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RESEARCH PAPER

Adrenaline reveals the torsadogenic effect of combined blockade of potassium channels in anaesthetized guinea pigs

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Background and purpose: Torsade de pointes (TdP) can be induced in several species by a reduction in cardiac repolarizing capacity. The aim of this study was to assess whether combined I_{Kr} and I_{Ks} blockade could induce TdP in anaesthetized guinea pigs and whether short-term variability (STV) or triangulation of action potentials could predict TdP.
Experimental approach: Experiments were performed in open-chest, pentobarbital-anaesthetized, adrenaline-stimulated male Dunkin Hartley guinea pigs, which received three consecutive i.v. infusions of either vehicle, the I_{Kr} blocker E-4031 (3, 10 and 30 nmol kg^{-1} min^{-1}), the I_{Ks} blocker HMR1556 (75, 250, 750 nmol kg^{-1} min^{-1}) or E-4031 and HMR1556 combined. Phenylephrine-stimulated guinea pigs were also treated with the K^+ channel blockers in combination. Arterial blood pressure, ECGs and epicardial monophasic action potential (MAP) were recorded.
Key results: TdP was observed in 75% of adrenaline-stimulated guinea pigs given the K^+ channel blockers in combination, but was not observed in guinea pigs treated with either I_K blocker alone, or in phenylephrine-stimulated guinea pigs. Salvos and ventricular tachycardia occurred with adrenaline but not with phenylephrine. No changes in STV or triangulation of the MAP signals were observed before TdP.
Conclusions and implications: Combined blockade of both I_{Kr} and I_{Ks} plus the addition of adrenaline were required to induce TdP in anaesthetized guinea pigs. This suggests that there must be sufficient depletion of repolarization reserve and an appropriate trigger for TdP to occur.
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Keywords: E-4031; guinea pig; HMR1556; I_{Kr}; I_{Ks}; K^+ channel blockers; proarrhythmia; repolarization reserve; torsade de pointes

Abbreviations: APD_{90}, action potential duration at 90% repolarization; AV, atrioventricular; I_K, delayed rectifier potassium current; I_{Kr}, rapidly activating delayed rectifier potassium current; I_{Ks}, slowly activating delayed rectifier potassium current; MAP, monophasic action potential; PEG, polyethyleneglycol; STV, short-term variability; TdP, torsade de pointes; VPB, ventricular premature beat; VT, ventricular tachycardia

Introduction

Torsade de pointes (TdP) is a potentially lethal ventricular arrhythmia associated with prolongation of the QT interval and can be induced by drugs that block cardiac repolarizing currents, such as the delayed rectifier potassium current (I_K). There are relatively few *in vivo* animal models in which TdP can be studied. The two most commonly used are an α-adrenoceptor-stimulated rabbit model (Carlsson *et al*., 1990; Batey and Coker, 2002) and a canine model of chronic atrioventricular (AV) block (Weissenburger *et al*., 1991; Vos *et al*., 1995). The induction of TdP in a whole animal model was first described in the early 1990s (Carlsson *et al*., 1990, 1993), and numerous blockers of the rapidly activating component of I_K (I_{Kr}) have been tested using Carlsson's anaesthetized rabbit model as described originally (Buchanan *et al*., 1993; Carlsson *et al*., 1997; Brooks *et al*., 2000; Lu *et al*., 2000; Wu *et al*., 2005), or with slight modifications to the anaesthesia (Bril *et al*., 1996), or α_1-adrenoceptor agonist (Farkas *et al*., 1998). A further modification was introduced by Batey and Coker (2002) who used escalating doses of the α_1-adrenoceptor agonist, phenylephrine, in pentobarbital-anaesthetized rabbits. This variation of the model has also successfully demonstrated the torsadogenic activity of several drugs that block I_{Kr} (Batey and Coker, 2002; Farkas and Coker, 2002, 2003; Michael *et al*., 2007).

Species differences do occur in the density of I_{Kr} and also the slowly activating component of I_K (I_{Ks}). Guinea pigs

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exhibit a higher density of I_{Ks} over I_{Kr} than rabbits, giving rise to a larger total I_K in guinea pigs (Lu *et al*., 2001). *In vivo* guinea pig models have been used recently to predict the torsadogenic potential of drugs from their effects on cardiac repolarization (Hamlin *et al*., 2003; Fossa *et al*., 2004; Testai *et al*., 2004; Hauser *et al*., 2005), and guinea pig models are commonly used for toxicology and safety pharmacological assessment of QT prolongation. Previous work has shown that drugs that block I_{Kr}, such as cisapride and sotalol, prolonged repolarization in anaesthetized guinea pigs but did not cause TdP or other arrhythmias (Testai *et al*., 2004; Hauser *et al*., 2005) even in the presence of AV block (Batey and Coker, 2002). In contrast to I_{Kr} blockers, little work has been performed *in vivo* in guinea pigs using potent inhibitors of I_{Ks}. Chromanol 293B has been shown to prolong QT intervals in anaesthetized guinea pigs (Yang *et al*., 2004), and HMR1556 prolonged monophasic action potential (MAP) duration in guinea pig isolated, paced hearts (Gogelein *et al*., 2000). Despite all the convincing data that I_{Kr} and I_{Ks} blockers prolong repolarization in this species, there are only two studies in which TdP has been shown to occur in guinea pigs. One required the destruction of the sinus node and programmed electrical stimulation to reveal the torsadogenic actions of I_{Kr} blockers (Fossa *et al*., 2004) and the other described arrhythmias resembling TdP induced by the antihistamine, terfenadine, but no examples of this arrhythmia were shown (Hey *et al*., 1995).

The first aim of this study was to test the hypothesis that TdP will occur when cardiac repolarizing capacity is severely limited by combined blockade of I_{Kr} and I_{Ks} in the α_1-adrenoceptor-stimulated, open-chest, pentobarbital-anaesthetized guinea pig. As I_{Ks} is activated by β-adrenoceptor stimulation (Volders *et al*., 2003; Lu *et al*., 2005), a second hypothesis was tested, that β-adrenoceptor stimulation in addition to α-adrenoceptor stimulation is required to reveal the torsadogenic potential of these drugs in the anaesthetized guinea pig. In addition, the potential of changes in short-term variability (STV) of repolarization (Thomsen *et al*., 2004) and triangulation of action potentials (Hondeghem *et al*., 2001) to predict TdP have also been examined.

Methods

Animal preparation

All animal experiments were performed in accordance with the UK Animals (Scientific Procedures) Act 1986 and conducted under the authority of Project Licence number 40/2548. Male Dunkin Hartley guinea pigs (320–700 g) were purchased from B&K Universal (Hull, UK) and Harlan (Bicester, UK). Guinea pigs were anaesthetized by intraperitoneal administration of sodium pentobarbital (40–60 mg kg^{-1}). Most guinea pigs required on average a further 24 mg kg^{-1} of sodium pentobarbital administered in small doses at 15 min intervals to achieve a suitable level of anaesthesia for surgery. The trachea was cannulated to allow artificial ventilation. Initially, guinea pigs were ventilated with room air at 54 strokes per min and a stroke volume of 10 mL kg^{-1} body weight with an oxygen supplement. The ventilation rate and volume were then changed to 70 strokes per min and a stroke volume of 7 mL kg^{-1} body weight.

The right and left jugular veins were cannulated for drug administration, and the right carotid artery was cannulated for the purpose of measuring arterial blood pressure. ECG leads I, II and III were monitored using subcutaneous needle electrodes. An opening was made in the thorax by sectioning ribs three and four on the left side of the sternum. A pericardotomy was then performed to allow measurement of an epicardial MAP signal from the anterior surface of the left ventricle using a Hugo Sachs Elektronik spring-loaded Ag-AgCl epicardial MAP electrode (Linton Instrumentation, Diss, Norfolk, UK). All signals were recorded as described previously (Michael *et al*., 2007) using equipment detailed elsewhere (Farkas and Coker, 2002). Arterial blood gases, pH and K^+ were monitored at the end of the surgical preparation. There was an equilibration period of at least 10 min after the completion of the surgical preparation.

Experimental protocols

The arrhythmia induction protocol consisted of three cycles of drug administration. In the first study, the α-adrenoceptor agonist phenylephrine was infused at a rate of 75 nmol kg^{-1} min^{-1} for 15 min, then the dose of phenylephrine was increased to 150 nmol kg^{-1} min^{-1} for 3 min followed by further increases to 225 then to 300 nmol kg^{-1} min^{-1}. Five minutes into the first cycle, a concurrent infusion of the K^+ channel blockers was started. At the end of the cycle, all infusions were switched off and there was a 10 min drug-free interval. The doses of phenylephrine used in the second and third cycle were the same as in the first cycle, whereas the infusion rate of the K^+ channel blockers was increased threefold in the second cycle and tenfold in the third cycle. This drug infusion protocol has been illustrated in previous publications (Batey and Coker, 2002; Farkas and Coker, 2002). In the first study (phenylephrine study), guinea pigs received either the vehicle (saline and polyethyleneglycol 400 (PEG-400), $n=3$), E-4031 and HMR1556 combined, at the lower doses (E-4031: 1, 3 and 10 nmol kg^{-1} min^{-1}; HMR1556: 25, 75, 250 nmol kg^{-1} min^{-1}, $n=3$) or at the standard doses (E-4031: 3, 10 and 30 nmol kg^{-1} min^{-1}; HMR1556: 75, 250, 750 nmol kg^{-1} min^{-1}, $n=6$).

In the second study (adrenaline study), phenylephrine was replaced with adrenaline, which was infused at rates of 30, 60, 90 and 120 nmol kg^{-1} min^{-1} for 15, 3, 3 and 3 min, respectively. Guinea pigs were assigned randomly to receive either E-4031 alone at the standard doses (3, 10 and 30 nmol kg^{-1} min^{-1}, $n=8$), HMR1556 alone at the standard doses (75, 250, 750 nmol kg^{-1} min^{-1}, $n=8$), both drugs combined ($n=8$) or vehicle (saline infused at the same rate as E-4031 and PEG-400 infused at the same rate as HMR1556, $n=4$).

ECG analysis and arrhythmia diagnosis

ECG intervals were measured manually in beats originating from the sinoatrial node that were not preceded or followed by ectopic beats or conduction block, in the manner described previously (Farkas *et al*., 2004). At some time

points, ECG intervals could not be measured from all animals because of marked changes in morphology or arrhythmic activity. Normally, ECG intervals were measured from lead II, but in some experiments, ECG intervals were measured from lead III. All measurements were made from the same lead throughout the protocol for each animal. Ventricular premature beats (VPBs), bigeminy, salvos and ventricular tachycardia (VT) were defined according to the Lambeth Conventions (Walker *et al*., 1988). TdP was defined as a polymorphic VT of four or more beats, where twisting of the QRS complex around the isoelectric baseline was visible in at least one ECG lead. It coincided with a decline in arterial blood pressure towards zero with relatively little pulsatile activity. Conduction block, either intraventricular such as bundle branch block, or AV block was also identified and quantified.

QT interval correction

QT values were corrected for heart rate using a correction factor derived from baseline heart rate and QT intervals from each animal included in each study. The correction factor was based on that originally described by Carlsson *et al*. (1993) and subsequently modified by others (Batey and Coker, 2002; Farkas and Coker, 2002, 2003). For the phenylephrine study, the rate-corrected QT interval (QTc) was $QT-0.699(RR-254)$, and for the adrenaline study, the formula was $QTc = QT-0.747(RR-220)$, which corrected QT intervals to the mean baseline heart rate observed in that study.

Beat-to-beat variability of repolarization

The epicardial MAP signal was analysed using Electrophysiology Data Recorder (EDR) software (version 2.8; John Dempster, University of Strathclyde, Glasgow, UK) as described in detail previously (Michael *et al*., 2007). MAP durations were measured at 30 and 90% repolarization. To assess beat-to-beat variability of MAP duration at 90% repolarization (APD_{90}), values for 30 consecutive beats of sinus origin were measured before the start of the experimental protocol, that is at baseline, immediately before the first VPB, during each of the three cycles and before the first episode of TdP (or at an equivalent time point in guinea pigs that did not have TdP). From APD_{90}, Poincaré plots were produced by plotting each value against the former value. STV was determined as the mean orthogonal distance from the diagonal to the points of the plots and calculated according to the formula described previously (Thomsen *et al*., 2004; Detre *et al*., 2005) where $STV = \Sigma|D_{n+1}-D_n|/[30 \times \sqrt{2}]$ and D represents APD_{90}. Triangulation of the MAP ($APD_{90}-APD_{30}$), as defined by Hondeghem *et al*. (2001), was also calculated at these time points.

Pilot studies: dose selection and study design

Based on previous work in anaesthetized rabbits (Michael *et al*., 2007), preliminary studies with E-4031 in anaesthetized guinea pigs investigated the effects of two consecutive cycles of 24 nmol kg^{-1} min^{-1} E-4031 + phenylephrine ($n=3$). QT intervals were increased by 100%, one animal had a few VPBs and another had conduction block but no TdP was seen. With two consecutive cycles of 250 nmol kg^{-1} min^{-1} HMR1556 + phenylephrine ($n=3$), the QT interval was prolonged by 20% but arrhythmias only occurred in one animal and there was no TdP. It was therefore decided that to allow direct comparison with data obtained in the anaesthetized α_1-adrenoceptor-stimulated rabbit model (Michael *et al*., 2007), the doses of E-4031 and HMR1556 used previously in rabbits should also be used in guinea pigs. As the extent of QT and QTc prolongation was then found to be less in the guinea pigs than in the rabbits, additional experiments with three fold higher doses of both drugs were performed in the phenylephrine study and these higher doses were also chosen for use in the adrenaline study.

Statistics

Continuous data are expressed as mean ± s.e.mean. One-way ANOVA, with *post hoc* Tukey's tests, was used to compare haemodynamic and QT interval data at baseline and the 15-min time point in each cycle within and among groups. The General Linear Model with interaction was used to compare differences in profiles among treatment groups. The incidences of arrhythmias were compared by Fisher's exact probability test. STV and triangulation were compared with one-way ANOVA. Differences were considered statistically significant when $P<0.05$.

Drugs

HMR1556 ((3R,4S)-(+)-*N*-[3-hydroxy-2,2-dimethyl-6-(4,4,4-trifluorobutoxy)chroman-4-yl]-*N*-methylmethanesulphonamide; a gift from Sanofi-Aventis, Frankfurt, Germany) was dissolved at 3 mg mL^{-1} in PEG-400 each day. Stock solutions of E-4031 (1-[2-(6-methyl-2-pyridyl)ethyl]-4-(4-methylsulphonylaminobenzoyl)piperidine; purchased from Wako, Neuss, Germany) in normal saline (0.9% w/v NaCl in distilled H_20) were prepared in advance and stored in aliquots at −20 °C, then diluted to 51 μg mL^{-1} (lower dose) or 204 μg mL^{-1} (standard dose) with saline before use. L-Phenylephrine HCl and adrenaline bitartrate (Sigma, Poole, UK) were dissolved in saline at 1 and 0.612 mg mL^{-1}, respectively, and prepared freshly each day.

Results

Effects of E-4031 combined with HMR1556 in phenylephrine-stimulated guinea pigs

TdP was not observed in phenylephrine-stimulated guinea pigs receiving the combination of E-4031 and HMR1556 at the lower doses or at the standard doses. Conduction block was observed in all guinea pigs given K^+ channel blockers and included both bundle branch block and 2:1 AV block. The total duration of conduction block was 269 ± 135 s with the lower doses and 2099 ± 358 s with the standard doses. Apart from a few VPBs that occurred in one guinea pig receiving the standard doses, and the conduction block

already described, no other arrhythmias were seen in guinea pigs treated with the K^+ channel blockers in combination, in the presence of rising doses of phenylephrine.

Phenylephrine had no significant effect on heart rate over the course of the experimental protocol. Heart rate declined in guinea pigs treated with the K^+ channel blockers in combination at the standard doses, falling significantly from baseline during the second cycle. However, there were no differences in heart rate values between the treatment groups at the midpoint of the second cycle (Figure 1a). Phenylephrine did not prolong QT intervals significantly, whereas the K^+ channel blockers caused some prolongation of QT and QTc intervals (Figures 1b and c). The maximum percentage changes in QT intervals were $20 \pm 22\%$ with the vehicle, $44 \pm 17\%$ with the lower doses of the K^+ channel blockers and $76 \pm 6\%$* with the standard doses. The corresponding QTc changes were $5 \pm 8\%$, $18 \pm 6\%$ and $36 \pm 10\%$* (*$P<0.05$ compared with vehicle).

Marked phenylephrine-induced increases in mean arterial blood pressure were observed in guinea pigs receiving the vehicle in all three drug administration cycles, with recovery to baseline when the phenylephrine infusion was stopped (Figure 2a). Similar changes were observed in the presence of the combination of E-4031 and HMR1556, except during the third cycle where there appeared to be some attenuation of the phenylephrine-induced increase in mean arterial blood pressure with the standard doses of the combination. Statistical analysis of each cycle as a whole, revealed that

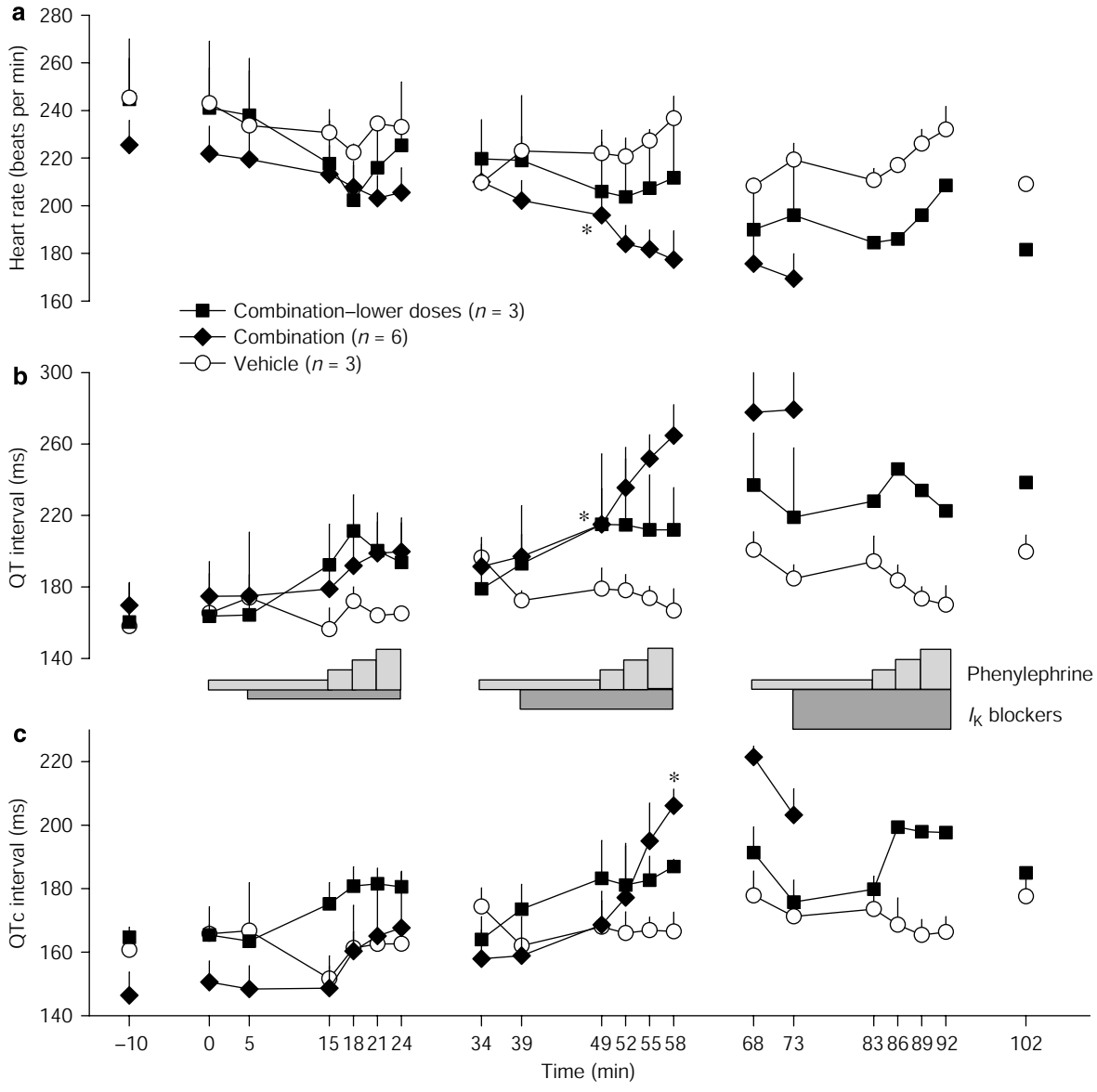

Figure 1 (**a**) Heart rate, (**b**) QT intervals and (**c**) QTc intervals in anaesthetized guinea pigs in the phenylephrine study. Guinea pigs received phenylephrine in the presence of E-4031 combined with HMR1556 at the lower doses (E-4031: 1, 3, 10 nmol kg^{-1} min^{-1}; HMR1556: 25, 75, 250 nmol kg^{-1} min^{-1}) or at the standard doses (E-4031: 3, 10, 30 nmol kg^{-1} min^{-1}; HMR1556: 75, 250, 750 nmol kg^{-1} min^{-1}). The light grey bars indicate when phenylephrine was administered and the dark grey bars indicate administration of I_K blockers. Values are expressed as mean ± s.e.mean. *$P<0.05$ compared with baseline (−10 min), one-way ANOVA. At some time points, there are no data or *n* is less than the stated values due to arrhythmias, which prevented accurate measurement of heart rate and ECG intervals. I_K, delayed rectifier potassium current.

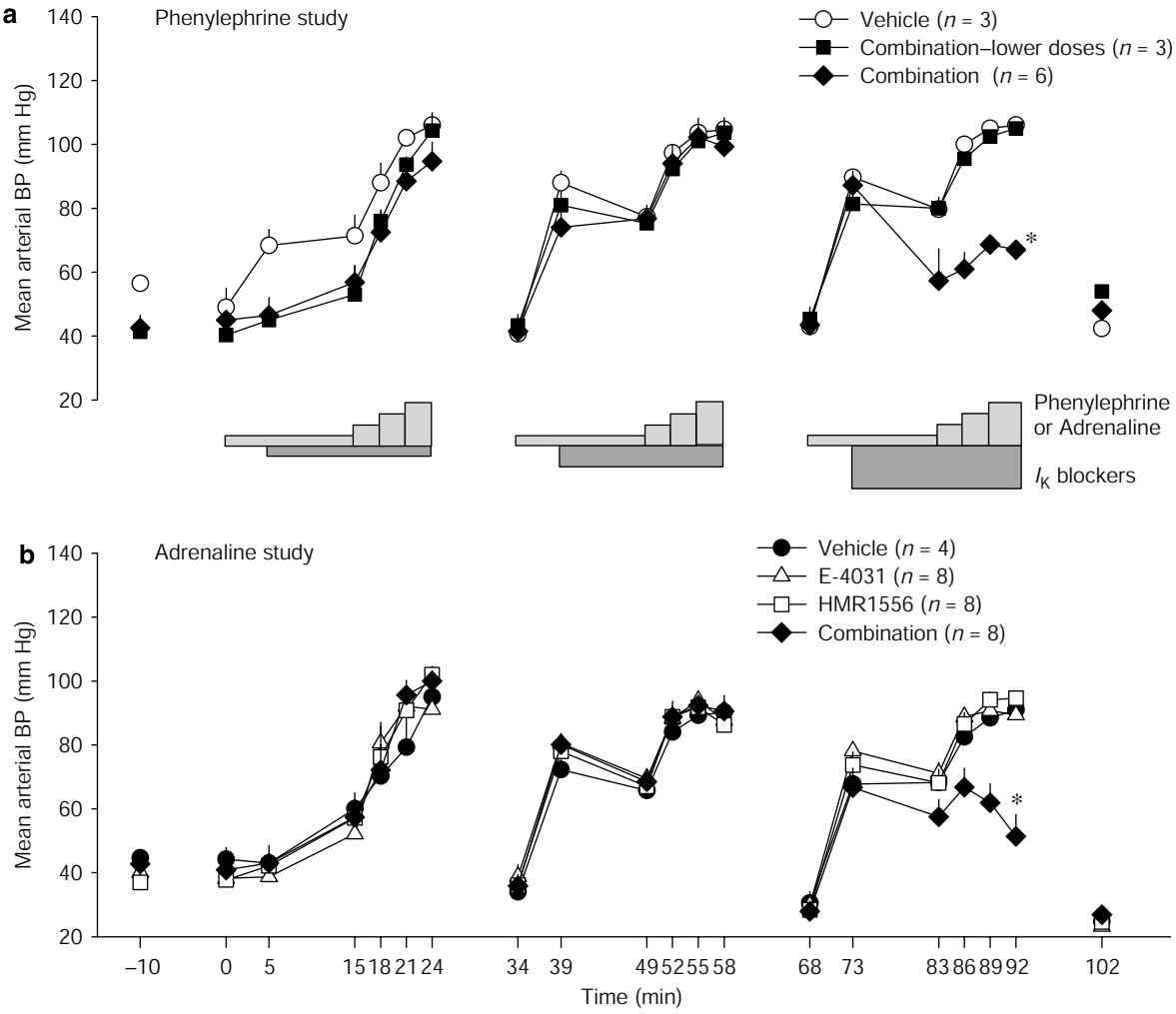

Figure 2 Mean arterial blood pressure (BP) in (**a**) the phenylephrine study where anaesthetized guinea pigs received phenylephrine and either E-4031 combined with HMR1556 at the lower doses (E-4031 1, 3, 10 nmol kg^{-1} min^{-1}; HMR1556: 25, 75, 250 nmol kg^{-1} min^{-1}) or at the standard doses (E-4031: 3, 10, 30 nmol kg^{-1} min^{-1}; HMR1556: 75, 250, 750 nmol kg^{-1} min^{-1}) and in (**b**) the adrenaline study where anaesthetized guinea pigs received adrenaline and either vehicle (saline + PEG-400), E-4031 (3, 10, 30 nmol kg^{-1} min^{-1}), HMR1556 (75, 250, 750 nmol kg^{-1} min^{-1}) or the combination of both drugs. The light grey bars indicate when phenylephrine or adrenaline was administered and the dark grey bars indicate administration of I_K blockers. Values are expressed as mean ± s.e.mean. *$P<0.05$ for the profile of all values in the third cycle in the combination group compared with other groups, General Linear Model with interaction. To aid clarity, symbols for the within-group statistical analyses have been omitted. At baseline and at the midpoint of each cycle, there were no significant differences among groups, one-way ANOVA. BP, arterial blood pressure. I_K, delayed rectifier potassium current.

in the third cycle, giving both drugs in combination at the standard doses gave rise to a significantly different profile of mean arterial blood pressure values than those in other treatment groups (Figure 2a). At this time, there was persistent conduction block resulting in reduced heart rate and therefore reduced cardiac output, which could explain why arterial blood pressure was lower.

Baseline blood gas, pH and K^+ values were $PO_2 = 97 \pm 11$ mm Hg, $PCO_2 = 30 \pm 2$ mm Hg, pH 7.57 ± 0.03 units, $K^+ = 3.25 \pm 0.38$ mmol L^{-1} ($n = 12$), and there were no significant differences among the treatment groups.

Effects of E-4031 alone, and in combination with HMR1556, on blood pressure in adrenaline-stimulated guinea pigs

The changes in mean arterial blood pressure throughout the course of the experimental protocol in the adrenaline study were similar to those seen in the phenylephrine study (Figure 2). Adrenaline significantly increased blood pressure in all three cycles with recovery to baseline when the adrenaline infusion was stopped. At the midpoint of each dosing cycle, the K^+ channel blockers, when administered alone or in combination, had no effect on the adrenaline-induced increase in blood pressure. Towards the end of the third cycle, however, E-4031 and HMR1556 in combination attenuated the effects of adrenaline on blood pressure (Figure 2b). As in the phenylephrine study, there was persistent conduction block in the adrenaline study at this time.

Effects of E-4031 alone, and in combination with HMR1556, on arrhythmias in adrenaline-stimulated guinea pigs

Figure 3 shows an example of an episode of drug-induced TdP from an adrenaline-stimulated guinea pig. TdP was

observed in a total of 75% of guinea pigs receiving E-4031 and HMR1556 in combination, but not with the vehicle or either K^+ channel blocker given alone (Figure 4a). TdP only occurred during the third cycle of drug infusion and guinea pigs that had TdP had on average a total duration of 52 ± 18 s of this arrhythmia.

Conduction block was observed in half of the guinea pigs given E-4031 alone, in one guinea pig given HMR1556 alone and in none of the guinea pigs given the vehicle (Figure 4b). Almost all the guinea pigs given the combination of both K^+ channel blockers had conduction block, and the total duration of this was 255 ± 110 s ($n = 7$). Figure 4 also summarizes the incidences of various other arrhythmias observed in anaesthetized guinea pigs in the adrenaline study. In contrast to the data from the phenylephrine study described above, almost all guinea pigs in the adrenaline study had VPBs and the majority also had salvos. All guinea pigs that had TdP also had bigeminy and VT.

Effects of E-4031 alone, and in combination with HMR1556, on heart rate and ECG intervals in adrenaline-stimulated guinea pigs

Adrenaline dose-dependently increased heart rate in all three drug administration cycles, with recovery to baseline values when the adrenaline infusion was stopped (Figure 5a). The K^+ channel blockers attenuated the adrenaline-induced increase in heart rate during the third cycle. Statistical comparison performed among the groups at the midpoint of the third cycle revealed that E-4031 significantly attenuated the adrenaline-induced increase in heart rate when administered alone, and HMR1556 potentiated the effects of E-4031 as heart rate was lowered further when these drugs were administered in combination (Figure 5a). This effect is probably a consequence of sustained conduction block.

Adrenaline abbreviated QT intervals in all three drug administration cycles with recovery to baseline values when the adrenaline infusion was stopped (Figure 5b). Similar changes were observed in the presence of the K^+ channel blockers, except during the third cycle where the QT-prolonging effects of these drugs were apparent. At the midpoint of the third cycle, QT intervals were significantly longer in guinea pigs administered E-4031 alone, or in combination with HMR1556, compared with the vehicle (Figure 5b). Within-group analysis also revealed that QT intervals were significantly prolonged compared with baseline in guinea pigs receiving the combination at this time point. At the midpoint of the third cycle, QT intervals changed from baseline by $-11 \pm 3\%$, $4 \pm 3\%$, $-8 \pm 3\%$ and $51 \pm 7\%$* (*$P < 0.05$ compared with vehicle) in the presence of the vehicle, E-4031 alone, HMR1556 alone and the combination, respectively (Figure 5b). However, when QT intervals were rate-corrected, no changes in QTc intervals were observed in any of the treatment groups and there were no differences among treatment groups at the midpoint of each cycle (Figure 5c).

Baseline PR and QRS intervals were 52 ± 1 and 44 ± 2 ms ($n = 28$), respectively, and there were no differences among the treatment groups. No changes were observed in PR intervals as the experimental protocol progressed, and QRS intervals were only significantly prolonged during the third cycle in the presence of the combination of both K^+ channel blockers.

Effects of E-4031 alone, and in combination with HMR1556, on action potential duration, variability of repolarization and triangulation

The pattern of changes in epicardial monophasic APD_{90} in each drug treatment group with time was similar to the pattern of changes in QT intervals shown in Figure 5b. Beat-

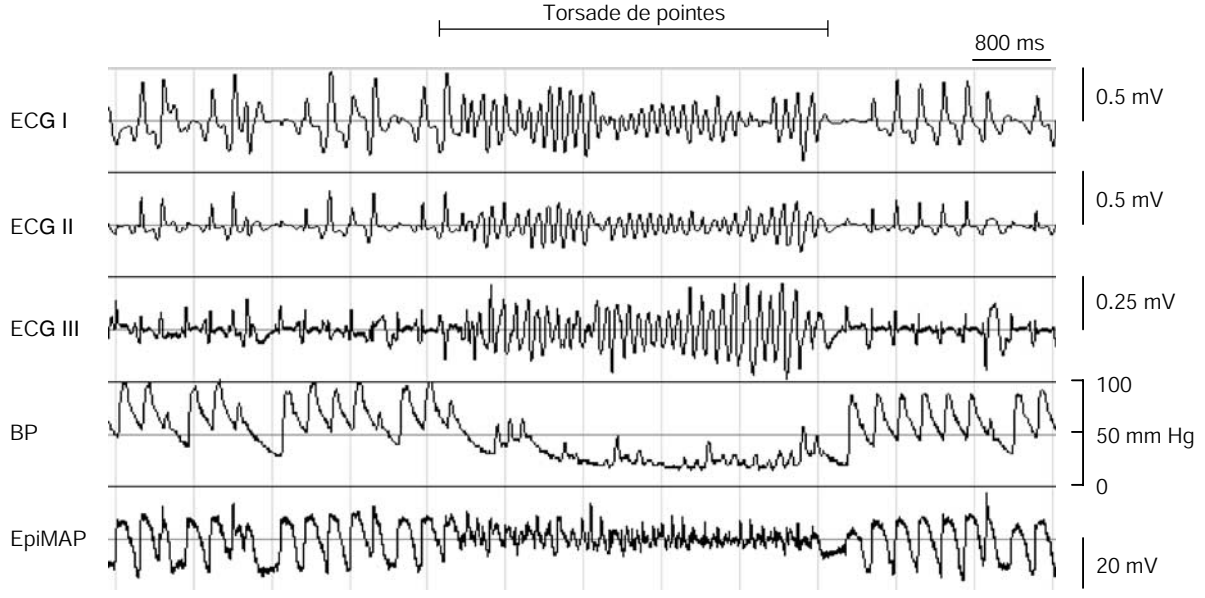

Figure 3 An example of drug-induced torsade de pointes in an anaesthetized guinea pig receiving the combination of E-4031 (3, 10, 30 nmol kg^{-1} min^{-1}) and HMR1556 (75, 250, 750 nmol kg^{-1} min^{-1}) in the presence of adrenaline. ECG I–III, lead I, lead II and lead III limb lead electrocardiograms; BP, arterial blood pressure; EpiMAP, epicardial monophasic action potential.

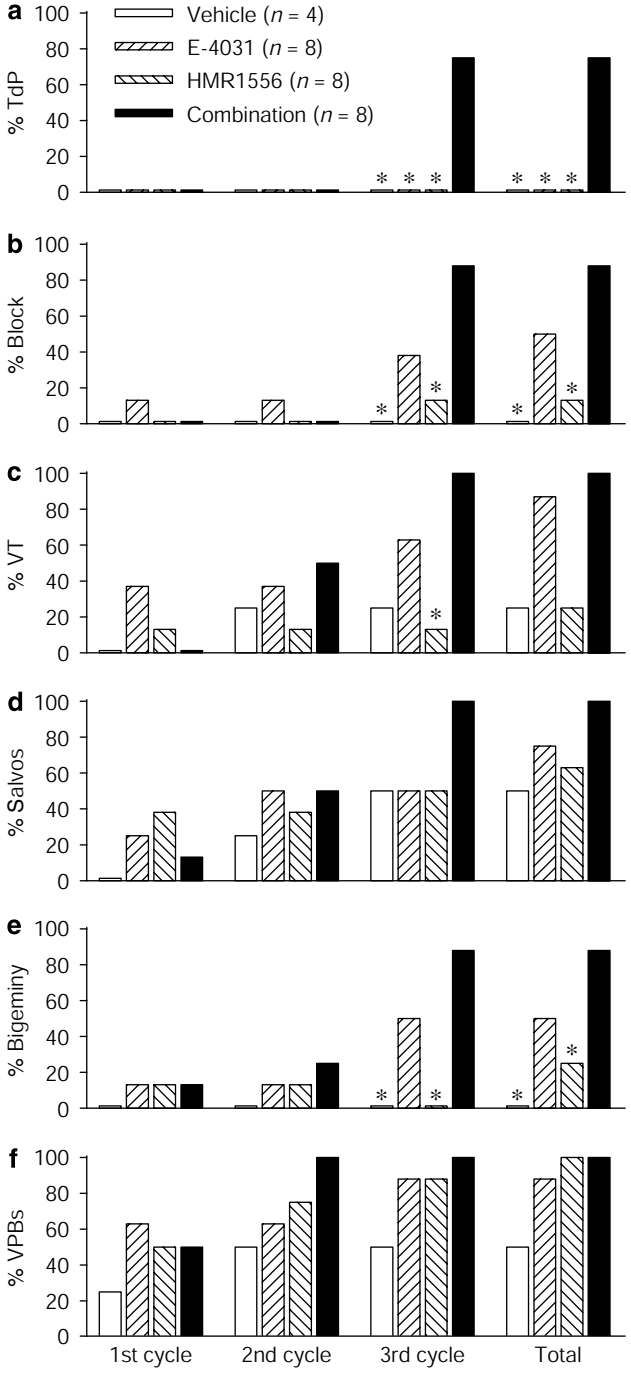

Figure 4 The incidence (expressed as percentage animals treated) of (**a**) torsade de pointes (TdP), (**b**) block, (**c**) VT, (**d**) salvos, (**e**) bigeminy and (**f**) VPBs in anaesthetized guinea pigs that received adrenaline and either vehicle (saline + PEG-400), E-4031 (3, 10, 30 nmol kg^{-1} min^{-1}), HMR1556 (75, 250, 750 nmol kg^{-1} min^{-1}) or the combination of both drugs. *$P<0.05$ compared with combination, Fisher's Exact test. Block, conduction block; VPBs, ventricular premature beats; VT, ventricular tachycardia.

to-beat variability of repolarization was assessed by calculating STV from Poincaré plots of the epicardial MAP. Figures 6a–d illustrate examples of Poincaré plots of APD_{90} from guinea pigs with and without TdP. Mean baseline STV of APD_{90} was calculated as 6.4 ± 0.8 ms in all guinea pigs in the adrenaline study, but neither of the K^+ channel blockers altered STV when given alone or in combination (Figure 6e). Even when the data were plotted by whether or not TdP occurred, there were no significant differences either within or among the groups (Figure 6f).

In the Poincaré plots in Figure 6, the longer term variation in APD_{90} can also be seen. In the presence of the lower doses of the I_K blockers, adrenaline tended to shorten APD_{90}, resulting in the measurements taken just before the occurrence of the first VPB being lower than the baseline values (Figures 6a–d). Despite the continued presence of adrenaline, infusion of the higher doses of both of the I_K blockers in the combination group appeared to lengthen APD_{90} when measured just before TdP usually occurred (Figure 6g), but this apparent increase just failed to be significantly different from the baseline or pre-VPB values ($P = 0.057$). However, the APD_{90} values in the combination group, at this time point, were significantly greater than the values in the other groups (Figure 6g). When the data were sorted according to the presence or absence of TdP, there was no difference in baseline APD_{90}; 133 ± 6 and 137 ± 6 ms, respectively, whereas in the guinea pigs that had TdP, there was significant lengthening of APD_{90} shortly before TdP occurred. In contrast, in the guinea pigs that did not have TdP, APD_{90} was still shorter than at baseline (Figure 6h).

Possible changes in action potential morphology were assessed by calculating triangulation of the MAP ($APD_{90}-APD_{30}$) at the same time points as STV had been measured. Mean baseline triangulation was 50 ± 5 ms for all guinea pigs in the adrenaline study. No clear change in triangulation was observed from baseline with the K^+ channel blockers administered alone or in combination (Figure 7a). When the data were sorted by the presence or absence of TdP, no changes in triangulation were observed in guinea pigs before the first arrhythmia, or before the first episode of TdP (Figure 7b).

Effects of E-4031 alone, and in combination with HMR1556, on blood gases and K^+ in adrenaline-stimulated guinea pigs

Baseline blood gas, pH and K^+ values were $PO_2 = 105 \pm 6$ mm Hg, $PCO_2 = 22 \pm 1$ mm Hg, pH = 7.52 ± 0.02 units, $K^+ = 3.97 \pm 0.18$ mmol L^{-1} ($n = 28$). There were no significant differences in blood gas and K^+ values among the treatment groups.

Discussion

This study is the first to clearly illustrate drug-induced TdP in intact anaesthetized guinea pigs with normal baseline heart rates. The experiments have shown that concomitant infusion of adrenaline was necessary to reveal the torsadogenic action of K^+ channel blockers. In addition, blockade of both I_{Kr} and I_{Ks} was required to decrease repolarization reserve sufficiently to induce TdP in anaesthetized guinea pigs.

Comparison of combined I_{Kr} and I_{Ks} blockade in phenylephrine-stimulated guinea pigs and rabbits

The combination of the K^+ channel blockers did not induce TdP in phenylephrine-stimulated guinea pigs. In a total of 15

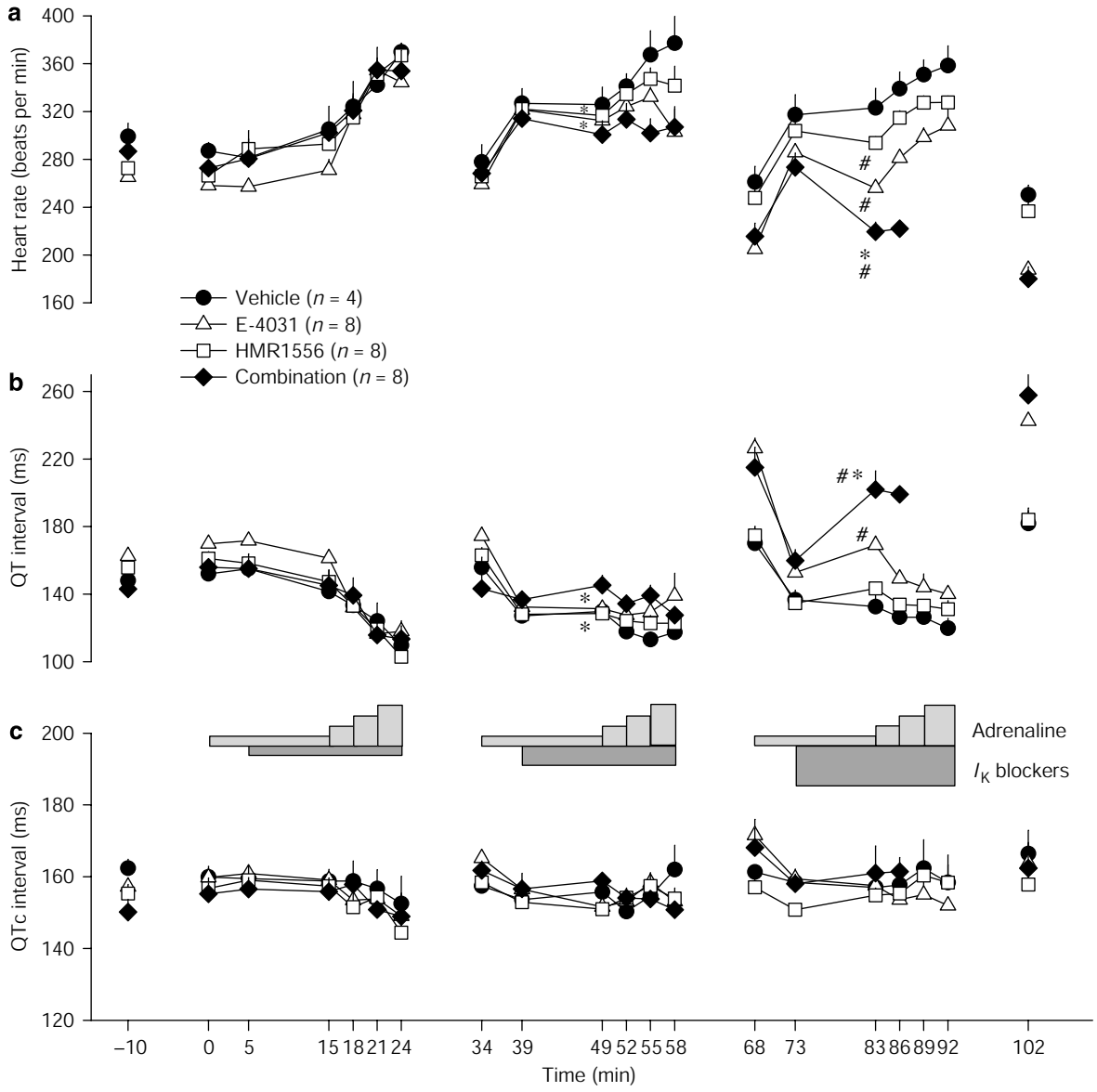

Figure 5 (**a**) Heart rate, (**b**) QT intervals and (**c**) QTc intervals in anaesthetized guinea pigs in the adrenaline study. Guinea pigs received adrenaline in the presence of either vehicle (saline + PEG-400), E-4031 (3, 10, 30 nmol kg^{-1} min^{-1}), HMR1556 (75, 250, 750 nmol kg^{-1} min^{-1}) or the combination of both drugs. The light grey bars indicate when adrenaline was administered and the dark grey bars indicate administration of I_K blockers. Values are expressed as mean ± s.e.mean. *$P<0.05$ compared with baseline (−10 min), $^{\#}P<0.05$ compared with vehicle group, one-way ANOVA. At some time points, *n* is less than the stated values due to arrhythmias, which prevented accurate measurement of heart rate and ECG intervals. I_K, delayed rectifier potassium current.

guinea pigs given phenylephrine with either E-4031 or HMR1556 alone (pilot studies), or in combination, TdP was never seen. This is in contrast to the effects of these drugs in phenylephrine-stimulated, anaesthetized rabbits (Michael *et al.*, 2007) where HMR1556 potentiated the torsadogenic effect of E-4031. Indeed, the lower doses of the K^+ channel blockers used in combination in this phenylephrine guinea pig study were torsadogenic in the rabbit model. This raises the question of why TdP was not observed in phenylephrine-stimulated guinea pigs but was observed in phenylephrine-stimulated rabbits.

Combined I_{Kr} and I_{Ks} blockade, at the standard doses, prolonged QT intervals to a greater extent in phenylephrine-stimulated guinea pigs (76 ± 6 ms) compared with phenylephrine-stimulated rabbits receiving the drugs at the lower doses (57 ± 6 ms) (Michael *et al.*, 2007). Thus, despite there being substantial prolongation of repolarization in both species, TdP did not occur in guinea pigs. It is clear from this study that the extent of QT prolongation is not the only factor involved in the development of TdP, a finding that is in agreement with others (Hondeghem *et al.*, 2001; Milberg *et al.*, 2002; Thomsen *et al.*, 2004). Thus when considering the use of *in vivo* guinea pig models to identify potential QT interval-prolonging effects of drugs (Hamlin *et al.*, 2003; Testai *et al.*, 2004; Hauser *et al.*, 2005), caution should be exercised when predicting the torsadogenic potential solely from the ability of a drug to prolong QT intervals.

Comparable to the findings from an α_1-adrenoceptor-stimulated rabbit model (Michael *et al.*, 2007), guinea pigs

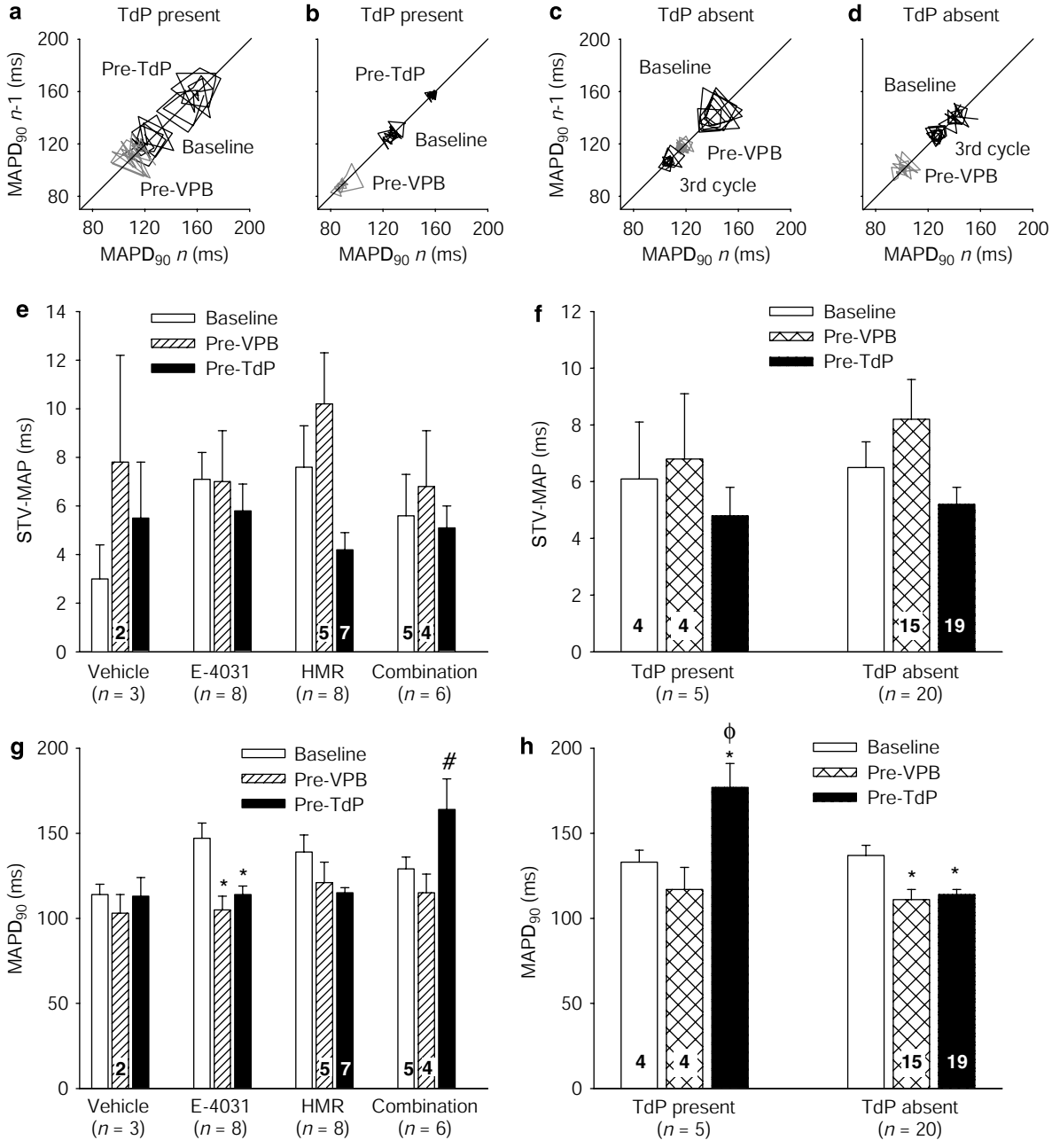

Figure 6 Adrenaline study: (**a**–**d**) examples of Poincaré plots of $MAPD_{90}$ at baseline, immediately before the first ventricular premature beat (Pre-VPB) and (**a** and **b**) before the first episode of torsade de pointes (Pre-TdP) or (**c** and **d**) at similar time points in guinea pigs that did not have TdP (3rd cycle). (**a** and **b**) Illustrate data from the combination group; (**c** and **d**) illustrate data from the E-4031 group. (**e**) STV-MAP by treatment group. (**f**) STV-MAP by presence or absence of TdP. (**g**) $MAPD_{90}$ by treatment group. (**h**) $MAPD_{90}$ by presence or absence of TdP. (**e**–**h**) Values are mean ± s.e.mean. $^{*}P<0.05$ compared with baseline values, $^{\#}P<0.05$ compared with vehicle, E-4031 and HMR1556 groups, $^{\phi}P<0.05$ compared with group with no TdP, one-way ANOVA. Numbers in columns indicate *n* values where these are less than the group value. $MAPD_{90}$, monophasic action potential duration at 90% repolarization; STV-MAP, short-term variability of the $MAPD_{90}$.

developed long periods of sustained conduction block when given with the combination of HMR1556 and E-4031. Despite conduction block being observed, TdP did not occur in phenylephrine-stimulated, anaesthetized guinea pigs. Thus, conduction block *per se* did not produce the experimental conditions required to cause TdP. It is interesting to note that, with the exception of conduction disturbances, very few arrhythmias were observed in phenylephrine-stimulated guinea pigs, which is in contrast with observations in rabbits (Batey and Coker, 2002; Farkas and Coker, 2002, 2003; Michael *et al*., 2007).

Although phenylephrine produced similar changes in arterial blood pressure in both species, it only induced bradycardia in rabbits (Batey and Coker, 2002; Farkas and Coker, 2002, 2003; Michael *et al*., 2007). Taken together, these findings suggest a similar response to phenylephrine at peripheral (vascular) α_1-adrenoceptors, but not at cardiac α_1-adrenceptors receptors, in rabbits and guinea pigs. Indeed,

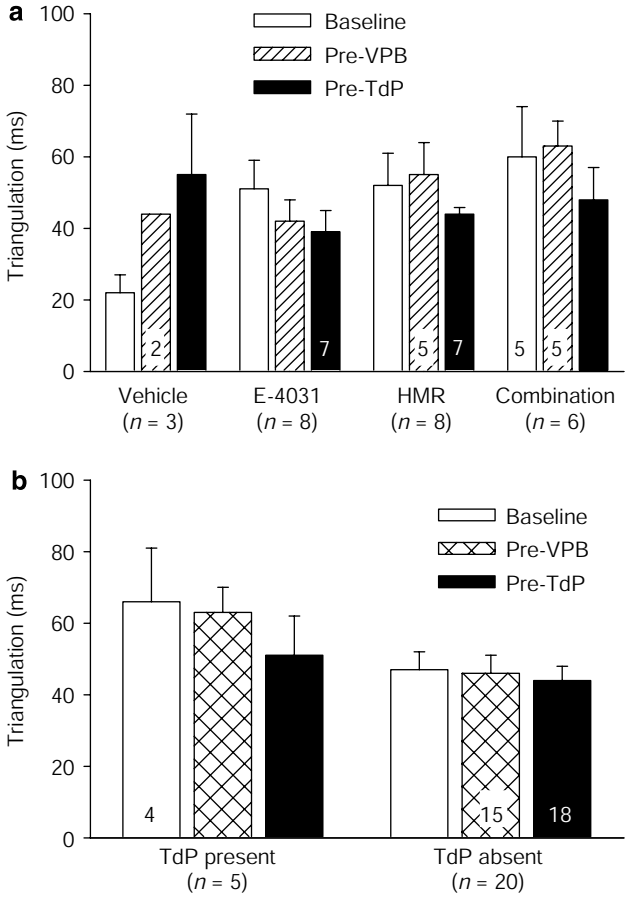

Figure 7 Action potential triangulation (APD_{90}–APD_{30}) in anaesthetized guinea pigs in the adrenaline study at baseline, immediately before the first ventricular premature beat (Pre-VPB) and before the first episode of torsade de pointes (Pre-TdP) or at similar time points in guinea pigs that did not have TdP. Values are expressed as mean ± s.e.mean (**a**) by treatment group and (**b**) by presence or absence of TdP. Numbers in columns indicate *n* values where these are less than the group value.

guinea pigs have few cardiac α_1-adrenoceptors (Hescheler *et al*., 1988; Chess-Williams *et al*., 1990), whereas in rabbit hearts the existence of cardiac α_1-adrenoceptors is of major importance (Schumann and Brodde, 1979). There is considerable evidence to suggest that stimulation of myocardial α_1-adrenoceptors increases intracellular Ca^{2+}, which can contribute to the generation of early afterdepolarizations (del Balzo *et al*., 1990; Molina-Viamonte *et al*., 1991) and triggered activity (Zipes, 2003). Thus, the relative lack of cardiac α_1-adrenoceptors may explain why phenylephrine could not provide the trigger that contributes to the induction of TdP in anaesthetized guinea pigs.

Adrenaline reveals the torsadogenic effect of combined I_{Kr} and I_{Ks} blockade in guinea pigs

It is evident from this study that adrenaline did provide the arrhythmogenic trigger that revealed the torsadogenic effect of E-4031 combined with HMR1556 in anaesthetized guinea pigs. The infusion of adrenaline induced many more arrhythmias (VPBs, bigeminy, salvos, VT) than were observed in phenylephrine-stimulated guinea pigs. The predominant adrenoceptor on guinea pig cardiac myocytes is the β_1-adrenoceptor (Broadley *et al*., 1986; Chess-Williams *et al*., 1990). It is likely, therefore, that adrenaline exerted direct effects on the myocardium via these receptors to cause the increase in intracellular Ca^{2+} (Hescheler *et al*., 1988) required for the initiation of triggered activity.

Adrenaline given alone increased heart rate and shortened QT intervals (see Figure 5). In the first two cycles of drug administration, this effect was evident in all the groups. By the beginning of the third cycle, however, QT intervals appeared to be longer in the groups that had received E-4031, and after starting the highest infusion rates of the K^+ channel blockers, there was significant bradycardia and QT intervals were prolonged by E-4031. Comparison of the percentage changes in the QT intervals from baseline to the middle of the third cycle revealed marked prolongation in the group receiving the combination of both I_K blockers. This may suggest that prolongation of QT intervals was important for the genesis of TdP. Analysis of the APD_{90} data confirmed that significant prolongation of repolarization did occur, but only in the guinea pigs which then had TdP. However, this cannot be the only factor contributing to the development of TdP, as extensive prolongation of repolarization was seen in the phenylephrine study but no TdP occurred. A major difference between the phenylephrine study and the adrenaline study was the much greater occurrence of arrhythmias in the guinea pigs treated with adrenaline. It seems likely that adrenaline promoted K^+ channel blocker-induced TdP because it favoured the occurrence of other arrhythmias, such as VPBs and salvos, which could trigger TdP. It has been shown previously in a rabbit model that animals that had more drug-induced VPBs were more likely to have TdP (Batey and Coker, 2002).

Another factor, which may be important for adrenaline to reveal the torsadogenic action of I_K blockade in this model, is the contribution of I_{Ks} to repolarization reserve in the guinea pig. Normally, activation of cardiac β-adrenoceptors by adrenaline will increase the activity of I_{Ks}, thus shortening ventricular repolarization and providing a physiological mechanism for protection against reentrant arrhythmias that may occur at fast heart rates (Schwartz *et al*., 2001). However, activation of cardiac β-adrenoceptors also increases L-type Ca^{2+} current (I_{CaL}). Under normal conditions, a greater increase in I_{Ks} rather than in I_{CaL} explains why action potential duration and QT intervals shorten, but when I_{Ks} is blocked then increased I_{CaL} is dominant, resulting in early afterdepolarizations that can trigger TdP (Shimizu and Antzelevitch, 1998). The lack of TdP in the guinea pigs treated with adrenaline and E-4031 indicates that the much larger I_{Ks} in the guinea pig compared with the rabbit (Lu *et al*., 2001) provided sufficient repolarization reserve to protect against the development of TdP. Combined blockade of both I_{Kr} and I_{Ks} and infusion of adrenaline were required for TdP to occur in anaesthetized guinea pigs.

Predictors of TdP: STV and triangulation of action potentials

The results of the present study indicate that TdP cannot be predicted by STV of APD_{90} or by triangulation of epicardial action potentials. A lack of predictive value for STV and

triangulation has also been reported previously in phenylephrine-stimulated, anaesthetized rabbits treated with K^+ channel blockers (Michael *et al*., 2007). This contrasts with findings that STV of left ventricular MAP predicted TdP in dogs with chronic AV block (Thomsen *et al*., 2004; Detre *et al*., 2005; Thomsen *et al*., 2006, 2007) and that triangulation was a predictor in studies in rabbit isolated perfused hearts (Hondeghem *et al*., 2001, 2003; Milberg *et al*., 2004). Bradycardia is a feature of both these models and it is possible that STV and action potential triangulation can only predict TdP that occurs at lower heart rates. However, in the present study, MAPs were only recorded from the epicardium of the left ventricle and it is possible that changes in triangulation and STV might have been detected from the endocardial surface, as this has been shown to be more sensitive to drug-induced changes *in vitro* (Liu *et al*., 2006) and *in vivo* (Gallacher *et al*., 2007).

In conscious dogs and anaesthetized rabbits, increased STV of QT intervals predicted TdP (Lengyel *et al*., 2007). However, in anaesthetized dogs (Thomsen *et al*., 2004) and anaesthetized α_1-adrenoceptor-stimulated rabbits (Michael *et al*., 2007; Vincze *et al*., 2008), no increases in STV of QT intervals were detected before TdP occurred. Although STV of QT intervals was not assessed in the guinea pigs, the lack of changes in STV of APD_{90} concurred with a similar finding in rabbits (Michael *et al*., 2007) and indicated that TdP cannot be predicted by changes in STV of repolarization in these models. Thus, in the absence of a positive control, if a drug does not alter STV of repolarization in any particular model it should not be assumed to be devoid of torsadogenic potential. The factors that did contribute to the development of TdP in the guinea pigs were reduction of repolarization reserve (QT interval and APD_{90} prolongation) and the occurrence of other arrhythmias.

Clinical implications

There are differences in the expression and function of I_{Kr} and I_{Ks} between rabbits and guinea pigs (Lu *et al*., 2001; Zicha *et al*., 2003). Total I_K is greater in guinea pigs than in rabbits, mainly because of the much greater expression of I_{Ks} in the former species. It has also been suggested that the expression of I_{Ks} in man is greater than in rabbits but less than in guinea pigs (Zicha *et al*., 2003). Although the kinetics of I_{Ks} activation and recovery in the human heart are more like those in dog and rabbit, rather than guinea pig (Jost *et al*., 2007), adrenaline does reveal the important contribution of I_{Ks} to repolarization reserve in the human heart (Jost *et al*., 2005). Thus, comparison of data from both guinea pig and rabbit models of drug-induced TdP could improve extrapolation to the human situation.

Benefits and limitations of the guinea pig model

The adrenaline-stimulated guinea pig model described here provides a novel method for inducing TdP in a small animal in which I_{Ks} is a major contributor to cardiac repolarization. To induce TdP in this model, blockade of I_{Ks}, in addition to blockade of I_{Kr}, and the introduction of a trigger, in the form of VPBs, was required. Recently, models of drug-induced TdP involving administration of an I_{Ks} blocker to either conscious (Lengyel *et al*., 2007) or anaesthetized dogs (Gallacher *et al*., 2007) have been described. The guinea pig model described here provides cost and ethical advantages over the use of dogs in safety pharmacology. The ability to induce TdP in a species other than the dog or rabbit, with a different balance of K^+ currents, will also be useful for those interested in exploring mechanisms involved in arrhythmogenesis. One limitation of the guinea pig model is the relatively high doses of drugs required to achieve a significant reduction of repolarization reserve and induce TdP.

Conclusions

This study has demonstrated that adrenaline revealed the torsadogenic effect of combined pharmacological blockade of I_{Kr} and I_{Ks} in anaesthetized guinea pigs. The model provides a novel and simple method for inducing TdP with drugs in a small animal, which may be useful in safety pharmacology and in the study of mechanisms contributing to the development of arrhythmias. These studies emphasize the importance of achieving sufficient reduction of repolarization reserve and the provision of an appropriate 'trigger' for the induction of TdP.

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Conflict of interest

The authors state no conflict of interest.

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