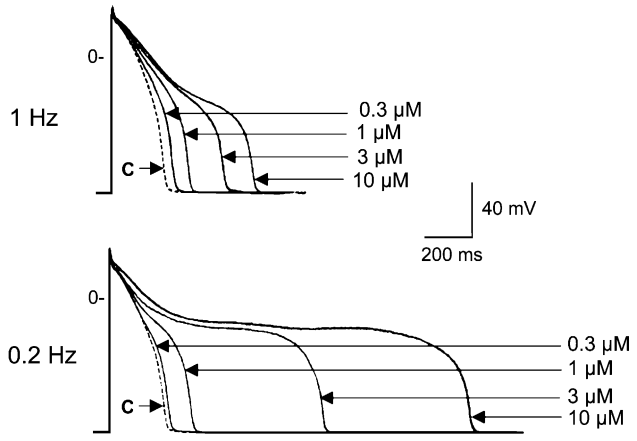

Fig. 3. Representative example of the lengthening effects exerted by increasing concentrations (0.3–10 μM) of risperidone on action potentials recorded from rabbit ventricular myocardium stimulated at 1 and 0.2 Hz. Microelectrode impalement was maintained in the same cell during the whole experiment. Control drug-free action potentials (C) are indicated in dotted line. Control action potential duration at 90% repolarization was 208 ms at 1 Hz and 190 ms at 0.2 Hz.

tion of V_{max} was observed from the concentration of 0.3 μM risperidone.

3.1.2. Risperidone effects on action potential recorded from ventricular myocardium

Fig. 3 illustrated the effects of increasing concentrations (0.3–10 μM) of risperidone on the action potential recorded from ventricular myocardium. Action potential duration was concentration dependently prolonged and this effect was intensified by decreasing the stimulation rate at 0.2 Hz. As described above in Purkinje fiber, the action potential plateau was slightly lowered by 10 μM risperidone under a stimulation rate of 1 Hz, whereas this effect was more marked at the concentrations of 3 and 10 μM at 0.2 Hz. In six of eight preparations, 10 μM risperidone caused the development of single and/or multiple early afterdepolarization as illustrated in Fig. 2C.

Table 3 summarized the effects obtained on action potential parameters in eight preparations. Action potential durations at 50% and at 90% repolarization were already significantly lengthened by 0.1 μM risperidone as compared to the control values: 200 ± 6 vs. 178 ± 5 ms and 246 ± 7 vs. 220 ± 4 ms, respectively. The plateau and the final repolarization were prolonged with the same intensity, reaching 143 ± 24% for the plateau and 144 ± 20% for the final repolarization at 10 μM risperidone.

As indicated in Table 3, risperidone did not alter significantly the other action potential parameters even at the highest concentration of 10 μM.

Expressed as percentage vs. the control values, in Fig. 4, the lengthening effect exerted by 0.3, 1 and 3 μM of risperidone on the action potential at 90% repolarization was more potent in Purkinje fibers than in ventricle and this difference in potency was accentuated with increasing concentrations: it was +33 ± 3% in Purkinje fiber vs. +24 ± 2% in ventricle at 0.3 μM risperidone and +193 ± 17% in Purkinje fiber vs. +91 ± 12% in ventricle at 3 μM.

3.2. Risperidone and K^+ currents

3.2.1. Risperidone effects on the delayed rectifier current recorded in ventricular myocytes

In mammalian species, I_K consists of at least two components, the rapid and the slow delayed rectifying currents, I_{Kr} and I_{Ks}, respectively. I_{Kr} is selectively blocked by E4031 at the concentration of 5 μM (Sanguinetti and Jurkiewicz, 1990). In our experimental set up, I_K tail current was completely abolished after 5 min of exposure to 5 μM E4031 (data not shown), suggesting that the I_K tail current recorded in our conditions was mainly composed of I_{Kr}.

Three concentrations of risperidone were tested: 0.003, 0.03 and 0.3 μM, these concentrations being applied each for 5 min in cumulative manner. Representative current traces obtained in control and after perfusion of the three concentrations are illustrated in Fig. 5A. The averaged tail current–voltage relationships are plotted as a function of test potentials in Fig. 5B. Risperidone decreased dose-dependently I_K tail current amplitude which was significantly reduced at 0.03 μM from +30 mV to more positive

Table 3
Concentration-dependent effects induced by risperidone on action potential parameters recorded in ventricular myocardium stimulated at the frequency of 1 Hz

	Control	Risperidone (μM)					
		0.03	0.1	0.3	1	3	10
RMP (mV)	−85 ± 1	−83 ± 1	−84 ± 1	−84 ± 1	−85 ± 1	−85 ± 1	−86 ± 1
APA (mV)	118 ± 2	118 ± 3	120 ± 2	119 ± 2	121 ± 2	120 ± 2	121 ± 3
OS (mV)	34 ± 2	35 ± 2	36 ± 1	35 ± 2	36 ± 2	35 ± 2	34 ± 2
V_{max} (V/s)	151 ± 13	138 ± 7	144 ± 6	135 ± 8	134 ± 7	129 ± 7	123 ± 9
APD_{50} (ms)	178 ± 5	189 ± 5	200[a] ± 6	222[a] ± 6	264[a] ± 10	343[a] ± 28	436[a] ± 49
APD_{90} (ms)	220 ± 4	232 ± 5	246[a] ± 7	272[a] ± 8	324[a] ± 13	422[a] ± 31	544[a] ± 53

RMP: resting membrane potential; APA: action potential amplitude; OS: overshoot; V_{max}: maximal rate of depolarization; APD_{50} and APD_{90}: action potential duration at 50% and 90% repolarization, respectively. $n = 8$.

[a] $P < 0.05$ vs. control.

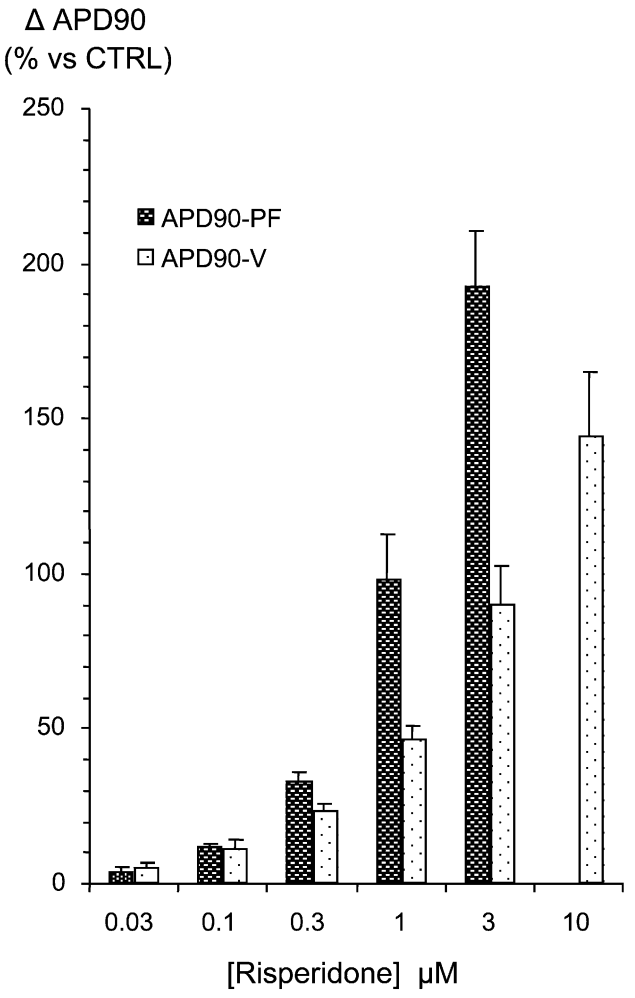

Fig. 4. Comparison of risperidone prolonging effects on action potential duration recorded from rabbit Purkinje fibers (PF, $n=7$) and ventricular myocardium (V, $n=8$). APD_{90}: action potential duration at 90% repolarization. ΔAPD_{90}: prolonging effect expressed as percentage versus control (drug-free) values.

potentials (0.58 ± 0.06 to 0.30 ± 0.02 pA/pF at $+50$ mV, $n=6$) and at 0.3 μM from $+20$ mV to more positive potentials (0.58 ± 0.06 to 0.23 ± 0.03 pA/pF at $+50$ mV, $n=6$).

3.2.2. Risperidone effects on the transient outward current recorded in atrial myocytes

Two cumulative concentrations of risperidone, 3 and 30 μM, were tested on the transient outward current I_{to}, each concentration being applied for 5 min. Representative current traces obtained in control and after 5 min of risperidone superfusion are illustrated in Fig. 6A. The concentration of 3 μM of risperidone did not significantly decrease the peak current and the sustained current while the concentration of 30 μM caused a strong reduction both in the peak and in the sustained current and an apparent acceleration of the time course of inactivation. The time constants of the fast and slow components of I_{to} inactivation have been calculated from the I_{to} trace evoked at $+60$ mV depolarizing pulse. In the presence of risperidone 30 μM, the time constants of the two components of I_{to} inactivation are significantly accelerated: 4.0 ± 0.6 vs. 25.8 ± 1.4 ms under control condition ($n=6$) for the fast component and 30.0 ± 12.1 vs. 78.0 ± 10.9 ms under control condition ($n=6$) for the slow component, suggesting an open channel blockade. Fig. 6B showed the averaged current density–voltage relationships of I_{to} obtained in control and after 3 and 30 μM of risperidone. I_{to} current was measured as the difference between the peak current and the sustained current at the end of the pulse. Risperidone decreased in dose-dependent manner the current density. At the concentration of 3 μM this effect was not significant whatever the potential. On the other hand, 30 μM risperidone reduced significantly the current density from -20 mV to more positive potentials.

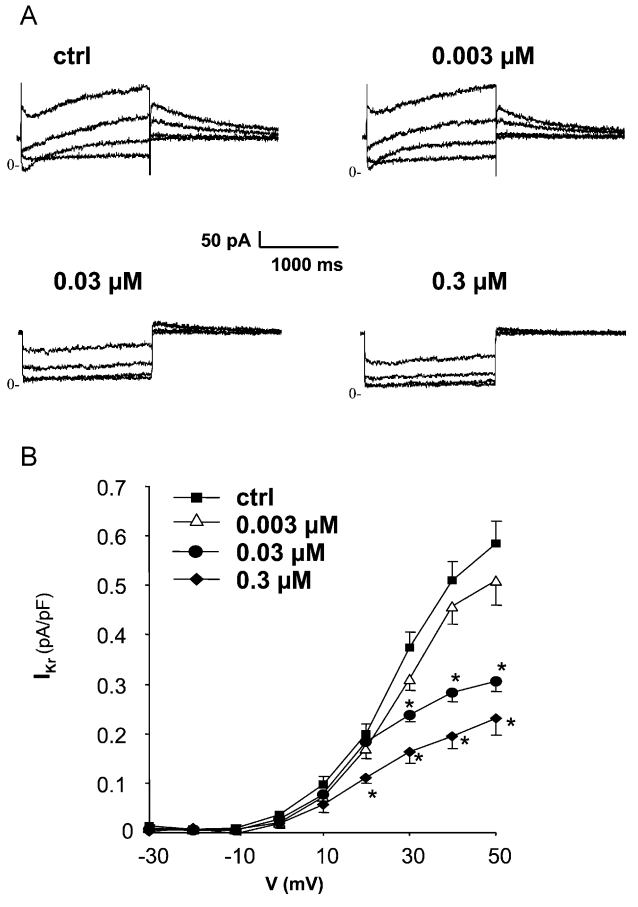

Fig. 5. Concentration-dependent effects of risperidone on I_{Kr} in rabbit ventricular myocytes. (A) Representative traces of I_{Kr} currents are shown in control and after 5-min superfusion of 0.003, 0.03 and 0.3 μM risperidone. I_{Ktail} current was measured, at the return to -40 mV, as the difference between the peak current and the steady-state current. (B) Averaged tail-current density–voltage relations of I_{Kr} in control and after exposure to 0.003, 0.03 and 0.3 μM risperidone ($n=6$, $*P<0.05$ vs. control, ctrl). Risperidone 0.03 and 0.3 μM reduced significantly the current density of I_{Kr} from $+30$ mV (0.03 μM) and $+20$ mV (0.3 μM) to more positive potentials.

The maximum current density of I_{to} measured at +60 mV decreased from 7.72 ± 0.65 to 6.98 ± 0.61 pA/pF at 3 μM, $P > 0.05$ ($n = 6$) and from 7.72 ± 0.65 to 5.06 ± 0.28 pA/pF at 30 μM, $P < 0.05$ ($n = 6$).

3.2.3. Risperidone effects on the inward rectifier current recorded in ventricular myocytes

Fig. 7A gives representative current records in control and after 5 min of exposure to risperidone at the cumulative concentrations of 3 and 30 μM. Both concentrations decreased the initial peak as well as the steady-state I_{K1} amplitudes. Steady-state current densities (in a potential range of −140 to +10 mV) are plotted in Fig. 7B, for the control and after superfusion of increasing concentrations of risperidone (0.03, 0.3, 3 and 30 μM) respectively.

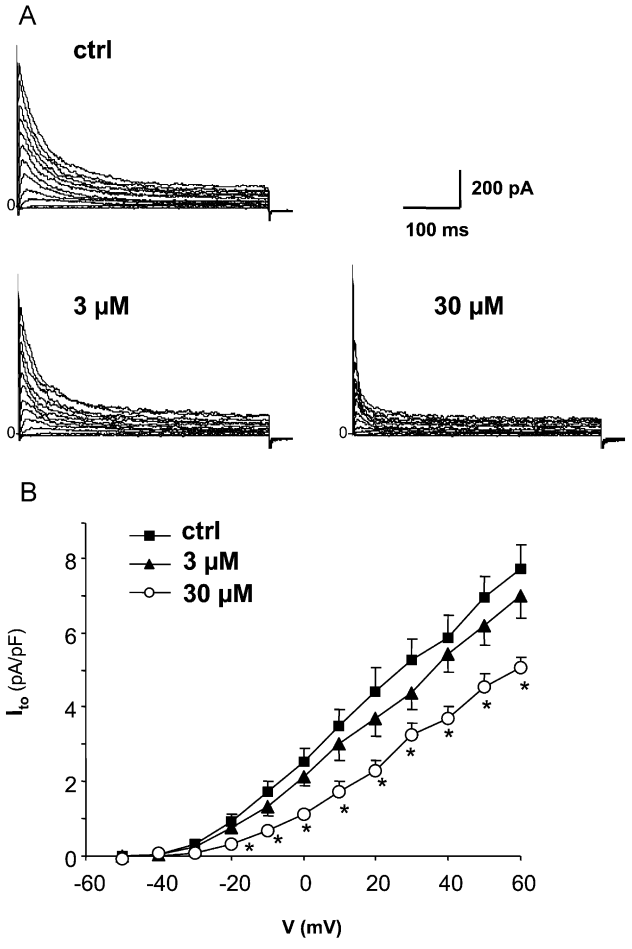

Fig. 6. Concentration-dependent effects of risperidone on I_{to} in rabbit atrial myocytes. (A) Representative traces of I_{to} currents were shown before and after 5-min superfusion of 3 and 30 μM risperidone. I_{to} current was measured as the difference between the peak current and the steady-state current at the end of the pulse. (B) Averaged current density–voltage relationships of I_{to} in control and after 5-min exposure to 3 and 30 μM risperidone ($n = 6$, * $P < 0.05$ vs. control, ctrl). Risperidone 30 μM reduced significantly the current density of I_{to} from −20 mV to more positive potentials.

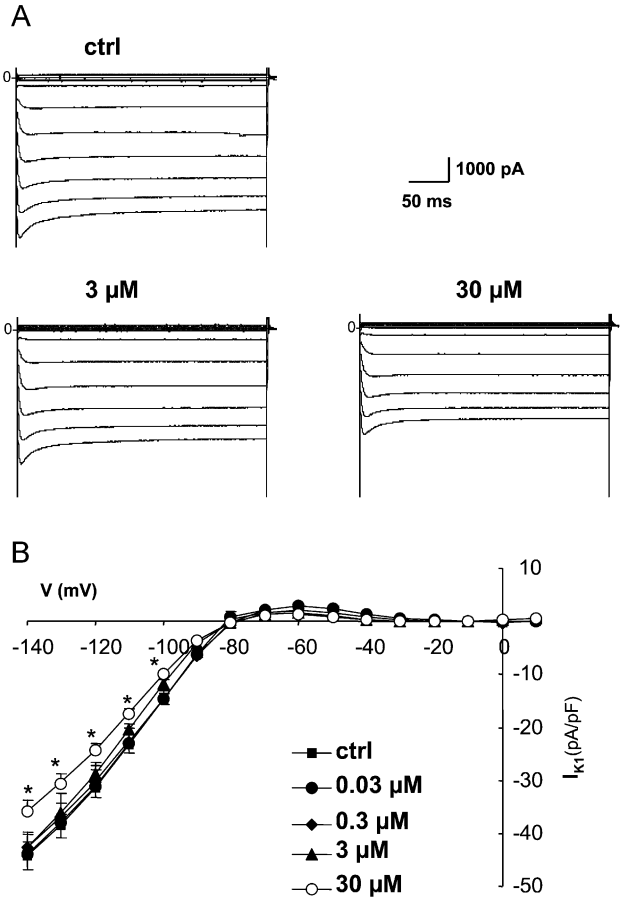

Fig. 7. Concentration-dependent effects of risperidone on I_{K1} in rabbit ventricular myocytes. (A) Representative examples of currents are shown in control and after 5-min superfusion of 3 and 30 μM risperidone. Steady-state current amplitude of I_{K1} was evaluated at the end of the pulse. (B) Averaged current density–voltage relationships of steady-state component of I_{K1} in control (ctrl) and after 5-min exposure to 0.03, 0.3, 3 and 30 μM risperidone ($n = 6$, * $P < 0.05$ vs. control). Risperidone 30 μM reduced significantly the steady-state current in a potential range of −140 to −100 mV.

Risperidone decreased the steady-state current density I_{K1} in a concentration-dependent manner. This effect was significant only for the strongest concentration, 30 μM, in a potential range of −140 to −100 mV. At −140 mV, the current density decreased by −44.0 ± 3.2 to −35.9 ± 4.9 pA/pF, $P < 0.05$ ($n = 6$).

4. Discussion

The results obtained in this study provide evidence that risperidone exerts direct cardiac electrophysiological effects. In action potentials recorded from rabbit Purkinje fibers and ventricular myocardium, risperidone lengthened the action potential duration in concentration- and reverse frequency-dependent manner and caused the development of early afterdepolarizations. Patch-clamp experiments revealed blocking effects on I_{Kr} and at a lesser degree on I_{to} and

I_{K1}. These effects can give an explanation for the prolonged QT interval observed in some patients treated with the drug.

A concentration-dependent lengthening of repolarization without noticeable modification of other action potential parameters and further intensified by low stimulation rate characterizes the typical class III antiarrhythmic effects (according to the classification of antiarrhythmic drugs of Vaughan-Williams) and confers to the drug proarrhythmic potential (Haverkamp et al., 2000; Malik and Camm, 2001; Yang et al., 2001). Our results showed that risperidone exerted typical class III antiarrhythmic effects at concentrations lower than 1 μM both in Purkinje fibers and in contractile myocardium. The lengthening effect on action potential duration was more marked in Purkinje fiber than in ventricular myocardium. As already reported (Lu et al., 2001), rabbit Purkinje fibers are a highly sensitive tissue for the detection of drug-induced lengthening effects on repolarization. This property has been attributed to the larger density in I_{Kr} over I_{Ks} as previously described (Veldkamp, 1998).

As already widely reported, nonantiarrhythmic drugs that have been associated with prolongation of QT interval and torsades de pointes incorporate I_{Kr} blockade in their spectrum of effects (Haverkamp et al., 2000). Our study indicates that risperidone induced potent blocking effects on I_{Kr} current at low concentrations since 0.03 μM was able to already reduce significantly this current which was further diminished by approximately 55% at 0.3 μM. The peak plasma concentration after an oral dose of 4 mg risperidone was close to 0.17 μM. Taking into account its volume of distribution (1 to 2 l/kg), this experimental concentration of 0.3 μM risperidone can be considered clinically relevant. In contrast, the blocking effects on I_{to} and I_{K1} current were found at 100 times higher concentration (30 μM) which could occasionally occur in cases of suicidal overdose.

In a previously reported study carried out on the HERG K^+ channel that expresses the delayed rectifier current I_{Kr} in human, risperidone was found to inhibit 50% of this current at the concentration of 1.6 μM (Frederiksen and Adamantidis, 2000). Therefore, it appears that the blocking effect is more potent in native rabbit I_{Kr} than in cloned human channels, this confirms the high sensitivity of rabbit cardiac tissues to drug effects and their suitability in detecting drug-induced electrophysiological properties.

As a consequence of I_{Kr} blockade, single and multiple early afterdepolarizations were elicited by stimulation at low rate which exaggerated the prolonging effect on action potential duration. This observation was already reported with potent I_{Kr} blocking drugs (Dumotier et al., 1999) and also with noncardiac drugs such as the antipsychotics (Adamantidis et al., 1993; Adamantidis et al., 1994), the prokinetic drug cisapride (Puisieux et al., 1996) or the quinolone antibiotic sparfloxacin (Adamantidis et al., 1998). The general consensus is that early afterdepolarizations constitute the trigger mechanism for the initiation of torsades de pointes (Asano et al., 1997). A great number of studies have demonstrated that all conditions known to predispose to torsades de pointes in patients are also known to induce early afterdepolarization in the experimental settings such as bradycardia, hypokalemia and/or hypomagnesemia (for review, see Volders et al., 2000). Interestingly, in our study, risperidone was able to cause early afterdepolarizations as well in Purkinje fibers as in ventricular myocardium, this being rather uncommon in multicellular endocardial preparations (Antzelevitch et al., 1996).

It is now established that the HERG channel constitutes the main molecular target for drugs which produce prolongation of the QT interval (so-called acquired long QT syndrome) and that this interaction may contribute to the generation of the polymorphic ventricular tachycardia torsades de pointes. Such noncardiovascular drugs included antipsychotics which block HERG with different degree of block: the concentrations required to produce 50% block of HERG (IC_{50}) were in nanomolar range for sertindole and haloperidol and in micromolar range for risperidone (Frederiksen and Adamantidis, 2000). In contrast, these IC_{50} induced drastic prolonging effects on action potential duration with haloperidol and risperidone and only minimal effect with sertindole (Adamantidis et al., 2000). Similar differences in the intensity for prolonging QT interval (haloperidol>risperidone>sertindole) were found in isolated perfused feline heart (Drici et al., 1998). This may be partly explained by the concomitant depression of other action potential parameters, especially the maximal rate of rise of phase 0, action potential amplitude and plateau duration with sertindole (Adamantidis et al., 2000) which indicates Ca^{2+} and Na^+ current reduction, which makes the prolonging effect less marked (Dumotier et al., 1999) and proarrhythmic effects of QT prolongation less likely to occur (Bril et al., 1996). As recently confirmed by other authors (Yang et al., 2001; Malik and Camm, 2001), there is not a clear relation between the I_{Kr} (or HERG) block and the development of torsades de pointes, and the multiple pharmacologic effects of a drug can modulate the extent to which it prolongs repolarization phase.

4.1. Clinical implications

Several cases of widening QRS complex and lengthening of QTc interval were reported during clinical studies undertaken with risperidone at therapeutic dose (Barnes and Mc Phillips, 1999; Reilly et al., 2000) or in case of overdosage (Brown et al., 1993; Lo Vecchio et al., 1996; Laroussinie et al., 1997; Kopala et al., 1998), but no case of torsades de pointes with risperidone has been reported. Only one case of death following cardiac arrest was described with a lengthening of QT interval at 480 ms, possibly related to a QRS widening at 160 ms (Ravin and Levenson, 1997) after administration of a therapeutic dose (4 mg/day) and in the absence of cardiovascular antecedent.

Unexpectedly, risperidone was found to strongly prolong repolarization and to block the I_{Kr} current similarly to the

so-called "pure" class III antiarrhythmic drugs. Usually such effects are considered as potentially proarrhythmic (Haverkamp et al., 2000). Several features may be involved to explain this apparent paradox. Firstly, risperidone has antagonistic effects on α_1-adrenoceptors which cause tachycardia, thus indirectly minimizing the prolonging effects through the reverse frequency-dependent mechanisms (Hondeghem and Snyders, 1990). Furthermore, a recent report demonstrates that treatment with α_1-adrenoceptor blocking drug was beneficial in preventing life-threatening cardiac events in patients with congenital long QT syndrome (Furushima et al., 2001). On the other hand, pharmacokinetic studies of risperidone have revealed a small volume of distribution (1–2 l/kg) that indicates low tissue binding particularly at cardiac level. In addition, risperidone is predominantly metabolized by the cytochrome P450 isoenzyme 2D6 (CYP2D6) and at a lesser degree by the isoenzyme 3A4 (Fang et al., 1999). Although the enzymatic activity of the CYP2D6 is the subject of genetic polymorphism, it has been found that the active antipsychotic fraction (risperidone plus 9-hydroxyrisperidone) was almost similar between extensive and poor metabolizers (Huang et al., 1993). Finally, the poor lipophilicity of risperidone may contribute to the rapid reversal of adverse cardiac effects (Harry, 1997; Capel et al., 2000; Feifel et al., 2000). Thus, in agreement with a recent review on the potential cardiac risk of antipsychotic drugs (Glassman and Bigger, 2001), our study confirms that several factors other than cardiac direct electrophysiological effects have to be taken into consideration for evaluating the potential for serious cardiac adverse events.

In conclusion, the present study demonstrates that in rabbit cardiac tissues, risperidone potently prolongs ventricular repolarization in rabbit cardiac tissues by blocking the rapid component of the delayed rectifier potassium current. This effect may be involved in the QT prolongation reported in some patients and kept in mind in case of overdosage.

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