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Limited induction of torsade de pointes by terikalant and erythromycin in an in vivo model

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Abstract

The proarrhythmic activities of the selective I_{Kr} blocker erythromycin and the less selective K^+ channel blockers, terikalant and clofilium, have been compared in an α_1-adrenoceptor-stimulated, anaesthetized rabbit model. Terikalant (2.5, 7.5 and 25 nmol kg^{-1} min^{-1}; $n=10$), erythromycin (133, 400 and 1330 nmol kg^{-1} min^{-1}; $n=8$), clofilium (20, 60 and 200 mg kg^{-1} min^{-1}; $n=10$) or vehicle ($n=8$) was infused intravenously over 19 min and there was a 15-min interval between each infusion. QT and QTc intervals, and epicardial monophasic action potential duration were prolonged significantly (and to a similar extent) only by clofilium and terikalant. The total incidences of torsade de pointes were 60%*, 20%, 0% and 0% in clofilium-, terikalant-, erythromycin- and vehicle-treated animals, respectively (* $P<0.05$ compared to vehicle control). In conclusion, terikalant exerted mild proarrhythmic activity though it prolonged repolarisation markedly. Despite being given in high doses, erythromycin neither prolonged repolarisation nor induced proarrhythmia.

Keywords: QT interval; K^+ channel blocker; Proarrhythmia; Torsade de pointes; α_1-Adrenoceptor stimulation; Clofilium; Erythromycin; Terikalant

1. Introduction

Torsade de pointes is a life-threatening form of polymorphic ventricular tachycardia which can be evoked by several antiarrhythmic and non-antiarrhythmic drugs (Haverkamp et al., 2000). These drugs are thought to exert their anti- and proarrhythmic effects by prolonging the repolarisation phase of action potential of ventricular myocytes. Prolongation of repolarisation can be brought about either by reduction of outward K^+ currents or enhancement of inward Na^+ and Ca^{2+} currents. Predisposing factors for the generation of torsade de pointes are bradycardia, electrolyte abnormalities (i.e. hypokalaemia and hypomagnesaemia), prolonged repolarisation, depressed left ventricular function and/or a history of life-threatening arrhythmias. The underlying mechanism of torsade de pointes is still not known fully, but early afterdepolarisations and dispersion of ventricular repolarization are suspected to be the main electrophysiologic substrates for generation of torsade de pointes (Haverkamp et al., 2000).

Terikalant, a novel antiarrhythmic agent, prolongs repolarisation by inhibiting K^+ currents, e.g. the inward rectifier K^+ current (I_{K1}) (Escande et al., 1992), the transient outward K^+ current (I_{to}) (McLarnon and Xu, 1995) and the rapid component of the delayed rectifier K^+ current (I_{Kr}) (Jurkiewicz et al., 1996). In guinea pig isolated hearts (Williams et al., 1999) and in anaesthetized rats (Schultz et al., 1998), terikalant prolonged repolarisation.

Erythromycin, a macrolide antibiotic, has been reported to prolong repolarisation in man (Oberg and Bauman, 1995) as well as in several different experimental animal models (Drici et al., 1999; Fazekas et al., 1998; Hanada et al., 1999; Morey et al., 1997). The drug has been associated with the induction of torsade de pointes in man (Drici et al., 1998; Haverkamp et al., 2000; Oberg and Bauman, 1995). The possible mechanism underlying erythromycin-induced torsade de pointes is prolongation of repolarisation either by blocking I_{Kr} directly (Antzelevitch et al., 1996; Daleau et al., 1995; West et al., 1998) or by slowing the metabolism of other drugs, e.g. terfenadine or quinidine, via inhibition of cytochrome *P*450 3A4 enzyme (Dresser et al., 2000).

Clofilium is a multiple K^+ channel blocker as it affects I_{Kr}, (Li et al., 1996), the slow component of the delayed rectifier K^+ current (I_{Ks}) (Varnum et al., 1993), I_{to} (Castle,

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1991), the ATP-dependent K^+ current (I_{KATP}) (Sakuta et al., 1993) and to some extent I_{K1} (Li et al., 1996) in a similar concentration range. Inward Na^+ current (I_{Na}) and L-type Ca^{2+} current ($I_{Ca\text{-}L}$) are also reduced by clofilium in the same concentration range (Li et al., 1996). The drug prolonged repolarisation and the QT interval in man (Greene et al., 1983; Platia and Reid, 1984) and in experimental animals, e.g. dogs (Echt et al., 1989) and rabbits (Carlsson et al., 1990). Clofilium was shown to evoke torsade de pointes in rabbits (Carlsson et al., 1990) and now the drug is widely used as a positive control agent as it is capable of inducing a high incidence of torsade de pointes in both in vitro (D'Alonzo et al., 1999; Johna et al., 1998) and in vivo (Batey and Coker, 2002; Buchanan et al., 1993; Carlsson et al., 1990) rabbit experiments.

The proarrhythmic effects of terikalant have not been examined to date and only little is known about the proarrhythmic activity of erythromycin in vivo. Therefore, the present study compared the proarrhythmic effects of terikalant and erythromycin to that of clofilium in a modified α_1-adrenoceptor-stimulated anaesthetized rabbit model (Batey and Coker, 2002). Our main reason for doing the present experiments was not to establish a model that would be predictive for man, but to look at a wider range of drugs to see what effects they have in vivo and to try to understand how torsade de pointes arises in this rabbit model.

2. Materials and methods

2.1. Animals

The experiments were performed on male New Zealand White rabbits weighing 2.6 to 3.1 kg. The animal handling procedures were in accordance with the Guidance on the Operation of the Animals (Scientific Procedures) Act 1986, London, UK and also adhered to European Community guidelines for the use of experimental animals. The experiments were conducted under the authority of Project Licence no. 40/1702 and approved by the University of Liverpool Animal Welfare Committee.

2.2. Animal preparation

The proarrhythmic activity of the drugs was examined by using a modification of the method of Carlsson et al. (Batey and Coker, 2002; Carlsson et al., 1990). Animals were anaesthetized with Na^+ pentobarbitone (30 mg kg^{-1} i.v. via the marginal vein of the left ear). The trachea was cannulated to allow artificial ventilation. A cannula was introduced into the right femoral artery for arterial blood pressure measurement. The cannula was filled with heparin-treated saline (0.9% w/v NaCl containing 15 i.u. ml^{-1} heparin) and connected to a Bell and Howell type 4-422 transducer, which was linked to a Gould 6615-30 DC bridge amplifier. The amplified arterial blood pressure signal was recorded at a sampling rate of 250 Hz by a Po-Ne-Mah P3 computerised data acquisition system (Linton, Diss, Norfolk, UK). The right femoral vein and the marginal vein of the right ear were also cannulated for i.v. administration of drugs. After performing a sternal split and pericardiotomy, an electrode (EP Technologies model no. 225, Linton) connected to a Gould 6615-58 bioelectric amplifier was positioned on the epicardial surface of the left ventricle in order to monitor monophasic action potentials. Subcutaneous needle electrodes were inserted in all four limbs, connected to Gould 6615-65 ECG or 6615-58 bioelectric amplifiers and Leads I, II and III of the electrocardiogram (ECG) were recorded simultaneously, along with the monophasic action potential signal, at a sampling rate of 1000 Hz by the Po-Ne-Mah data acquisition system.

Immediately after performing the sternal split, artificial ventilation was started (Bioscience pump) with room air at a rate of 38 stroke min^{-1}, and a stroke volume of ~ 6 ml kg^{-1} body weight and a positive end expiratory pressure of 1–2 cm H_2O. Blood gases were monitored with a Ciba Corning 850 pH/blood gas analyser (Bayer, Newbury, Berks, UK). If necessary, the stroke volume of the ventilation pump and the positive end expiratory pressure were adjusted to maintain blood gases within the normal range. Anaesthesia was maintained as necessary by administration of further doses of Na^+ pentobarbitone (3–6 mg kg^{-1} i.v.). Preparation was followed by a minimum 20 min stabilising period.

2.3. Experimental protocol

Rabbits were assigned randomly to receive three consecutive i.v. infusion rates of either erythromycin (133, 400 and 1330 nmol kg^{-1} min^{-1}, $n=8$), terikalant (2.5, 7.5 and 25 nmol kg^{-1} min^{-1}, $n=10$), clofilium (20, 60 and 200 nmol kg^{-1} min^{-1}, $n=10$) or vehicle (0.81% NaCl solution, $n=8$). Each drug infusion was administered for 19 min. Five minutes prior to each drug infusion, phenylephrine infusion was started and given i.v. for 24 min in increasing rates (i.e. 75, 150, 225 and 300 nmol kg^{-1} min^{-1} for 15, 3, 3 and 3 min, respectively). All three 24-min dosing cycles were followed by a 10-min drug-free interval. The drug administration protocol is illustrated in Fig. 1.

2.4. Arrhythmia diagnosis and electrocardiogram analysis

Blood pressure, the duration of the monophasic action potential and ECG intervals were measured at predetermined time points. After completing experiments, the data was replayed and the RR, PR, QRS and QT intervals were measured by manual positioning of on screen markers. At least four complexes of each variable were measured and averaged at each time point. The QT interval was defined as the time between the first deviation from the isoelectric line during the PR interval until the end of the TU wave. T (or U) waves frequently overlapped the P wave of the following

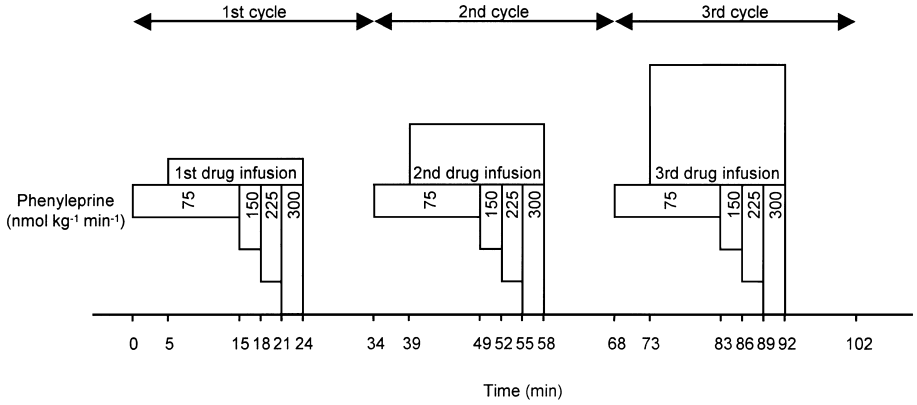

Fig. 1. Drug administration protocol: 1st drug infusion, clofilium 20 nmol kg^{-1} min^{-1}, terikalant 2.5 nmol kg^{-1} min^{-1}, erythromycin 133 nmol kg^{-1} min^{-1} or saline; 2nd drug infusion, clofilium 60 nmol kg^{-1} min^{-1}, terikalant 7.5 nmol kg^{-1} min^{-1}, erythromycin 400 nmol kg^{-1} min^{-1} or saline; 3rd drug infusion, clofilium 200 nmol kg^{-1} min^{-1}, terikalant 25 nmol kg^{-1} min^{-1}, erythromycin 1330 nmol kg^{-1} min^{-1} or saline.

sinus-origin beat due to the relatively high heart rate of the rabbit or to substantial QT prolongation. In these cases the end of the TU wave was extrapolated from the curve of the TU wave to the isoelectric line under the P wave. The values for QT interval were corrected for heart rate using the equation: $QTcL = QT - 0.704(RR - 250)$, which has been shown previously to be appropriate for these pentobarbitone-anaesthetized rabbits in our laboratory (Batey and Coker, 2002).

From the ECG, the incidence, the time to onset and the duration of ventricular arrhythmias were obtained. Ventricular premature beats, bigeminy, salvos and ventricular fibrillation were defined according to the Lambeth Conventions (Walker et al., 1988). When continuous ventricular fibrillation lasted longer than 120 s, then the experiment was terminated and ventricular fibrillation was defined as lethal. Torsade de pointes was defined as a polymorphic ventricular tachycardia where clear twisting of the QRS complexes around the isoelectric axis could be seen in at least one ECG lead. Runs of four or more ventricular premature beats without the torsade-like twisting QRS morphology were differentiated from torsade de pointes and were defined as ventricular tachycardia. Blocks in the conduction system were also monitored. Conduction disturbances included atrioventricular block and intraventricular conduction defects (right or left bundle branch block).

2.5. Drugs

Terikalant (terikalant fumarate) was a gift from Aventis (formerly Rhone-Poulenc Rorer, Vitry Sur Seine Cedex, France). All other drugs were purchased from the following sources: clofilium (clofilium tosylate) and phenylephrine (L-phenylephrine HCl) from Sigma (Poole, Dorset, UK); Na^+ pentobarbitone (Sagatal®) from National Veterinary Supplies (Stoke-on-Trent, UK), erythromycin (erythromycin lactobionate, Erythrocin I.V. Lactobionate®) and heparin–Na^+ (Multiparin®) from the Royal Liverpool University Hospital Pharmacy Department. The stock solution for infusion of the highest dose of terikalant was obtained by dissolving 1.97 mg terikalant fumarate in 0.5 ml warm water then adding 4.5 ml of 0.9% w/v NaCl solution (saline). The

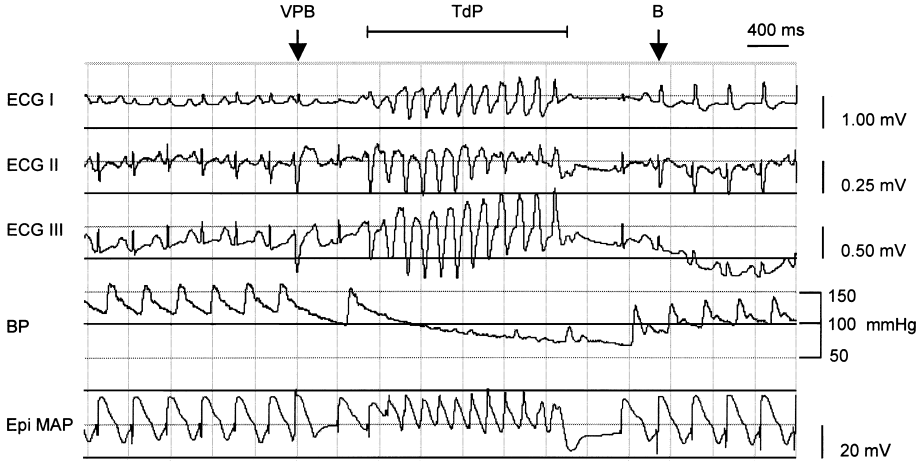

Fig. 2. An example of clofilium-induced torsade de pointes (TdP). ECG I–III, electrocardiogram leads I–III; BP, arterial blood pressure; Epi MAP, epicardial monophasic action potential; VPB, ventricular premature beat; B, intraventricular conduction block.

stock solution of clofilium was obtained by dissolving 16.16 mg clofilium tosylate in 5 ml 0.81% w/v saline. For the lower infusion rates, these drugs were diluted further with 0.81% w/v saline, which also served as the vehicle for the control group. Each terikalant, clofilium and vehicle infusion was administered in a volume of 0.6 ml kg^{-1} over 19 min. The stock solution for erythromycin infusion was prepared by adding 2.5 ml water containing 200 mg Erythrocin I.V. Lactobionate® to 2.5 ml 0.9% w/v saline. Subesquent dilutions were prepared with 0.45% w/v saline. Each erythromycin infusion was administered in a volume of 0.8 ml kg^{-1} over 19 min. Phenylephrine solution was prepared by dissolving 9 mg of L-phenylephrine HCl in 10 ml 0.9% w/v saline. Increasing doses of phenylephrine, i.e. 75, 150, 225 and 300 nmol kg^{-1} min^{-1}, were administered by infusing the same phenylephrine solution at rates of 16.67, 33.33, 50.00 and 66.67 μl kg^{-1} min^{-1}, respectively. Each solution was prepared freshly on the day of the experiment.

2.6. Statistical evaluation

Continuous data were expressed as mean ± standard error of the mean (S.E.M.). All data from independent samples, except arrhythmia incidences, were compared with Kruskal–Wallis tests. Continuous data from the same sample were compared with Friedman tests. Arrhythmia incidences were compared by using Fisher's exact probability test. Differences were considered statistically significant when $P < 0.05$.

3. Results

3.1. Arrhythmia incidences

Torsade de pointes was evoked only by clofilium (Fig. 2) and terikalant. However, the incidence of this arrhythmia was significantly higher compared to control only in the clofilium-treated group (Fig. 3). The incidence of torsade de pointes was not directly proportional to the dose or infusion rate of clofilium, since the middle infusion rate of this drug evoked most torsade de pointes (Fig. 3). A similar lack of relationship between infusion rate and torsade de pointes occurrence was found with terikalant, i.e. both the middle and the highest infusion rates of terikalant failed to increase the low incidence of torsade de pointes that was evoked by the lowest infusion rate of the drug (Fig. 3). In one clofilium-treated animal, torsade de pointes degenerated into ventricular fibrillation once during the 1st and once during the 3rd infusion. The latter episode of ventricular fibrillation lasted longer than 120 s and was classified as lethal. Clofilium evoked the most blocks in the conduction system, and this effect of the drug was dose-dependent (Fig. 3). Ventricular tachycardia that was not torsade de pointes occurred frequently during the first dosing cycle in each group and the incidence of this arrhythmia declined gradually in the following dosing cycles in each group (Fig. 3). A similar gradual decrease of the incidence of salvos was found during the three cycles in each group. Terikalant exerted a protective effect against salvos as the 3rd infusion of this drug reduced significantly the incidence of this

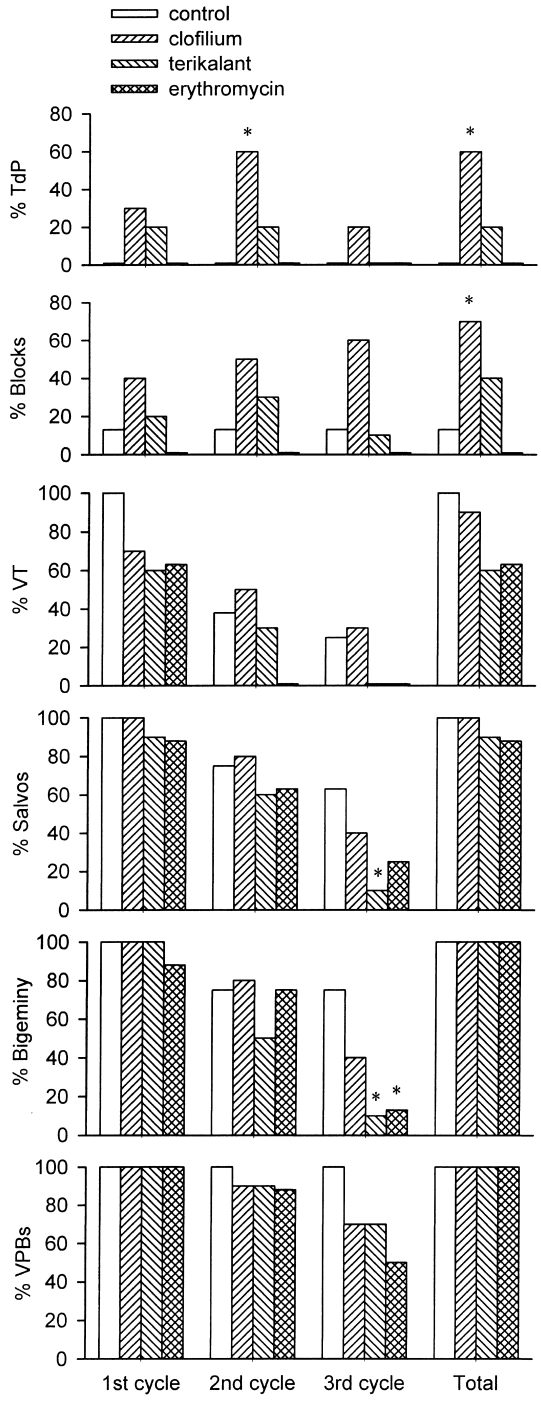

Fig. 3. Percent incidence of torsade de pointes (TdP), conduction blocks (blocks), ventricular tachycardia (VT), salvos, bigeminy and ventricular premature beats (VPBs) in rabbits treated with clofilium, terikalant and erythromycin during the three dosing cycles (see Fig. 1 for protocol). * $P < 0.05$ vs. control.

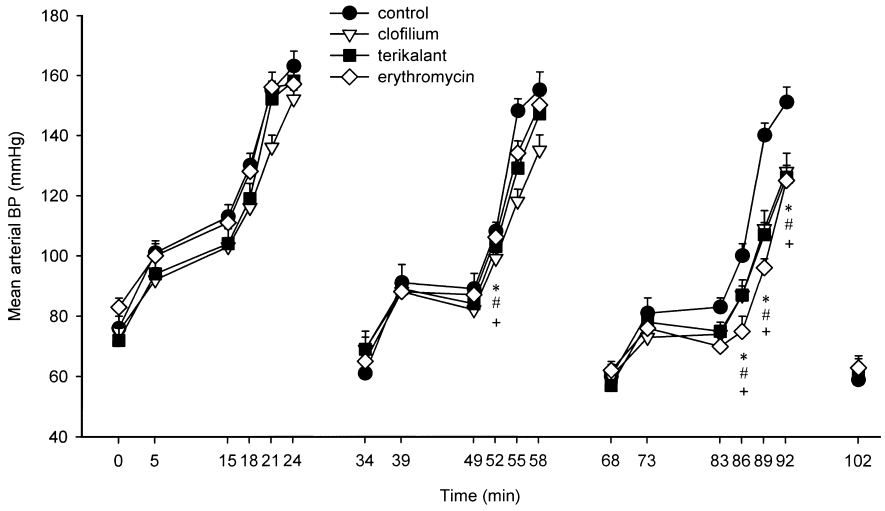

Fig. 4. Mean arterial blood pressure in rabbits treated with clofilium, terikalant, erythromycin or vehicle (control) during the three dosing cycles (see Fig. 1 for protocol). All values shown as mean ± S.E.M. $^{*}P<0.05$ clofilium vs. control; $^{\#}P<0.05$ terikalant vs. control; $^{+}P<0.05$ erythromycin vs. control, Kruskal–Wallis test.

arrhythmia compared to control (Fig. 3). The incidence of bigeminy was decreased significantly by the highest doses of terikalant and erythromycin (Fig. 3). The incidence of ventricular premature beats was not altered significantly by any of the drug treatments (Fig. 3).

3.2. Blood pressure and heart rate

Phenylephrine increased blood pressure in a dose-dependent manner and this effect was reproducible in all three cycles in the control group (Fig. 4). The low and middle infusion rates of all three drugs (clofilium, erythromycin and terikalant) had no or only a mild effect on mean arterial blood pressure, whereas their greatest rates lowered pressure significantly compared to control (Fig. 4).

Phenylephrine also reduced heart rate in a dose-dependent manner in all three dosing cycles, though there was only partial recovery from bradycardia during the 10 min drug-free intervals following each infusion in the control group (Fig. 5). Clofilium and terikalant had no effect on heart rate, whereas erythromycin attenuated the phenylephrine-induced bradycardia but only at the highest infusion rate (Fig. 5).

3.3. ECG intervals and epicardial monophasic action potential duration

Phenylephrine prolonged QT intervals markedly in the 1st dosing cycle in the control group (Fig. 6). In the following two dosing cycles, phenylephrine infusions caused only mild further prolongation of QT intervals

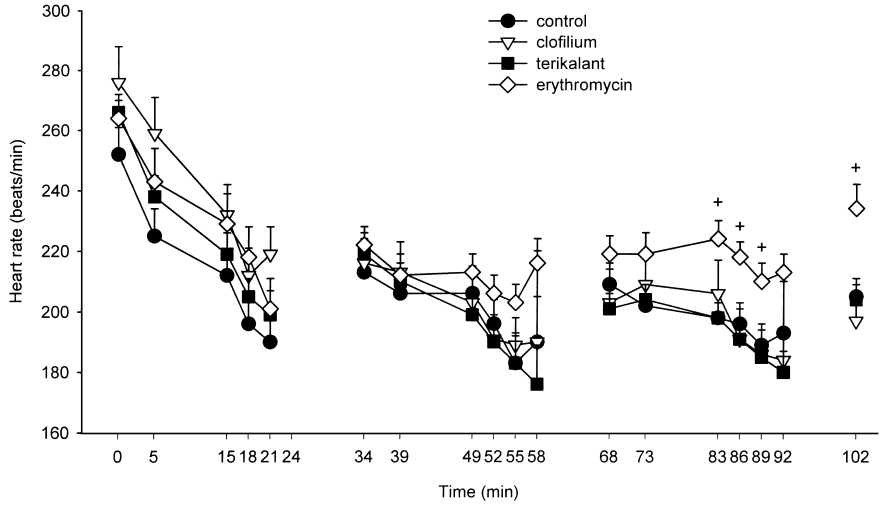

Fig. 5. Heart rate in rabbits treated with clofilium, terikalant and erythromycin during the three dosing cycles (see Fig. 1 for protocol). All values shown as mean ± S.E.M. $^{+}P<0.05$ erythromycin vs. control, Kruskal–Wallis test. Heart rate values were not determined from 18 to 24 min and from 55 to 58 min due to frequent arrhythmias.

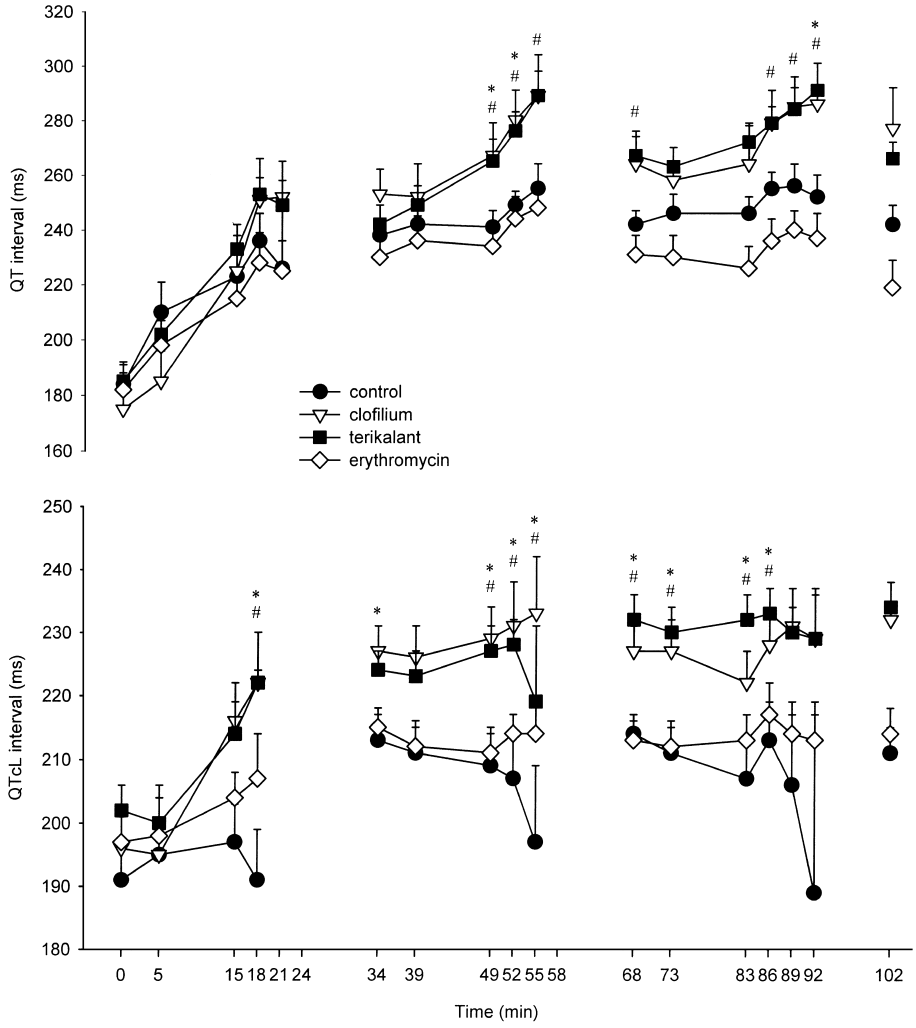

Fig. 6. QT intervals and rate corrected QT (QTcL) intervals in rabbits treated with clofilium, terikalant, erythromycin or vehicle (control) during the three dosing cycles (see Fig. 1 for protocol). All values shown as mean ± S.E.M. * $P<0.05$ clofilium vs. control; # $P<0.05$ terikalant vs. control, Kruskal–Wallis test. QT and QTcL values were not measured from 18 to 24 min and from 55 to 58 min due to frequent arrhythmias.

(Fig. 6). Both the middle and highest infusion rates of clofilium and terikalant increased QT intervals significantly compared to control. In contrast, erythromycin had no effect on QT intervals (Fig. 6). Qualitatively, similar results were gained when QT intervals were corrected for heart rate. All three doses of clofilium and terikalant prolonged QTc significantly compared to control, whereas erythromycin had no effect on QTc (Fig. 6).

There were no differences in PR or QRS intervals at any time point among the groups. However, within group analysis (Friedman test) revealed that clofilium increased the duration of the PR and QRS intervals modestly, e.g. the PR interval increased from 62 ± 1 ms at baseline to 71 ± 3 ms at 49 min and the QRS interval increased from 39 ± 3 to 51 ± 4 ms over the same time period. Terikalant did not alter QRS intervals and only increased the PR interval significantly at one time point, from a baseline value of 63 ± 2 ms to 67 ± 1 ms at the end of the second cycle of drug administration (58 min). Erythromycin caused a small increase in QRS interval, e.g. from 38 ± 3 to 44 ± 4 ms at 58 min, but did not alter PR intervals. There were no significant changes in PR or QRS intervals within the control group.

Phenylephrine prolonged the duration of the epicardial monophasic action potential mildly in the control group (Fig. 7). However, even the lowest infusion rate (20 nmol kg^{-1} min^{-1}) of clofilium prolonged epicardial monophasic action potential duration, such that it was significantly different from all the other groups (Fig. 7). Terikalant also prolonged epicardial monophasic action potential duration significantly but only at the middle and high infusion rates. In contrast, erythromycin did not alter the duration of the epicardial monophasic action potential (Fig. 7).

3.4. Noncardiac effects, body temperature, blood gases, pH^+ and K^+

Erythromycin exerted marked gastrointestinal prokinetic activity, inducing defaecation in six out of eight animals. In

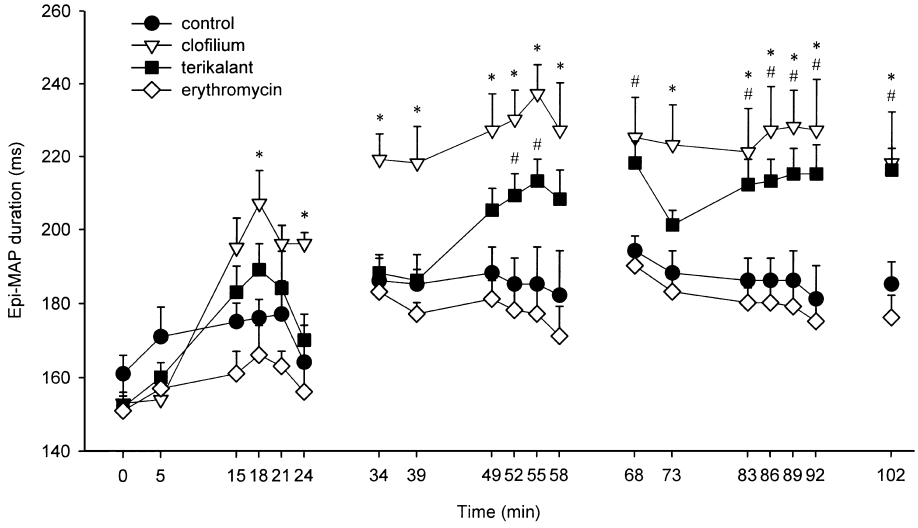

Fig. 7. Duration of epicardial monophasic action potential in rabbits treated with clofilium, terikalant, erythromycin or vehicle (control) during the three dosing cycles (see Fig. 1. for protocol). All values shown as mean ± S.E.M. *$P<0.05$ clofilium vs. control; #$P<0.05$ terikalant vs. control, Kruskal–Wallis test.

four animals this prokinetic effect occurred during the 2nd infusion of erythromycin (400 nmol kg^{-1} min^{-1}), whereas in one animal it occurred during the 1st infusion of erythromycin (133 nmol kg^{-1} min^{-1}), and in another one during the 3rd infusion of the drug (1330 nmol kg^{-1} min^{-1}).

Interestingly, as a result of anaesthesia and artificial ventilation, markedly low baseline blood K^+ values were measured. Baseline blood gas and K^+ values were $P_{O_2}=102\pm2$ mm Hg, $P_{CO_2}=24\pm1$ mm Hg, $pH^+=7.43\pm0.01$, $K^+=1.19\pm0.07$ mmol l^{-1} ($n=36$). There was a strong correlation between baseline K^+ and P_{CO_2} values ($r=0.758$, $P<0.05$). Blood K^+ values were measured in 18 conscious rabbits and then after pentobarbitone anaesthesia and surgical preparation and the K^+ values were 5.26 ± 0.05 and 1.30 ± 0.02 mmol l^{-1}, respectively ($P<0.05$). There were no significant differences between the blood gases, pH^+ and K^+ values of the groups at any time during the experiments and, apart from K^+, all of these variables were mostly within the physiological ranges (Table 1). Although blood K^+ increased following a rise in P_{CO_2} and a slight decrease of P_{O_2} and pH^+ in each group at the end of the 1st dosing cycle, it still remained at a low level during the whole experiment (Table 1). Body temper-

Table 1
Temperature, arterial blood gases, pH^+ and blood K^+ values measured before protocol and after the 1st, 2nd and 3rd infusions of drugs

	Rate (nmol kg^{-1} min^{-1})	n	Temperature (°C)	pH^+ (units)	P_{O_2} (mm Hg)	P_{CO_2} (mm Hg)	K^+ (mmol l^{-1})
Baseline							
Control	0	8	38.9 ± 0.3	7.44 ± 0.02	112 ± 3	23 ± 1	1.13 ± 0.12
Clofilium	0	10	39.2 ± 0.4	7.44 ± 0.01	96 ± 5	24 ± 2	1.23 ± 0.10
Terikalant	0	10	38.7 ± 0.3	7.42 ± 0.02	101 ± 5	26 ± 2	1.31 ± 0.16
Erythromycin	0	8	38.8 ± 0.3	7.44 ± 0.02	99 ± 5	23 ± 1	1.04 ± 0.14
After 1st infusion							
Control	0	8	38.7 ± 0.3	7.39 ± 0.02	99 ± 2	34 ± 2	2.54 ± 0.23
Clofilium	20	10	39.1 ± 0.3	7.37 ± 0.01	92 ± 3	36 ± 3	2.58 ± 0.24
Terikalant	2.5	10	38.5 ± 0.2	7.38 ± 0.02	94 ± 4	34 ± 2	2.34 ± 0.26
Erythromycin	133	8	38.6 ± 0.3	7.37 ± 0.03	93 ± 4	39 ± 2	2.61 ± 0.18
After 2nd infusion							
Control	0	8	38.5 ± 0.4	7.35 ± 0.02	99 ± 3	34 ± 3	2.64 ± 0.32
Clofilium	60	10	39.0 ± 0.3	7.33 ± 0.01	90 ± 4	37 ± 2	2.54 ± 0.08
Terikalant	7.5	10	38.4 ± 0.3	7.36 ± 0.02	93 ± 4	34 ± 2	2.39 ± 0.16
Erythromycin	400	8	38.4 ± 0.3	7.31 ± 0.04	90 ± 3	39 ± 1	2.76 ± 0.15
After 3rd infusion							
Control	0	8	38.3 ± 0.4	7.33 ± 0.02	101 ± 3	35 ± 2	2.78 ± 0.27
Clofilium	200	10	38.9 ± 0.3	7.28 ± 0.02	89 ± 4	37 ± 2	2.81 ± 0.08
Terikalant	25	10	38.2 ± 0.3	7.34 ± 0.02	94 ± 4	36 ± 1	2.46 ± 0.18
Erythromycin	1330	8	38.1 ± 0.3	7.31 ± 0.03	94 ± 3	35 ± 1	2.52 ± 0.16

n, number of animals. All data shown as mean ± S.E.M.

ature of the animals ranged between 38.1 and 39.2 °C and there were no significant differences between the groups (Table 1).

4. Discussion

The present data indicate that despite being given in high doses, erythromycin neither prolonged repolarisation (i.e. QT interval, QTc interval or epicardial monophasic action potential duration) nor evoked torsade de pointes in α_1-adrenoceptor-stimulated anaesthetized rabbits. Furthermore, torsade de pointes was evoked only by clofilium and terikalant. However, the incidence of this arrhythmia was significantly higher compared to control only in the clofilium-treated group, although terikalant widened QT and QTc intervals to a similar extent to clofilium. Moreover, the present results showed that the incidence of torsade de pointes was not directly proportional to the infusion rate or dose of clofilium or terikalant.

4.1. Mild proarrhythmic activity of terikalant

The proarrhythmic effects of terikalant have been examined for the first time and the results show that despite prolonging the QTc interval and monophasic action potential duration markedly, terikalant evoked torsade de pointes infrequently in α_1-adrenoceptor-stimulated anaesthetized rabbits. Presumably, the reason for this low proarrhythmic activity of the drug is not related to an inhibitory effect on α_1-adrenoceptors or to any effect on heart rate or blood pressure, since the marked haemodynamic effects of phenylephrine, i.e. bradycardia and high blood pressure, were not or only mildly affected by terikalant and the changes in haemodynamics did not differ from those induced by the highly proarrhythmic clofilium. This is supported by the notion that there has been no data published yet showing a α_1-adrenoceptor inhibitory effect of terikalant.

Terikalant is the active enantiomer of the racemic compound RP58866 (1-[-2-(3,4-dihydro-2*H*-1-benzopyran-4-yl)ethyl]-4-(3,4-dimethoxyphenyl)-piperidine hydrochloride). Previously, both RP58866 and clofilium have been reported to cause a similar incidence of torsade de pointes (Bril et al., 1996). The dose of RP58866 used by Bril et al. (1996) was equivalent to the middle dose of terikalant used here, so it is unlikely that the lesser proarrhythmic activity of terikalant reported here is due to differences in dosage. It is possible, however, that differences in the relative abilities of the compounds to block I_{Kr} and I_{K1} may influence their proarrhythmic potential. The IC_{50} values for blockade of I_{Kr} by RP58866 and terikalant were 22 and 31 nmol l^{-1}, respectively, whereas the corresponding IC_{50} values for blockade of I_{K1} were 8 and 6 μmol l^{-1} (Jurkiewicz et al., 1996). Thus, the ratio of I_{Kr} to I_{K1} blockade is higher for RP58866 than for terikalant, thus providing one possible explanation for differences in their proarrhythmic activity.

Terikalant evoked fewer conduction blocks than clofilium did, which suggests that the drug exerted a milder effect on the effective refractory period of the conductive system of the heart than clofilium did. Conduction blocks may play a role in torsade de pointes generation both in man (Houltz et al., 1998) and in rabbits (Farkas et al., 2002). Therefore, the infrequent occurrence of terikalant-induced conduction blocks may have contributed to the low proarrhythmic profile of the drug.

There was no significant difference between the effects of terikalant and clofilium on the QT and QTc intervals, which implies that the overall repolarisation prolonging effects of the two drugs were equivalent in the major part of the heart in the circumstances of the present study. However, the difference observed between the left ventricular epicardial monophasic action potential duration-prolonging effects of terikalant and clofilium and the differences between the incidences of block show that the repolarisation-prolonging effects of these two drugs vary considerably in different cells and areas of the rabbit heart. This difference in activity in different areas may result from the differences in molecular targets of these drugs. Terikalant blocks I_{K1} (Escande et al., 1992), I_{to} (McLarnon and Xu, 1995) and I_{Kr} (Jurkiewicz et al., 1996), whereas clofilium inhibits I_{Kr}, (Li et al., 1996), I_{Ks} (Varnum et al., 1993), I_{to} (Castle, 1991), I_{KATP} (Sakuta et al., 1993) and to some extent I_{K1} (Li et al., 1996). Clofilium also reduces I_{Na} and $I_{Ca\text{-}L}$ in the same concentration range (Li et al., 1996). This different ion channel selectivity and the resulting difference between the effects of the two drugs on the repolarisation phase of different types of myocytes (e.g. Purkinje fibres, M cells, endocardial and epicardial cells) may be a reason for the lesser proarrhythmic activity of terikalant compared to clofilium in the present study. However, a low proarrhythmic activity of a drug in α_1-adrenoceptor-stimulated anaesthetized rabbits does not necessarily mean that the drug is harmless in man. Quinidine, a highly proarrhythmic drug in man, does not evoke torsade de pointes in α_1-adrenoceptor-stimulated anaesthetized rabbits (Lu et al., 2000; Farkas et al., 2002). Likewise, terfenadine and cisapride, two noncardiac drugs known to evoke torsade de pointes in man, showed low proarrhythmic activity in α_1-adrenoceptor-stimulated anaesthetized rabbits (Batey and Coker, 2002; Carlsson et al., 1997; Lu et al., 2000). Therefore, further investigations are needed to prove that the use of terikalant is safe enough in man.

4.2. Lack of proarrhythmia with erythromycin

Despite being given in high doses, erythromycin neither prolonged repolarisation (i.e. QT, QTc or epicardial monophasic action potential duration) nor evoked torsade de pointes in the present study. The marked gastrointestinal prokinetic activity and the heart rate and blood pressure effects of the drug give encouraging evidence that the drug was successfully administered to the circulation and the

applied doses (infusion rates) were great enough. Despite these ancillary effects, erythromycin did not lengthen myocardial repolarisation in the present study. On the contrary, the drug prolonged repolarisation in both of the in vivo studies that examined the cardiac effects of erythromycin previously (Hanada et al., 1999; Rubart et al., 1993). However, in the study of Rubart et al. (1993), repolarisation, measured as the duration of the monophasic action potential, was prolonged and early afterdepolarisations (but not torsade de pointes) were induced in anaesthetized dogs only by extremely high intravenous doses of erythromycin (40–120 mg kg^{-1}). These doses are approximately 1.5–5 times higher than the large cumulative dose (26 mg kg^{-1}) that was administered to the animals in the present study. Interestingly, erythromycin infusion prolonged the QT interval at the rate of 4–8 mg kg^{-1} h^{-1} in anaesthetized rats in the study of Hanada et al. (1999), though the drug is reported to block only I_{Kr} (Antzelevitch et al., 1996; Daleau et al., 1995; West et al., 1998) and rat lacks functional delayed rectifier current (I_K) (Rees and Curtis, 1993; Varro et al., 1993). Erythromycin exerted a class III antiarrhythmic effect, i.e. prolonged repolarisation in several in vitro investigations. Clinically relevant concentrations of the drug (~ 100 μM) lengthened action potential duration as well as induced early afterdepolarisations in canine Purkinje fibres (Fazekas et al., 1998; Rubart et al., 1993; West et al., 1998) and M cells (Antzelevitch et al., 1996). Similar concentrations of erythromycin (100 μM) prolonged action potential duration in isolated Langendorff perfused guinea pig hearts (Daleau et al., 1995; Morey et al., 1997) and in isolated Langendorff perfused rabbit hearts (Drici et al., 1998, 1999; Eckardt et al., 1998; Hondeghem et al., 2001b). Furthermore, spontaneous torsade de pointes occurred due to erythromycin (100 μM) in isolated Langendorff perfused rabbit hearts but only when perfusing K^+ was very low (1.5 mM) and marked bradycardia was present due to total atrioventricular conduction block (Eckardt et al., 1998).

In the present study male rabbits were used. Drici et al. (1998) showed that perfusion with erythromycin caused significantly greater QT prolongation in female rabbit hearts than in male hearts. This was attributed to greater baseline and drug-induced changes in QT intervals in female rabbit hearts compared to male hearts. I_{Kr} and I_{Kl} are found to be significantly lower in ventricular myocytes from female rabbits compared with those from males (Ebert et al., 1998). Thus, a gender difference may be a reason for ineffectiveness of erythromycin in terms of lacking effect on QT intervals and epicardial monophasic action potential duration and lacking significant proarrhythmic activity in the present study. However, Carlsson et al. (1990), who developed the anaesthetized rabbit model of torsade de pointes, and other authors (Batey and Coker, 2002; Buchanan et al., 1993; Farkas et al., 1998; Mazur et al., 1999) have used male rabbits successfully for examination of proarrhythmic activity of repolarisation prolonging agents. This suggests that even if erythromycin blocks I_{Kr} and prolongs repolarisation in vitro, the drug possesses significantly lower potency to lengthen repolarisation in rabbits in vivo than either clofilium or terikalant, which may be the main reason for the lack of erythromycin induced proarhythmia in the present study. This is in accordance with the clinical observation that erythromycin-induced prolongation of repolarisation and torsade de pointes are very rare in man (Drici et al., 1998).

4.3. QT interval prolongation and torsade de pointes

Although there was no significant difference between the effects of terikalant and clofilium on the QT and QTc intervals, clofilium evoked more torsade de pointes than terikalant did. Likewise, ibutilide and H 345/52 (3,7-diazabicyclo(3.3.1)nonane-3-carboxylic acid,7-((2*S*)3-(4-cyanophenoxy)-2-hydroxypropyl)-1,1-dimethylethyl ester) lengthened QT interval to the same extent, but the former induced significantly more torsade de pointes in α_1-adrenoceptor-stimulated anaesthetized rabbits (Amos et al., 2001). Similarly, E-4031 (*N*-(4-(1-[2-(6-methyl-2-pyridyl)ethyl]-4-piperidyl)-carbonyl]phenyl)), clofilium, dofetilide and RP58866 induced significantly more torsade de pointes than BRL-32872 (*N*-(3,4-dimethoxyphenyl)-*N*-[3[[2-(3,4-dimethoxyphenyl)ethyl]propyl]-4-nitrobenzamide hydrochloride) in α_1-adrenoceptor-stimulated anaesthetized rabbits, although BRL-32872 caused an increase in QT interval that was comparable to that observed with the other four Class III antiarrhythmic agents (Bril et al., 1996). This indicates that QT prolongation is not the only contributing factor to generation of torsade de pointes in these models. This is in accordance with both clinical (Julian et al., 1997) and experimental (Hondeghem et al., 2001a, 2001b) results showing that prolongation of repolarisation is not proarrhythmic per se.

4.4. Blood K^+ and blood gases

The very low baseline K^+ concentrations in this study were a consequence of pentobarbitone anaesthesia and ventilation status since blood K^+ was within the physiological range in conscious animals and the K^+ concentration directly correlated with P_{CO_2} after anaesthesia. In other studies with pentobarbitone-anaesthetized and artificially ventilated rabbits, blood K^+ concentrations of approximately 1.0–1.5 mmol l^{-1} (Lightbown et al., 2001) and 2.5 mmol l^{-1} (Barrett et al., 1997; Barrett and Walker, 1998) have been reported previously. Recently, we have measured blood K^+ values in another 27 conscious rabbits and then after pentobarbitone anaesthesia and surgical preparation. The K^+ values were 4.50 ± 0.12 and 2.30 ± 0.07 mmol l^{-1}, respectively (A. Farkas and S.J. Coker, unpublished observations). This blood K^+ value measured after anaesthesia is higher than that of the present study but

this higher K^+ concentration was a consequence of a higher P_{CO_2} value. The arterial blood gases were $P_{O_2} = 94 \pm 2$ mm Hg, $P_{CO_2} = 37 \pm 1$ mm Hg, $pH^+ = 7.42 \pm 0.01$ ($n = 27$) and there was a strong correlation between baseline K^+ and P_{CO_2} values ($r = 0.701$, $P < 0.05$) similar to that observed in the present study. The baseline K^+ values of our unpublished study, where higher baseline P_{CO_2} values were measured, are similar to those reported by Barrett et al. (1997) and Barrett and Walker (1998), whereas the baseline blood K^+ values as well as the P_{CO_2} values of the present study are similar to those reported by Lightbown et al. (2001). This illustrates the influence of blood P_{CO_2} on K^+ concentrations and emphasises the need for detailed reporting of blood gas and K^+ values in proarrhythmia experiments performed in anaesthetized and artificially ventilated rabbits.

According to the data of the present and our unpublished experiments (see above), absolute blood K^+ concentrations were still low ($\sim 2.3–2.8$ mmol l^{-1}) even when P_{CO_2} values were at the higher end of the physiological range and other parameters connected to ventilation (e.g. P_{O_2}, pH^+) were normal in anaesthetized rabbits. This and the fact that blood K^+ values were normal in conscious animals illustrates that pentobarbitone anaesthesia affected blood K^+ concentration on its own independently from ventilation status.

Hypokalaemia enhances the effects of I_{Kr} blocking drugs and predisposes to torsade de pointes (Yang and Roden, 1996). Thus, the low K^+ values measured in the present study might contribute to the sensitisation of the animals to proarrhythmic effects. However, the observed differences between the proarrhythmic activity of the examined drugs cannot be related to differences between anaesthesia or ventilation status, as these were not different between groups.

4.5. Conclusions

The present results show that only clofilium induced torsade de pointes frequently in α_1-adrenoceptor-stimulated anaesthetized rabbits, whereas terikalant evoked torsade de pointes infrequently, though the drug prolonged repolarisation to a similar extent to clofilium. This indicates that prolongation of repolarisation is not the only contributing factor to generation of torsade de pointes in this model. Furthermore, despite being given in high doses, erythromycin did not evoke torsade de pointes. The low potency of the drug to prolong repolarisation in vivo in rabbits may be the main reason for the lack of erythromycin-induced proarrhythmia in the present study.

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