eppendorf

# Reach Your 100%

**Your success is our purpose**

Your time is precious not only in terms of cost. However, a drug's journey from concept to patient takes years. You need to rely on products and services that ensure maximum efficiency at every step.

Our high-quality solutions are all engineered around the kind of functionality and intuitive performance necessary to speed up workflows, secure reproducibility, and accelerate the desired results.

Let's work together to make our world a better place.

**www.eppendorf.com/pharma**

British Journal of Pharmacology (2008) 153, 75–89

0007–1188/08 $30.00
www.brjpharmacol.org

RESEARCH PAPER

# Relevance of anaesthesia for dofetilide-induced torsades de pointes in $\alpha_1$-adrenoceptor-stimulated rabbits

D Vincze[1], AS Farkas[2], L Rudas[1], P Makra[3], N Csík[4], I Leprán[5], T Forster[2], M Csanády[2], JG Papp[5,6], A Varró[5,6] and A Farkas[2]

[1]*Department of Anaesthesiology and Intensive Care, University of Szeged, Szeged, Hungary;* [2]*2nd Department of Internal Medicine and Cardiology Centre, University of Szeged, Szeged, Hungary;* [3]*Department of Experimental Physics, University of Szeged, Szeged, Hungary;* [4]*Department of Electrical Engineering and Cybernetics, Faculty of Mechanical Engineering and Automation, Kecskemét College, Kecskemét, Hungary;* [5]*Department of Pharmacology and Pharmacotherapy, University of Szeged, Szeged, Hungary and* [6]*Division of Cardiovascular Pharmacology, Hungarian Academy of Sciences, Szeged, Hungary*

**Background and purpose:** No information is available concerning the effects of anaesthetics in the most frequently used *in vivo* pro-arrhythmia model. Accordingly, in this study we examined the effect of pentobarbital, propofol or α-chloralose anaesthesia on the pro-arrhythmic activity of the class III anti-arrhythmic dofetilide in $\alpha_1$-adrenoceptor-stimulated rabbits.
**Experimental approach:** Rabbits anaesthetized intravenously with pentobarbital, propofol or α-chloralose were infused simultaneously with the $\alpha_1$-adrenoceptor agonist phenylephrine (15 $\mu g\,kg^{-1}\,min^{-1}$, i.v.) and dofetilide (0.04 $mg\,kg^{-1}\,min^{-1}$, i.v.). The electrocardiographic QT interval, the $T_{peak}$–$T_{end}$ interval and certain QT variability parameters were measured. The heart rate variability and the baroreflex sensitivity were utilized to assess the vagal nerve activity. The spectral power of the systolic arterial pressure was calculated in the frequency range 0.15–0.5 Hz to assess the sympathetic activity.
**Key results:** Pentobarbital considerably reduced, whereas propofol did not significantly affect the incidence of dofetilide-induced torsades de pointes (TdP) as compared with the results with α-chloralose (40% ($P=0.011$) and 70% ($P=0.211$) vs 100%, respectively). In additional experiments, neither doubling of the rate of the dofetilide infusion nor tripling of the rate of phenylephrine infusion elevated the incidence of TdP to the level seen with α-chloralose. None of the repolarization-related parameters predicted TdP. The indices of the parasympathetic and sympathetic activity were significantly depressed in the α-chloralose and propofol anaesthesia groups.
**Conclusions and implications:** In rabbits, anaesthetics may affect drug-induced TdP genesis differently, which must be considered when results of different studies are compared.
*British Journal of Pharmacology* (2008) **153,** 75–89; doi:10.1038/sj.bjp.0707536; published online 29 October 2007

**Keywords:** intravenous anaesthesia; pentobarbital; propofol; α-chloralose; torsades de pointes; pro-arrhythmia; dofetilide; $\alpha_1$-adrenoceptor stimulation; phenylephrine; rabbit

**Abbreviations:** ECG, electrocardiogram; PNN8, percentage of successive QT intervals that differ by more than 8 ms; RMSSD, root mean square of the successive differences in the RR or QT intervals; SAP MF, spectral power of the systolic arterial pressure in the mid-frequency range

## Introduction

The long QT syndrome is caused either by inherited 'channelopathies' or by acquired factors, for example cardiac or non-cardiac drugs that hinder the process of repolarization of the myocytes. The most dangerous consequence of the disturbed repolarization is the occurrence of a polymorphic ventricular tachycardia known as torsades de pointes, which can deteriorate into ventricular fibrillation. Thus, the pro-arrhythmic liability of any drug under development must be assessed so as to avoid the occurrence of drug-induced life-threatening arrhythmias, for example torsades de pointes, during pharmacotherapy (Thomsen *et al.*, 2006).

The most commonly used animal model for the *in vivo* screening of drug-induced pro-arrhythmia is the $\alpha_1$-adrenoceptor-stimulated anaesthetized rabbit model of the acquired

Correspondence: Dr A Farkas, 2nd Department of Internal Medicine and Cardiology Centre, Faculty of Medicine, University of Szeged, Korányi fasor 6, Szeged H-6720, Hungary.
E-mail: farkasa@in2nd.szote.u-szeged.hu
Received 15 June 2007; revised 7 September 2007; accepted 18 September 2007; published online 29 October 2007

long QT syndrome developed by Carlsson *et al*. (1990). During the development of the model, it was found that low doses of the class III anti-arrhythmic almokalant readily evoked torsades de pointes in conscious rabbits but when rabbits were anaesthetized with α-chloralose, torsades de pointes was rarely seen and if so, only at very high doses of almokalant (Carlsson *et al*., 1993). This anti-arrhythmic effect of α-chloralose anaesthesia was independent of its effect on the heart rate and blood pressure and was successfully counterbalanced by the administration of the $\alpha_1$-adrenoceptor-stimulant methoxamine (Carlsson *et al*., 1993). Later, the rabbit model with the combination of α-chloralose anaesthesia and $\alpha_1$-adrenoceptor-stimulation was also used by other authors (see Buchanan *et al*., 1993; Farkas *et al*., 1998). However, the poor solubility of α-chloralose and its very slow induction of anaesthesia led others to try different intravenous anaesthetics, for example pentobarbital (Bril *et al*., 1996; Batey and Coker, 2002) or a mixture of ketamine and xylazine (Mazur *et al*., 1999). Although the $\alpha_1$-adrenoceptor-stimulated anaesthetized rabbit model of the acquired long QT syndrome has been widely used to assess the pro-arrhythmic liability of novel anti-arrhythmics and other non-cardiac agents, direct comparison of the effect of the applied anaesthetics on the pro-arrhythmic activity of the test drug has not been performed as yet.

Pentobarbital is a frequently used intravenous anaesthetic in experimental studies *in vivo* as it provides fast induction and the reliable maintenance of anaesthesia. In earlier studies, pentobarbital reduced the incidence of drug-induced torsades de pointes in arterially perfused canine left ventricular wedge preparations (Shimizu *et al*., 1999) and in dogs *in vivo* (Weissenburger *et al*., 2000; Yamamoto *et al*., 2001; Voss *et al*., 2002). This suggests that pentobarbital anaesthesia might well reduce the incidence of drug-induced torsades de pointes in rabbits too, but this has not been studied to date.

Propofol is one of the most frequently used short-acting intravenous anaesthetics in intensive care units. A recent case report described that propofol abolished recurrent ventricular tachycardia called 'electrical storm' in an elderly patient, probably by suppressing sympathetic activity (Burjorjee and Milne, 2002). In the review by Booker *et al*. (2003), it was suggested that, despite the very limited number of data available on the drug, propofol might be a useful agent for the anaesthesia of patients with the long QT syndrome. However, the effect of propofol on drug-induced torsades de pointes has never been tested.

The aim of the present study was to examine whether pentobarbital anaesthesia or propofol anaesthesia reduces the pro-arrhythmic activity of the class III anti-arrhythmic dofetilide as compared with that seen with standard α-chloralose anaesthesia in the rabbit model of the acquired long QT syndrome. An attempt was made to determine whether the effects of the anaesthetics on the pro-arrhythmic activity of dofetilide were related to any effect on the basic haemodynamics (that is, the blood pressure and the heart rate), the repolarization-related parameters or the autonomic nervous system. The assessment of the repolarization-related parameters of the animals included measurement of the electrocardiographic QT and the rate-corrected QT intervals, the $T_{peak}$–$T_{end}$ intervals and the calculation of certain QT variability parameters. The activity of the parasympathetic arm of the autonomic nervous system of the animals was assessed via measurement of the 'heart rate variability' and the baroreflex sensitivity of the spontaneous baroreflex sequences. The activity of the sympathetic nervous system of the animals was estimated by calculating the spectral power of the systolic arterial pressure in the appropriate frequency range.

Since pentobarbital reduced the incidence of dofetilide-induced torsades de pointes in the present study, a second set of experiments was performed to examine whether increase of the dose (and the rate of infusion) of dofetilide or the sensitizing $\alpha_1$-adrenoceptor stimulant phenylephrine could elevate the pro-arrhythmic activity of dofetilide in pentobarbital-anaesthetized rabbits.

## Methods

*Animals*

The animals were handled in accordance with the European Community guidelines for the use of experimental animals, and the protocol was reviewed and approved in advance by the Ethical Committee for the Protection of Animals in Research at the University of Szeged, Hungary. The effects of different intravenous anaesthetics on the pro-arrhythmic activity of dofetilide were examined by using the method of Carlsson *et al*. (1990) with minor modifications. The experiments were performed on female New Zealand White rabbits weighing $2.61 \pm 0.04$ kg.

*Anaesthesia of the rabbits*

The animals were randomly allocated to one or other of the three anaesthesia groups, with 10 rabbits in each group. A catheter was introduced into the marginal vein of the left ear for blood sampling and anaesthesia induction. One millilitre of blood was withdrawn via the catheter to determine serum $K^+$ concentration of the conscious animal. After blood sampling, the animals were anaesthetized intravenously with α-chloralose, or propofol, or pentobarbital. The planned dose of α-chloralose was $90\,mg\,kg^{-1}$ i.v. (Carlsson *et al*., 1990), but $147 \pm 5\,mg\,kg^{-1}$ ($10\,mg\,ml^{-1}$, $1\,ml\,min^{-1}$) of anaesthetic was needed to achieve sufficiently deep anaesthesia. Propofol anaesthesia was induced with an i.v. bolus of $10\,mg\,kg^{-1}$ of propofol (Aeschbacher and Webb, 1993a), which was followed by an i.v. maintenance infusion of the drug. The planned rate of the maintenance infusion of propofol was $1\,mg\,kg^{-1}\,min^{-1}$ (Aeschbacher and Webb, 1993b), but it was necessary to elevate the rate of infusion of the drug to $1.29 \pm 0.10\,mg\,kg^{-1}\,min^{-1}$ so as to achieve sufficiently deep anaesthesia. Similarly, the planned dose of pentobarbital for anaesthesia was $30\,mg\,kg^{-1}$ i.v. (Farkas and Coker, 2002), but $42 \pm 2\,mg\,kg^{-1}$ ($30\,mg\,ml^{-1}$; $1\,ml\,min^{-1}$) was needed for sufficiently deep anaesthesia. In the second set of experiments (for the details, see the Experimental protocol section), two additional groups of animals with 10 rabbits in each group were anaesthetized with intravenous pentobarbital; anaesthesia was induced with $34 \pm 2$ and

$33 \pm 3\,mg\,kg^{-1}$ of pentobarbital in the first ('double dofetilide') and the second ('triple phenylephrine') group, respectively.

The depth of the anaesthesia was carefully assessed throughout the experiment. The surgical preparation of the animals started only when the pedal withdrawal reflex had disappeared and there was no response to pinching of the ears of the animal. The dose of each anaesthetic administered for induction was adjusted to the need of the animal. Anaesthesia induction lasted much longer in the animals anaesthetized with α-chloralose as compared with the other anaesthesia groups (~40 min in the chloralose group vs 3–4 min in the propofol and the pentobarbital-anaesthetized groups). The induction of anaesthesia depressed the rate of spontaneous breathing from ~60 to 20–30 $min^{-1}$ in each animal, independently of the anaesthetic applied.

When there was any sign of pain or consciousness, extra boluses of the anaesthetics were administered during the surgical preparation and during the protocol. Only one of the 10 animals needed an additional 20 mg of α-chloralose after anaesthesia induction. An extra bolus of propofol (10–100 mg) was administered several times to the first four animals in the 'propofol' group during the experiments, which proved that the planned maintenance dose of propofol ($1\,mg\,kg^{-1}\,min^{-1}$) was insufficient. An extra bolus of 6–9 mg pentobarbital was administered to the animals when the pentobarbital anaesthesia was not deep enough after induction, which occurred in 20 of the 30 pentobarbital-anaesthetized rabbits; these animals needed a total of $25 \pm 2$ mg extra pentobarbital after induction during the experiments, overall.

*Surgical preparation*

A catheter was introduced into the right carotid artery to measure blood pressure. The catheter was filled with heparin-treated saline (0.9% w/v NaCl containing 15 IU $ml^{-1}$ heparin) and connected to a pressure transducer of a PowerLab computerized data acquisition system (ADInstruments Inc., Colorado Springs, CO, USA). Blood pressure signal was recorded at a sampling rate of 1000 Hz by the PowerLab System. Two other catheters were introduced into the right jugular vein and the marginal vein of the right ear for the infusion of drugs. After tracheal cannulation, artificial ventilation was started (Harvard rodent ventilator, model 683, Harvard Apparatus, South Natick, MA, USA) with room air at a rate of 40 strokes per min, a stroke volume of ~6 ml (kg body weight)$^{-1}$ and a positive end-expiratory pressure of 1–2 cm $H_2O$. Blood gases were monitored with a Radiometer ABL 725 pH/blood gas analyser (Radiometer Medical ApS, Copenhagen, Denmark). If necessary, the stroke volume of the ventilation pump and the positive end-expiratory pressure were adjusted to maintain the blood gases within the normal range. Subcutaneous needle electrodes were inserted in all four limbs, and Leads I–III of the electrocardiogram (ECG) were recorded simultaneously at a sampling rate of 1000 Hz with the PowerLab System. The preparation was followed by a minimum 10-min stabilizing period.

*Experimental protocol*

In the first set of experiments, the rabbits were anaesthetized randomly with α-chloralose, or propofol, or pentobarbital (there were 10 rabbits in each anaesthesia group; for the details see the Anaesthesia section). After a 10-min baseline period, infusion of the $\alpha_1$-adrenoceptor agonist phenylephrine was started at increasing rates (that is, 3, 6, 9, 12 and $15\,\mu g\,kg^{-1}\,min^{-1}$ for 3, 3, 3, 3 and 5 min, respectively). From the 27th minute, dofetilide ($0.04\,mg\,kg^{-1}\,min^{-1}$ i.v., for 45 min) was administered simultaneously with the background phenylephrine infusion (at a rate of $15\,\mu g\,kg^{-1}\,min^{-1}$) until the end of the experiments (Figure 1a). Although the infusion rate of dofetilide is far beyond the clinically relevant dosing, this extremely high infusion rate of the drug was chosen as dofetilide infusion at this rate induced torsades de pointes frequently and reproducibly in $\alpha_1$-adrenergically stimulated, α-chloralose-anaesthetized rabbits (Lu *et al.*, 2001).

In the second set of experiments, only pentobarbital was applied to anaesthetize the rabbits. In the first group of animals (10 rabbits), the previous phenylephrine infusion protocol was used, but the rate of dofetilide infusion was

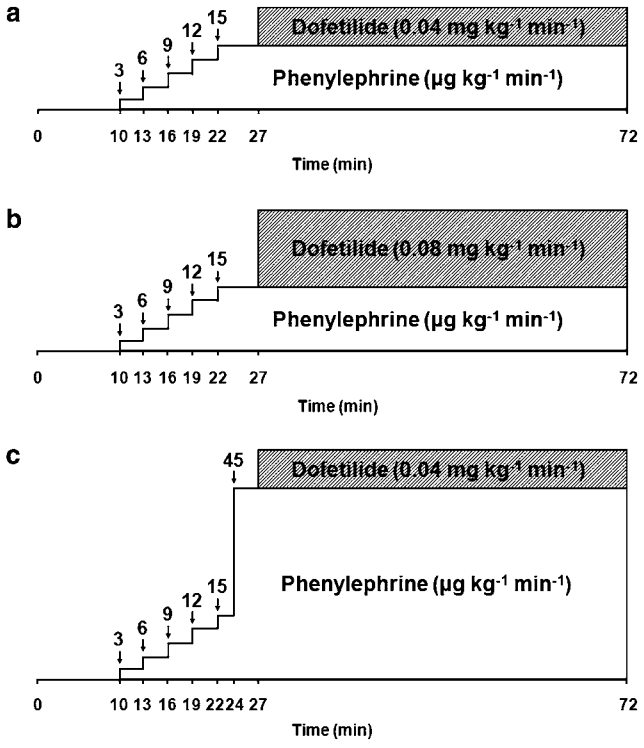


**Figure 1** Drug administration protocol. (**a**) The anaesthetic agent is α-chloralose, or propofol, or pentobarbital. Phenylephrine is administered at increasing rates (3, 6, 9, 12 and $15\,\mu g\,kg^{-1}\,min^{-1}$). Dofetilide is infused at a rate of $0.04\,mg\,kg^{-1}\,min^{-1}$. (**b**) Drug administration protocol in the 'double dofetilide' group. The anaesthetic agent is pentobarbital. Phenylephrine is administered at increasing rates (3, 6, 9, 12 and $15\,\mu g\,kg^{-1}\,min^{-1}$). Dofetilide is infused at a rate of $0.08\,mg\,kg^{-1}\,min^{-1}$. (**c**) Drug administration protocol in the 'triple phenylephrine' group. The anaesthetic agent is pentobarbital. Phenylephrine is administered at increasing rates (3, 6, 9, 12, 15 and $45\,\mu g\,kg^{-1}\,min^{-1}$). Dofetilide is infused at a rate of $0.04\,mg\,kg^{-1}\,min^{-1}$.

doubled (that is, 0.08 mg kg$^{-1}$ min$^{-1}$ for 45 min from the 27th minute; 'double dofetilide' group; Figure 1b). In the second group of animals (10 rabbits), the lower rate of dofetilide infusion (0.04 mg kg$^{-1}$ min$^{-1}$ for 45 min) was used, but the rate of the background phenylephrine infusion was tripled (45 μg kg$^{-1}$ min$^{-1}$; 'triple phenylephrine' group; Figure 1c).

*Electrocardiogram analysis and arrhythmia diagnosis*

The blood pressure and the ECG intervals were measured at predetermined time points. After completion of the experiments, the data were replayed and the RR, PQ, QRS and QT intervals were measured by the manual positioning of on-screen markers. The QT interval measurement was performed as described previously by Farkas *et al*. (2004). Briefly, the QT interval was defined as the time between the first deviation from the isoelectric line during the PQ interval until the end of the TU wave. The T (or U) waves frequently overlapped the P wave of the following sinus-origin beat, due to the relatively high heart rate of the rabbit or to substantial QT prolongation. In these cases, the extrapolation method was used (Farkas *et al*., 2004), that is the end of the TU wave was extrapolated from the curve of the TU wave to the isoelectric line under the P wave. All the values for the QT interval were corrected for the heart rate by two different methods, with the equation QTcC = QT−0.175(RR−300) developed to correct the QT intervals of rabbits anaesthetized with α-chloralose (Carlsson *et al*., 1993), or with the equation QTcL = QT−0.704(RR−250) developed for pentobarbital-anaesthetized rabbits (Batey and Coker, 2002). The $T_{peak}$–$T_{end}$ interval was measured according to Antzelevitch and Oliva (2006) in the standard limb Lead II of the ECG in the last minute before the start of the phenylephrine infusion (baseline), in the last minute before the start of the dofetilide infusion and in the 4th minute of the dofetilide infusion (further measurements were prevented by the frequent occurrence of dofetilide-induced arrhythmias). When the end of the $T_{peak}$–$T_{end}$ interval overlapped the following P wave, the extrapolation method (Farkas *et al*., 2004) was applied. Four consecutive $T_{peak}$–$T_{end}$ intervals were measured and averaged at each time point.

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, the experiment was terminated and the ventricular fibrillation was defined as lethal. Torsades 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 torsades-like twisting QRS morphology were differentiated from torsades de pointes and were defined as ventricular tachycardia. Blocks in the conduction system were also monitored. Conduction disturbances included atrioventricular blocks and intraventricular conduction defects (right or left bundle branch blocks).

*Measurement of the beat-to-beat variability of the QT intervals and the RR intervals*

The beat-to-beat variability of the QT intervals was determined from the manual measurement data on 30 consecutive QT intervals and the corresponding RR intervals in sinus rhythm at predetermined time points, that is in the last minute of the drug-free state, in the last minute of the phenylephrine infusion before the dofetilide administration and in the 2nd–4th minute of the dofetilide infusion, when arrhythmias were still infrequent. A computer program was developed in a NET environment to obtain the following parameters of the beat-to-beat variability of the QT intervals.

*RMSSD and SDSD.* One approach for characterization of the beat-to-beat variability of the RR and QT intervals is to take their successive differences and calculate the root mean square (RMSSD) and the standard deviation (SDSD) of these differences (Brennan *et al*., 2001).

*PNN8.* In human cardiovascular research, PNN50 (the percentage of successive RR intervals that differ by more than 50 ms) is a well-established measure of RR variability (Copie *et al*., 1996). We adopted this idea and tailored the difference criterion to the rabbit QT intervals. First, we chose the value of the criterion on the basis of the ratio of an average rabbit QT interval to a typical human RR interval duration, and then decreased it, because the QT interval variation for some specimens did not reach the original limit at all. Finally, we settled arbitrarily a value of 8 ms, that is PNN8 is the percentage of successive QT intervals that differ by more than 8 ms.

*Instability.* Following the definition by Hondeghem *et al*. (2001), the instability of the RR and QT intervals was calculated as the difference between the upper quartile (the upper boundary of the lowest 75% of the interval values) and the lower quartile (the upper boundary of the lowest 25% of the interval values).

*Short-term variability.* In terms of a Poincaré plot, which is a plot of RR or QT intervals ($d_{i+1}$) against the preceding RR or QT values ($d_i$), the short-term variability, introduced by Thomsen *et al*. (2004), can be visualized as the mean perpendicular distance between the points of the plot and the $d_{i+1} = d_i$ line.

*Long-term variability.* In the framework outlined above, long-term variability is the mean distance (measured parallel to the line $d_{i+1} = d_i$ in the Poincaré plot) between the individual RR or QT interval durations ($d_i$) and their mean value ($E(d)$) (Thomsen *et al*., 2004).

*QT variability index.* QT variability index, introduced by Berger *et al*. (1997), was calculated as the logarithmic ratio of the QT variance and the heart rate variance, each normalized by the respective mean.

The beat-to-beat variability of the RR interval was analysed with a computer software (WinCPRS; Absolute Aliens Oy, Turku, Finland); the analysis was performed in the ECG

section of the last 5 min of the drug-free period before the phenylephrine infusion and the last 1 min of each rate of phenylephrine infusion before the dofetilide administration (see the Experimental protocol section and Figure 1). Only the arrhythmia-free ECG sections were analysed. The software calculated the mean and the s.d. of the RR intervals together with RMSSD (see above) within a given experimental period.

*Analysis of the spontaneous baroreflex sequences*

The baroreflex sensitivity, an index of the vagal nerve activity, was evaluated by using the method of Bertinieri *et al*. (1988) with minor modifications. The beat-to-beat time series of the systolic arterial pressure and the RR interval were analysed with the computer software WinCPRS (Absolute Aliens Oy) to identify spontaneously occurring sequences of $\geqslant 3$ consecutive beats in which the systolic arterial pressure and the RR interval of the following (that is, lag 1) beat changed in the same direction, for example either increasing or decreasing (that is, hypertension and bradycardia, or hypotension and tachycardia, respectively). The threshold changes between two consecutive systolic arterial pressures and the RR interval values were set at 0.3 mm Hg and 0.1 ms, respectively. Linear regression was applied to each individual sequence, as in the Oxford technique, which uses bolus injections of vasoactive drugs. Only those sequences in which $r^2 > 0.7$ were accepted. The percentage incidence of the RR intervals that comprised spontaneous baroreflex sequences was calculated in each analysed ECG section. The mean individual slope of the baroreflex sequences, obtained by averaging all the slopes computed within a given experimental period, was calculated and taken as a measure of the integrated spontaneous baroreflex sensitivity for that period. Baroreflex analysis was performed in the last 5 min of the drug-free period and in the last 1 min of each rate of phenylephrine infusion before the dofetilide administration (see the Experimental protocol section and Figure 1). Few animals furnished spontaneous baroreflex sequences for each rate of phenylephrine infusion, and there were many arrhythmias during the phenylephrine infusion, which hindered the baroreflex analysis. These two factors together decreased the number of data obtained at each phenylephrine rate, which did not allow statistical comparison between the groups for every rate of phenylephrine infusion. Thus, only a single between-group analysis was performed, using the pooled baroreflex sensitivity data on all five 1-min time periods during the phenylephrine infusion.

*Spectral analysis of the variation in the systolic arterial blood pressure and the duration of the RR intervals*

Spectral analysis was performed with the arterial blood pressure and the ECG signal of the animals recorded in the last 5 min of the drug-free period and the 3 min of the $9\,\mu g\,kg^{-1}\,min^{-1}$ phenylephrine infusion rate (see the Experimental protocol section and Figure 1 for details). The examined frequency-domain parameters were the spectral powers in the low-, mid- and high-frequency ranges of the systolic arterial pressure and the RR interval spectra. The boundaries for the frequency ranges were as follows: the low-frequency domain ranged from 0.025 to 0.15 Hz, the mid-frequency domain from 0.15 to 0.5 Hz and the high-frequency domain from 0.5 to 3 Hz. These frequency ranges were chosen for the following reasons: in rabbits, an approximately 0.3 Hz oscillation in the arterial blood pressure has been identified, and spectral analysis revealed strong increases in the power at 0.3 Hz of the renal sympathetic nerve activity and of the blood pressure when sympathetic stimuli were applied (Malpas and Burgess, 2000). Thus, the spectral power of the systolic arterial pressure measured in the vicinity of 0.3 Hz (in our mid-frequency range) reflects the activity of the sympathetic nervous system in rabbits. Furthermore, the high-frequency component of the power spectrum of the RR interval variability reflected the magnitude of fluctuation in the cardiac vagal input associated with respiratory modulation (Iwao *et al*., 2000), and the spectral power of the variability of the RR interval was modulated by vagal influences at frequencies $<0.4373$ Hz (Moguilevski *et al*., 1996). Thus, the spectral power of the RR interval variability measured in our high-frequency range reflects the activity of the parasympathetic nervous system.

First, the R waves were identified in the ECG recordings and the systolic arterial pressure peaks were identified in the blood pressure signal to calculate the RR interval and the systolic arterial pressure spectrum, respectively. The fast discrete implementations of the Fourier transformation rely on an evenly spaced data set; however, this is naturally not the case with our RR interval and systolic arterial pressure sequences. To fulfil the conditions of even spacing, the data were 're-sampled' by using spline interpolation to obtain the intermediate RR interval or systolic arterial pressure values. The power spectral density of the re-sampled data was then calculated and the low-frequency (LF), mid-frequency (MF) and high-frequency (HF) spectral powers were obtained as follows:

$$\mathrm{LF} = \Delta f \sum_{i=i_{\min}}^{i_{\max}-1} S(i\,\Delta f)$$

$$\mathrm{MF} = \Delta f \sum_{j=j_{\min}}^{j_{\max}-1} S(j\,\Delta f)$$

$$\mathrm{HF} = \Delta f \sum_{k=k_{\min}}^{k_{\max}-1} S(k\,\Delta f)$$

where $\Delta f$ denotes the frequency resolution in the discrete spectrum and $S$ the power spectral density of either the RR interval or the systolic arterial pressure. The indices $i_{\min}$, $i_{\max}$, $j_{\min}$, $j_{\max}$, $k_{\min}$ and $k_{\max}$ correspond to the boundaries of the low-, mid- and high-frequency ranges:

$$i_{\min}\,\Delta f = 0.025\ \mathrm{Hz}$$
$$i_{\max}\,\Delta f = 0.15\ \mathrm{Hz} = j_{\min}\,\Delta f$$
$$j_{\max}\,\Delta f = 0.5\ \mathrm{Hz} = k_{\min}\,\Delta f$$
$$k_{\max}\,\Delta f = 3.0\ \mathrm{Hz}$$

The peak detection, the re-sampling and the calculation of the spectra were performed in a LabVIEW 7.1 (National

Instruments Corporation, Austin, TX, USA) programming environment.

*Statistical evaluation*

Continuous data were expressed as mean ± s.e.mean. All data from independent samples, with the exception of arrhythmia incidences, were compared with Kruskal–Wallis tests. Continuous data from the same sample were compared with Wilcoxon tests. Arrhythmia incidences were compared by using the Fisher's exact probability test. Differences were considered statistically significant when $P<0.05$.

*Drugs*

Dofetilide was a gift from EGIS Pharmaceuticals PLC (Budapest, Hungary). All other drugs were purchased from the following sources: α-chloralose and phenylephrine (L-phenylephrine HCl) from Sigma Chemical Co. (Budapest, Hungary); propofol (Diprivan 2%) from AstraZeneca (Mölndal, Sweden); pentobarbital (sodium pentobarbital, Nembutal) from Phylaxia-Sanofi (Budapest, Hungary); and heparin-sodium from Gedeon Richter Ltd (Budapest, Hungary).

The solution of α-chloralose was obtained by dissolving and boiling 400 mg of α-chloralose in 40 ml of 0.9% w/v saline at 50–60 °C. The propofol solution was the original 'Diprivan 2%' solution without any further dilution. The pentobarbital solution was prepared by adding 3 ml of 0.9% w/v saline to 3 ml of 'Nembutal' (6%) solution. The phenylephrine solution was prepared by dissolving 9 mg of L-phenylephrine HCl in 10 ml of 0.9% w/v saline. Increasing doses of phenylephrine, that is 3, 6, 9, 12, 15 and 45 $\mu g\,kg^{-1}\,min^{-1}$, were administered by infusing the given phenylephrine solution at rates of 3.33, 6.67, 10.00, 13.33, 16.67 and 50.00 $\mu l\,kg^{-1}\,min^{-1}$, respectively. The dofetilide solution was prepared by dissolving 9.6 mg of dofetilide in 4 ml of solvent (consisting of 3.6 ml of water + 0.4 ml of 1 M NaOH); infusion of the dofetilide solution at a rate of 16.67 $\mu l\,kg^{-1}\,min^{-1}$ resulted in the infusion of dofetilide at 0.04 $mg\,kg^{-1}\,min^{-1}$. The same dofetilide solution was infused into the animals in the 'double dofetilide' group, but the infusion rate was then doubled, that is infusion of the dofetilide solution at 33.33 $\mu l\,kg^{-1}\,min^{-1}$ resulted in the infusion of dofetilide at a rate of 0.08 $mg\,kg^{-1}\,min^{-1}$. Each solution was prepared freshly on the day of the experiment.

## Results

*Arrhythmia incidences and the onset time of torsades de pointes*

Torsades de pointes and ventricular fibrillation never occurred before the dofetilide infusion. In this period, the phenylephrine infusion induced only a few premature ventricular beats and no arrhythmias in the α-chloralose- and propofol-anaesthetized animals, respectively. In contrast, non-complex arrhythmias, for example ventricular premature beats and bigeminies, were evoked frequently in the pentobarbital-anaesthetized animals before the dofetilide administration. In the additional set of experiments with the pentobarbital-anaesthetized animals, the high rate of infusion of phenylephrine frequently induced both complex and non-complex arrhythmias, but did not trigger torsades de pointes or ventricular fibrillation before the dofetilide infusion (Table 1).

**Table 1** Incidence of arrhythmias before and during dofetilide infusion, onset time of torsades de pointes and pro-arrhythmic dose of dofetilide in anaesthetized rabbits

| | | *Incidence of arrhythmias (%)* | | | | | | | | *TdP onset (min)* | *TdP dose (mg kg$^{-1}$)* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | *VPB* | *BG* | *Salvo* | *Block* | *VT* | *TdP* | *VF* | *SVF* | | |
| *Before dofetilide* | | | | | | | | | | | |
| Chloralose | 10 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| Propofol | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| Pentobarbital | 10 | 50$^{\#}$ | 60*,$^{\#}$ | 20 | 0 | 0 | 0 | 0 | 0 | | |
| Dof2x | 10 | 50$^{\#}$ | 30 | 40 | 0 | 20 | 0 | 0 | 0 | | |
| Phe3x | 10 | 100$^{\dagger}$ | 80*,$^{\#}$ | 100$^{\dagger}$ | 70$^{\dagger}$ | 100$^{\dagger}$ | 0 | 0 | 0 | | |
| *During dofetilide* | | | | | | | | | | | |
| Chloralose | 10 | 100 | 100 | 100 | 90 | 100 | 100 | 20 | 20 | 7.79 ± 0.84 | 0.31 ± 0.03 |
| Propofol | 10 | 80 | 80 | 70 | 80 | 70 | 70 | 40 | 30 | 7.66 ± 2.31 | 0.31 ± 0.09 |
| Pentobarbital | 10 | 90 | 100 | 100 | 80 | 100 | 40* | 0 | 0 | 10.66 ± 3.45 | 0.43 ± 0.14 |
| Dof2x | 10 | 100 | 100 | 100 | 100 | 100 | 70 | 40 | 10 | 6.53 ± 2.68 | 0.52 ± 0.21 |
| Phe3x | 10 | 80 | 80 | 60 | 80 | 100 | 20* | 0 | 0 | 4.68 ± 1.15 | 0.19 ± 0.05 |

Incidence values are percentage incidences of arrhythmias; Before dofetilide, arrhythmia incidences when only phenylephrine infusion was administered; During dofetilide, arrhythmia incidences, torsades de pointes onset times and pro-arrhythmic doses of dofetilide when phenylephrine and dofetilide infusions were administered simultaneously; Chloralose, group of animals anaesthetized with α-chloralose; Propofol, group of animals anaesthetized with propofol; Pentobarbital, group of animals anaesthetized with pentobarbital; Dof2x, group of animals anaesthetized with pentobarbital, and dofetilide is infused at a rate of 0.08 $mg\,kg^{-1}\,min^{-1}$ ('double dofetilide' group; see Figure 1 for protocol); Phe3x, group of animals anaesthetized with pentobarbital, and the maximum phenylephrine infusion rate is 45 $\mu g\,kg^{-1}\,min^{-1}$ ('triple phenylephrine' group; see Figure 1 for protocol); VPB, ventricular premature beat; BG, bigeminy; Block, conduction block of any kind; VT, ventricular tachycardia different from torsades de pointes; TdP, torsades de pointes ventricular tachycardia; VF, ventricular fibrillation; SVF, sustained ventricular fibrillation, that is ventricular fibrillation that lasts >120 s; TdP onset, the onset time of torsades de pointes after commencing dofetilide infusion; TdP dose, the cumulative dose of dofetilide that triggered torsades de pointes; *n*, group size.
*$P<0.05$ vs the 'Chloralose' group; $^{\#}P<0.05$ vs the 'Propofol' group; $^{\dagger}P<0.05$ vs all other groups.

Dofetilide frequently evoked ventricular premature beats, bigeminies, salvos, conduction blocks and non-torsades de pointes ventricular tachycardias, independently of the anaesthetic applied (Table 1). Dofetilide induced torsades de pointes in all the animals anaesthetized with α-chloralose, and a majority of the propofol-anaesthetized animals exhibited this arrhythmia during the dofetilide infusion (Figure 2). In contrast, torsades de pointes was evoked in less than half of the pentobarbital-anaesthetized animals (Table 1). In the additional set of experiments with the pentobarbital-anaesthetized rabbits, neither the doubling of the dofetilide infusion rate nor the tripling of the phenylephrine infusion rate elevated the incidence of torsades de pointes to the level seen in the animals anaesthetized with α-chloralose (Table 1).

There was no statistical difference between the onset times of torsades de pointes in the various groups (Table 1). Similarly, there was no significant difference between the cumulative doses of dofetilide that triggered torsades de pointes in the various groups (Table 1).

*Blood pressure*

The baseline blood pressure values associated with the pentobarbital anaesthesia were significantly higher as compared with the values measured in the animals anaesthetized with α-chloralose or propofol (Table 2). Stepwise elevation of the rate of infusion of phenylephrine increased the blood pressure before the dofetilide infusion. Interestingly, the anaesthesia-related difference in the baseline of blood pressure values disappeared soon after the start of the phenylephrine infusion, that is the blood pressures rose more steeply in the α-chloralose- and the propofol-anaesthetized animals at low and intermediate phenylephrine infusion rates. After they had caught up, the blood pressure values rose similarly in all groups during the high rates of infusion of phenylephrine before the dofetilide administration (Table 2). In the additional set of experiments with the pentobarbital-anaesthetized rabbits, the application of the threefold phenylephrine infusion rate ($45\,\mu g\,kg^{-1}\,min^{-1}$) resulted in higher maximum blood pressure values relative to those measured in the animals that received the phenylephrine infusion at the earlier maximum rate of $15\,\mu g\,kg^{-1}\,min^{-1}$ (Table 2). During the dofetilide infusion, the blood pressures decreased in parallel in all the groups, but the blood pressure always remained higher in the 'triple phenylephrine' group as compared with all the other groups (Table 2).

*Heart rate and ECG intervals*

Unlike the blood pressure, the baseline heart rate values did not differ in the different anaesthesia groups. Phenylephrine mildly reduced the heart rates and prolonged the QT intervals in the α-chloralose- and pentobarbital-anaesthetized groups before the dofetilide infusion, but there were no differences in the heart rates and the QT intervals in the groups immediately before the start of the dofetilide infusions (Table 3). Arrhythmias occurred frequently in all animals approximately 5 min after the start of the dofetilide administration, which prevented measurement of the sinus heart rate and the ECG intervals thereafter. Following the start of the dofetilide administration, arrhythmias occurred instantly in the animals in the 'double dofetilide' group, preventing subsequent measurements of the sinus heart rate and the ECG intervals. Similarly, in consequence of the frequent occurrence of arrhythmias induced by the highest rate of phenylephrine infusion, sinus heart rates and ECG intervals could not be measured in the animals in the 'triple phenylephrine' group.

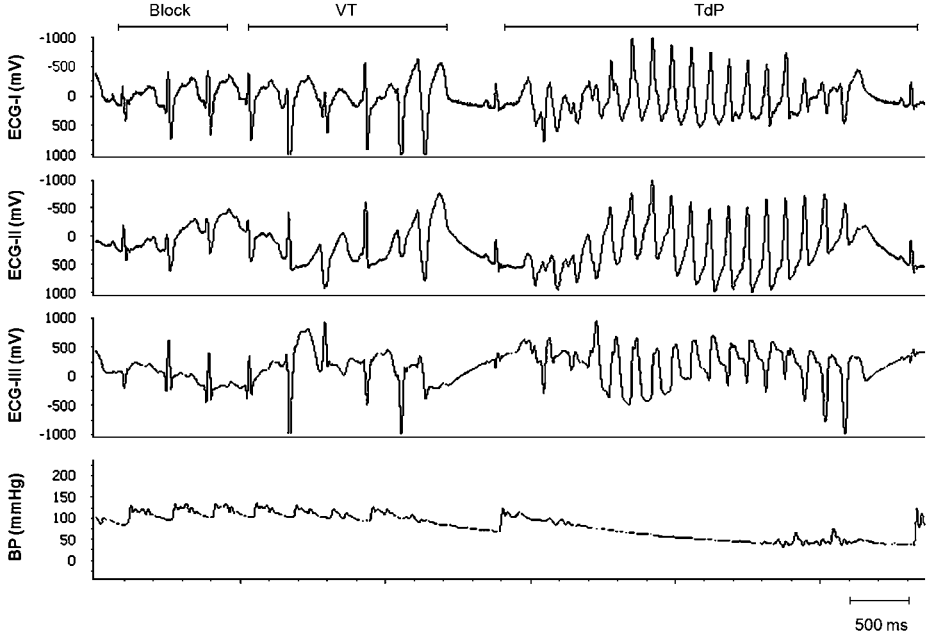


**Figure 2** An example of dofetilide-induced torsades de pointes ventricular tachycardia (TdP). ECG I–III, electrocardiogram Leads I–III; BP, arterial blood pressure; Block, intraventricular conduction block; VT, polymorphic ventricular tachycardia different from torsades de pointes.

**Table 2** Mean arterial blood pressures (mm Hg) in anaesthetized rabbits

| | n | *0 min* | *10 min* | *Phenylephrine* | | | | | *Phenylephrine + dofetilide* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *13 min* | *16 min* | *19 min* | *22 min* | *27 min* | *32 min* | *52 min* | *72 min* |
| Chlor | 10 | 61 ± 4 | 58 ± 3 | 61 ± 4 | 66 ± 6 | 67 ± 8 | 80 ± 7 | 93 ± 6 | 93 ± 5 | 78 ± 4 | 83 ± 5 |
| Prop | 10 | 65 ± 6 | 66 ± 7 | 67 ± 6 | 76 ± 8 | 77 ± 7 | 83 ± 7 | 98 ± 5 | 91 ± 5 | 87 ± 6 | 75 ± 7 |
| Pento | 10 | 86 ± 4*,# | 84 ± 4*,# | 84 ± 5*,# | 83 ± 5 | 87 ± 5 | 92 ± 5 | 95 ± 5 | 90 ± 6 | 82 ± 8 | 77 ± 8 |
| Dof2x | 10 | 88 ± 3*,# | 86 ± 2*,# | 80 ± 4* | 88 ± 3 | 94 ± 3*,# | 95 ± 5 | 107 ± 5 | 94 ± 4 | 84 ± 4 | 76 ± 5 |
| Phe3x | 10 | 84 ± 3*,# | 87 ± 2*,# | 87 ± 2*,# | 91 ± 3 | 94 ± 4*,# | 101 ± 4 | 131 ± 5† | 128 ± 4† | 107 ± 4† | 100 ± 4† |

Phenylephrine, time points when phenylephrine is infused at increasing rates; Phenylephrine + dofetilide, time points when phenylephrine and dofetilide are infused simultaneously (see Figure 1 for protocol); Chlor, group of animals anaesthetized with α-chloralose ('chloralose' group); Prop, group of animals anaesthetized with propofol; Pento, group of animals anaesthetized with pentobarbital ('pentobarbital' group); Dof2x, group of animals anaesthetized with pentobarbital, and dofetilide is infused at a rate of 0.08 mg kg$^{-1}$ min$^{-1}$ ('double dofetilide' group); Phe3x, group of animals anaesthetized with pentobarbital and the maximum phenylephrine infusion rate is 45 μg kg$^{-1}$ min$^{-1}$ ('triple phenylephrine' group); *n*, group size.
All values are means ± s.e.mean.
*$P<0.05$ vs 'chloralose' group; #$P<0.05$ vs 'propofol' group; †$P<0.05$ vs all other groups.

**Table 3** Heart rates, QT and $T_{peak}$–$T_{end}$ intervals in anaesthetized rabbits

| | n | *0 min* | *10 min* | *Phenylephrine* | | | | | *Phe + dof* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *13 min* | *16 min* | *19 min* | *22 min* | *27 min* | *29 min* | *32 min* |
| *Chlor* | | | | | | | | | | |
| HR | 10 | 279 ± 13 | 268 ± 11 | 266 ± 11 | 263 ± 10 | 257 ± 10 | 248 ± 12 | 231 ± 15* | 235 ± 18 | 245 ± 14 |
| QT | 10 | 151 ± 4 | 155 ± 4 | 156 ± 4 | 160 ± 4 | 162 ± 3 | 162 ± 4 | 178 ± 9* | 178 ± 16 | 196 ± 10# |
| TpTe | 10 | | 60 ± 6 | | | | | 64 ± 6 | 65 ± 8 | |
| *Prop* | | | | | | | | | | |
| HR | 10 | 258 ± 11 | 260 ± 10 | 260 ± 9 | 258 ± 10 | 258 ± 10 | 251 ± 12 | 232 ± 17 | 217 ± 15 | 227 ± 12 |
| QT | 10 | 157 ± 5 | 161 ± 4 | 161 ± 4 | 159 ± 4 | 161 ± 4 | 166 ± 7 | 164 ± 7 | 187 ± 11# | 200 ± 11# |
| TpTe | 10 | | 58 ± 5 | | | | | 61 ± 6 | 71 ± 9 | |
| *Pento* | | | | | | | | | | |
| HR | 10 | 279 ± 9 | 284 ± 8 | 279 ± 8 | 275 ± 7 | 276 ± 8 | 268 ± 7 | 252 ± 6* | 245 ± 11 | 228 ± 14 |
| QT | 10 | 151 ± 5 | 149 ± 4 | 151 ± 5 | 154 ± 5 | 156 ± 5 | 158 ± 5 | 156 ± 3* | 172 ± 6 | 205 ± 16# |
| TpTe | 10 | | 73 ± 6 | | | | | 69 ± 6 | 65 ± 10 | |
| *Dof2x* | | | | | | | | | | |
| HR | 10 | 292 ± 11 | 286 ± 12 | 284 ± 11 | 281 ± 12 | 274 ± 12 | 267 ± 12 | 248 ± 15* | 260 ± 16 | ND |
| QT | 10 | 147 ± 5 | 152 ± 5 | 151 ± 6 | 153 ± 5 | 158 ± 5 | 161 ± 5 | 170 ± 8* | 178 ± 7 | ND |
| TpTe | 10 | | 64 ± 7 | | | | | 70 ± 8 | 71 ± 8 | |
| *Phe3x* | | | | | | | | | | |
| HR | 10 | 278 ± 8 | 272 ± 7 | 272 ± 7 | 271 ± 8 | 264 ± 11 | 260 ± 16 | ND | ND | ND |
| QT | 10 | 157 ± 3 | 158 ± 3 | 161 ± 3 | 160 ± 3 | 161 ± 4 | 165 ± 5 | ND | ND | ND |
| TpTe | 10 | | 62 ± 4 | | | | | ND | ND | |

Phenylephrine, time points when phenylephrine is infused at increasing rates; Phe + dof, time points when phenylephrine and dofetilide are infused simultaneously (see Figure 1 for protocol); HR, sinus heart rate (min$^{-1}$); RR, the electrocardiographic RR interval (ms); QT, the electrocardiographic QT interval (ms); TpTe, the electrocardiographic $T_{peak}$–$T_{end}$ interval (ms); ND, value not determined due to the frequent occurrence of arrhythmias; *n*, group size.
All values are means ± s.e.mean.
*$P<0.05$ vs the value measured before the start of phenylephrine infusion at 10 min; #$P<0.05$ vs the value measured before the start of dofetilide infusion at 27 min. For further details see Table 2.

The dofetilide infusion quickly and markedly prolonged the QT and the rate-corrected QT intervals in all the groups, independently of the anaesthesia applied (Table 3); up until the time point at which these intervals could be measured (see above), there was no significant difference in the QT and the rate-corrected QT intervals in the various groups. There was no qualitative difference between the effects of the drugs on the QT and the rate-corrected QT interval at any time point in any group of animals (data not shown). Similarly, there was no qualitative difference between the results of the two different methods of QT correction (see Electrocardiogram analysis and arrhythmia diagnosis) at any time point in any group of animals (data not shown).

There was no significant difference between the $T_{peak}$–$T_{end}$ intervals in the various groups at any of the three time points of the measurement; phenylephrine did not prolong the $T_{peak}$–$T_{end}$ interval significantly as compared with the baseline in the various anaesthesia groups (Table 3). Interestingly, dofetilide infusion in the first 2 min did not prolong the $T_{peak}$–$T_{end}$ interval significantly as compared with the value measured before the start of this infusion in the various anaesthesia groups (Table 3).

The mean baseline PQ interval in the different anaesthesia groups ranged from 61 ± 2 to 69 ± 2 ms and remained stable in all groups up until the time point at which this parameter could be measured (see above); there was no significant

difference between the PQ values in the various groups at any time point of the measurement. The mean baseline QRS interval in the various groups ranged from 33 ± 1 to 34 ± 1 ms and remained stable up until the time point at which it could be measured (see above); there was no significant difference between the QRS values in the various groups at any time point of the measurement.

*Beat-to-beat variability of the QT and the RR interval*

Interestingly, none of the calculated QT variability data differed in the various groups at baseline, during the phenylephrine infusion and at the beginning of the dofetilide infusion (Table 4). Within-group analysis revealed that PNN8 and the short-term variability of the QT interval rose significantly during the phenylephrine infusion and at the beginning of the dofetilide infusion relative to the respective baseline values in the 'chloralose' group (Table 4). No similar increase in the QT variability parameters was seen in the other anaesthesia groups.

The values of the beat-to-beat variability of the RR interval did not differ statistically in the different anaesthesia groups at baseline. In contrast, significantly lower s.d. and RMSSD values were measured in the α-chloralose-anaesthetized animals as compared with those in the other anaesthesia groups during the stepwise elevation of the rate of infusion of phenylephrine, whereas the pentobarbital-anaesthetized animals tended to show the highest s.d. and RMSSD values (Table 5).

*Baroreflex sensitivity*

At baseline, a significantly higher mean down-baroreflex sensitivity was measured in the animals anaesthetized with pentobarbital ('pentobarbital', 'double dofetilide' and 'triple phenylephrine' groups) in comparison with those measured in the animals anaesthetized with α-chloralose or propofol (Table 6).

Very marked differences between the baroreflex sensitivities in the various anaesthesia groups were observed during the phenylephrine infusion, that is significantly higher up-baroreflex sensitivity and down-baroreflex sensitivity were measured in the animals anaesthetized with pentobarbital (the 'pentobarbital', 'double dofetilide' and 'triple phenylephrine' groups) as compared with those measured in the animals anaesthetized with α-chloralose or propofol (Table 6).

*The spectral power of the systolic arterial pressure and the RR interval*

The baseline mid-frequency spectral power of the systolic arterial pressure was significantly higher in the 'pentobarbital' group as compared with those in the 'chloralose' and

**Table 4** The beat-to-beat variability of the QT interval in anaesthetized rabbits

| | N | *MeanQT* | *s.d.* | *RMSSD* | *SDSD* | *PNN8* | *Instability* | *LTV* | *STV* | *QTVI* |
|---|---|---|---|---|---|---|---|---|---|---|
| *Chlor* | | | | | | | | | | |
| Basal | 10 | 166 ± 3 | 7.5 ± 1.9 | 7.4 ± 1.0 | 7.5 ± 1.0 | 9.7 ± 1.8 | 12.8 ± 4.5 | 7.2 ± 2.2 | 3.8 ± 0.4 | 1.2 ± 0.2 |
| Phe15 | 10 | 190 ± 9* | 8.1 ± 0.9 | 9.8 ± 1.0 | 9.9 ± 1.0 | 17.2 ± 2.4* | 9.5 ± 0.7 | 6.7 ± 0.8 | 5.4 ± 0.5* | 1.2 ± 0.2 |
| Dof | 10 | 198 ± 13* | 8.4 ± 1.1 | 10.2 ± 1.3 | 10.4 ± 1.3 | 18.3 ± 2.8* | 11.2 ± 1.8 | 7.2 ± 1.1 | 5.5 ± 0.7* | 1.0 ± 0.2 |
| *Prop* | | | | | | | | | | |
| Basal | 10 | 178 ± 5 | 7.9 ± 0.9 | 9.0 ± 1.3 | 9.2 ± 1.3 | 12.4 ± 2.2 | 10.4 ± 1.0 | 6.8 ± 0.7 | 4.8 ± 0.6 | 1.4 ± 0.1 |
| Phe15 | 10 | 183 ± 8 | 6.1 ± 0.6 | 7.8 ± 0.7 | 7.9 ± 0.7 | 13.8 ± 2.1 | 7.6 ± 1.0 | 5.0 ± 0.6 | 4.2 ± 0.3 | 0.9 ± 0.2 |
| Dof | 10 | 194 ± 7 | 8.0 ± 1.4 | 9.2 ± 1.2 | 9.4 ± 1.2 | 15.9 ± 2.7 | 10.3 ± 1.9 | 7.0 ± 1.6 | 5.1 ± 0.7 | 1.2 ± 0.2 |
| *Pento* | | | | | | | | | | |
| Basal | 8 | 168 ± 10 | 8.9 ± 1.5 | 11.0 ± 2.0 | 11.2 ± 2.0 | 16.4 ± 4.7 | 12.5 ± 2.1 | 7.3 ± 1.2 | 5.8 ± 1.1 | 1.3 ± 0.2 |
| Phe15 | 9 | 171 ± 4 | 7.7 ± 1.1 | 9.2 ± 1.2 | 9.4 ± 1.2 | 14.9 ± 3.1 | 10.6 ± 1.8 | 6.7 ± 1.0 | 5.0 ± 0.7 | 1.2 ± 0.1 |
| Dof | 8 | 190 ± 10*,# | 10.2 ± 1.7 | 11.9 ± 1.8 | 12.1 ± 1.9 | 20.7 ± 4.5 | 12.5 ± 2.2 | 8.6 ± 1.7 | 6.4 ± 1.0 | 1.3 ± 0.2 |
| *Dof2x* | | | | | | | | | | |
| Basal | 10 | 172 ± 10 | 5.8 ± 0.7 | 6.8 ± 0.9 | 6.9 ± 0.9 | 7.2 ± 2.6 | 7.1 ± 1.0 | 4.9 ± 0.6 | 3.7 ± 0.5 | 1.2 ± 0.1 |
| Phe15 | 10 | 179 ± 9 | 6.3 ± 0.7 | 7.2 ± 1.0 | 7.4 ± 1.0 | 11.0 ± 3.0 | 9.6 ± 0.9 | 5.7 ± 0.7 | 4.1 ± 0.6 | 1.2 ± 0.1 |
| Dof | 7 | 182 ± 11 | 5.9 ± 0.8 | 6.4 ± 0.7 | 6.5 ± 0.7 | 8.4 ± 1.8 | 7.7 ± 1.0 | 5.2 ± 0.8 | 3.5 ± 0.4 | 0.9 ± 0.3 |
| *Phe3x* | | | | | | | | | | |
| Basal | 10 | 171 ± 4 | 7.2 ± 1.0 | 8.3 ± 1.0 | 8.4 ± 1.0 | 13.8 ± 2.7 | 10.2 ± 1.3 | 6.4 ± 1.0 | 4.5 ± 0.6 | 1.3 ± 0.1 |
| Phe15 | 10 | 179 ± 4 | 6.6 ± 0.5 | 8.1 ± 0.7 | 8.2 ± 0.7 | 14.1 ± 2.5 | 9.1 ± 0.9 | 5.6 ± 0.5 | 4.2 ± 0.4 | 0.8 ± 0.2 |
| Dof | 3 | 158 ± 24 | 9.0 ± 2.2 | 7.8 ± 1.8 | 7.9 ± 1.9 | 12.6 ± 5.7 | 11.7 ± 5.2 | 8.4 ± 3.3 | 4.2 ± 0.9 | 1.1 ± 0.2 |

Basal, the value determined from 30 consecutive QT intervals before drug administration; Phe15, value determined from 30 consecutive QT intervals in the last minute of the 15 $\mu g\,kg^{-1}\,min^{-1}$ phenylephrine infusion rate; Dof, the value measured from 30 consecutive QT intervals shortly after the beginning of the dofetilide infusion (in the 2nd–4th minute of dofetilide infusion), when arrhythmias were still infrequent. *N*, number of animals in which the QT variability was determined as there was no arrhythmia at the time point of the measurement; MeanQT, the mean of the measured 30 consecutive QT intervals (ms); s.d., the standard deviation of the measured 30 consecutive QT intervals (ms); RMSSD, the root mean square of the successive difference of the QT intervals (ms); SDSD, standard deviation of successive differences of the QT intervals (ms); PNN8, percentage of QT intervals differing from the preceding one by more than 8 ms (%); Instability, QT instability (ms); LTV, long-term variability of the QT intervals (ms); STV, short-term variability of the QT intervals (ms); QTVI, QT variability index.
All values shown as means ± s.e.mean.
*$P<0.05$ vs Basal; #$P<0.05$ vs Phe. For further details see Table 2.

**Table 5** The beat-to-beat variability of the RR interval in anaesthetized rabbits

| | *Drug-free* | *Phe3* | *Phe6* | *Phe9* | *Phe12* | *Phe15* |
|---|---|---|---|---|---|---|
| *Chlor* | | | | | | |
| *N* | 10 | 9 | 9 | 8 | 8 | 8 |
| MeanRR | 223 ± 10 | 221 ± 11 | 224 ± 11 | 227 ± 12 | 228 ± 12 | 232 ± 10 |
| s.d. | 1.6 ± 0.2 | 1.4 ± 0.1 | 1.3 ± 0.1 | 1.3 ± 0.1$^{\#,*,\dagger,\ddagger}$ | 1.3 ± 0.1$^{*,\dagger,\ddagger}$ | 1.6 ± 0.2$^{\dagger,\ddagger}$ |
| RMSSD | 1.4 ± 0.2 | 1.3 ± 0.2 | 1.1 ± 0.1$^{*,\ddagger}$ | 1.2 ± 0.1 | 1.4 ± 0.2 | 1.5 ± 0.2 |
| *Prop* | | | | | | |
| *N* | 10 | 10 | 9 | 8 | 9 | 10 |
| MeanRR | 235 ± 9 | 235 ± 8 | 230 ± 8 | 233 ± 10 | 231 ± 9 | 234 ± 8 |
| s.d. | 2.1 ± 0.3 | 1.9 ± 0.1 | 1.8 ± 0.1 | 1.8 ± 0.1 | 1.6 ± 0.1 | 1.6 ± 0.1$^{\dagger,\ddagger}$ |
| RMSSD | 1.5 ± 0.1 | 1.6 ± 0.2 | 1.4 ± 0.2 | 1.6 ± 0.2 | 1.5 ± 0.2 | 1.4 ± 0.1 |
| *Pento* | | | | | | |
| *N* | 8 | 8 | 7 | 8 | 7 | 6 |
| MeanRR | 217 ± 7 | 212 ± 7 | 218 ± 8 | 215 ± 8 | 212 ± 5 | 219 ± 6 |
| s.d. | 2.1 ± 0.3 | 1.6 ± 0.2 | 1.9 ± 0.3 | 2.1 ± 0.2 | 2.1 ± 0.4 | 2.0 ± 0.3$^{\ddagger}$ |
| RMSSD | 1.9 ± 0.2 | 1.8 ± 0.2 | 1.7 ± 0.3 | 2.1 ± 0.4 | 1.7 ± 0.2 | 1.8 ± 0.3 |
| *Dof2x* | | | | | | |
| *N* | 10 | 10 | 10 | 9 | 9 | 7 |
| MeanRR | 212 ± 9 | 213 ± 8 | 217 ± 10 | 219 ± 11 | 228 ± 11 | 241 ± 15 |
| s.d. | 1.9 ± 0.4 | 2.0 ± 0.3 | 1.6 ± 0.2 | 2.2 ± 0.4 | 2.6 ± 0.4 | 3.2 ± 0.7 |
| RMSSD | 1.4 ± 0.1 | 1.6 ± 0.2 | 1.6 ± 0.2 | 1.6 ± 0.2 | 1.5 ± 0.1 | 1.9 ± 0.3 |
| *Phe3x* | | | | | | |
| *N* | 10 | 10 | 9 | 8 | 6 | 6 |
| MeanRR | 222 ± 6 | 222 ± 6 | 224 ± 7 | 230 ± 12 | 238 ± 21 | 253 ± 18 |
| s.d. | 2.2 ± 0.3 | 2.2 ± 0.2 | 1.9 ± 0.3 | 2.8 ± 0.6 | 2.5 ± 0.4 | 3.8 ± 0.8 |
| RMSSD | 2.0 ± 0.3 | 2.2 ± 0.3 | 2.2 ± 0.6 | 2.3 ± 0.8 | 1.8 ± 0.1 | 1.5 ± 0.2 |

*N*, number of animals in which variability of the RR interval was determined; MeanRR, mean duration of the RR intervals within the 5- or 1-min period when measurement was taken (ms); s.d., standard deviation of RR intervals within the 5- or 1-min period when measurement was taken (ms); RMSSD, root mean square of successive RR differences (ms); Drug-free, value determined from the consecutive RR intervals of the last 5-min period before phenylephrine infusion; Phe3, Phe6, Phe9, Phe12 and Phe15, values determined from the consecutive RR intervals for the last 1 min of the 3, 6, 9, 12 and 15 $\mu g\,kg^{-1}\,min^{-1}$ phenylephrine infusion rates, respectively.
All values shown as means ± s.e.mean.
$^{\#}P<0.05$ vs 'propofol' group; $^{*}P<0.05$ vs 'pentobarbital' group; $^{\dagger}P<0.05$ vs 'double dofetilide' group; $^{\ddagger}P<0.05$ vs 'triple phenylephrine' group. For further details see Table 2.

'propofol' groups (Figure 3a). Slightly lower values of the mid-frequency spectral power of the systolic arterial pressure were measured in all the groups during the phenylephrine infusion and there were no statistical differences between the values in the various groups at this time of the experiment (Figure 3b). The mid-frequency spectral power of the RR interval did not differ in the various groups (Figures 3c and d).

There was no statistical difference between the spectral powers of the systolic arterial pressure in the various groups, in either the low- or the high-frequency range, and similarly, there was no statistical difference between the spectral powers of the RR interval in the various groups in either the low- or the high-frequency range (data not shown).

## Discussion

This study is the first to report that pentobarbital anaesthesia differs substantially from α-chloralose anaesthesia in terms of anti-arrhythmic potency against drug-induced pro-arrhythmia. In the present study, pentobarbital anaesthesia reduced, whereas propofol anaesthesia did not affect, the incidence of dofetilide-induced torsades de pointes in rabbits relative to that seen with the standard α-chloralose anaesthesia. Although doubling of the dose of dofetilide increased the pro-arrhythmic activity of the drug in the pentobarbital-anaesthetized rabbits, the incidence of torsades de pointes still did not reach the level achieved with half the dose of dofetilide in the α-chloralose-anaesthetized rabbits. Interestingly, even tripling of the rate of the sensitizing background infusion of phenylephrine did not overcome the protective effect of the pentobarbital anaesthesia. The differences between the effects of the anaesthetics on the pro-arrhythmic activity of dofetilide were not related to the blood pressure, the heart rate or the measured parameters reflecting the ventricular repolarization. However, in the first set of experiments, significantly lower indices of the parasympathetic and sympathetic activities were found in the groups of animals anaesthetized with α-chloralose or propofol as compared with those measured in the group of animals anaesthetized with pentobarbital, which implies that a reduced autonomic activity may predispose to drug-induced torsades de pointes in rabbits.

*Electrophysiology of pentobarbital: the possible mechanisms of the effect against dofetilide-induced pro-arrhythmia*

Pentobarbital is an inhibitor of the inward $Na^+$ current ($I_{Na}$) (Wartenberg *et al.*, 2001). It also blocks the slow ($I_{Ks}$) and the

**Table 6** Baroreflex sensitivity in anaesthetized rabbits

| | *Chlor* | *Prop* | *Pento* | *Dof2x* | *Phe3x* |
|---|---|---|---|---|---|
| *Baseline* | | | | | |
| Up-BRS | | | | | |
| a | 8 | 9 | 7 | 7 | 10 |
| Mean | 3.59 ± 1.00 | 2.47 ± 0.24 | 2.97 ± 0.79 | 2.98 ± 0.51 | 3.75 ± 0.52 |
| Valid | 2.71 ± 1.26 | 5.86 ± 1.68 | 5.44 ± 3.00 | 2.13 ± 0.71 | 4.67 ± 2.17 |
| Down-BRS | | | | | |
| b | 5 | 8 | 7 | 7 | 8 |
| Mean | 1.74 ± 0.33 | 1.61 ± 0.17 | 3.25 ± 0.58*,# | 2.58 ± 0.40# | 2.65 ± 0.34# |
| Valid | 3.40 ± 0.87 | 4.39 ± 2.21 | 3.71 ± 1.04 | 0.76 ± 0.16 | 5.19 ± 1.86 |
| *Phenylephrine* | | | | | |
| Up-BRS | | | | | |
| c | 30 | 42 | 17 | 30 | 23 |
| Mean | 2.47 ± 0.14 | 2.76 ± 0.17 | 5.68 ± 1.09*,#† | 3.06 ± 0.32 | 3.74 ± 0.29*,#† |
| Valid | 5.52 ± 0.87 | 5.90 ± 0.73 | 6.05 ± 1.40 | 5.16 ± 1.25 | 3.53 ± 0.73 |
| Down-BRS | | | | | |
| d | 25 | 30 | 22 | 21 | 22 |
| Mean | 2.13 ± 0.28 | 2.00 ± 0.17 | 4.22 ± 0.64*,# | 3.95 ± 0.53*,# | 3.59 ± 0.48*,# |
| Valid | 4.78 ± 0.86 | 6.01 ± 1.07 | 5.43 ± 0.96 | 4.64 ± 0.85 | 4.37 ± 1.27 |

Baseline, baseline baroreflex sensitivity (BRS) data determined by spontaneous up- and down-BRS sequences found in the 5-min period before the start of phenylephrine infusion; Phenylephrine, pooled BRS data determined by spontaneous up- and down-BRS sequences found during phenylephrine infusion before the start of dofetilide administration; Up-BRS, sequence of consecutive, constantly widening RR intervals, where the RR interval duration widening was induced by an increase in the systolic arterial blood pressure; Down-BRS, sequence of consecutive, constantly shortening RR intervals, where the RR interval duration shortening was induced by a decrease in the systolic arterial blood pressure; a, number of animals in which spontaneous up-BRS sequences were found before phenylephrine infusion; b, number of animals in which spontaneous down-BRS sequences were found before phenylephrine infusion; c, number of 1-min periods (out of the possible 50 in each group) in which spontaneous up-BRS sequences were found during stepwise elevation of the phenylephrine infusion rate; d, number of 1-min periods (out of the possible 50 in each group) in which spontaneous down-BRS sequences were found during stepwise elevation of the phenylephrine infusion rate; Mean, mean baroreflex sensitivity value (ms (mm Hg)$^{-1}$); Valid, the percentage incidence (%) of RR intervals displaying spontaneous baroreflex sequences used to calculate baroreflex sensitivity data.
All values shown as means ± s.e.mean.
*$P<0.05$ vs 'chloralose' group; #$P<0.05$ vs 'propofol' group; †$P<0.05$ vs 'double dofetilide' group. For further details see Table 2.

rapid ($I_{Kr}$) component of the delayed rectifier $K^+$ current and the inward rectifier $K^+$ current ($I_{K1}$) with the highest affinity for $I_{Ks}$ (Bachmann *et al*., 2002). Simultaneous blockade of the sarcolemmal $Na^+$ and $K^+$ channels can provide a beneficial effect against drug-induced torsades de pointes via suppression of the early afterdepolarizations (Wu *et al*., 2005).

Pentobarbital homogeneously prolonged the duration of the action potential of the canine endo-, epi- and mid-myocardium, which significantly reduced the ability of repolarization-prolonging drugs to increase transmural dispersion of the repolarization (Shimizu *et al*., 1999), and thus, similarly to our results, pentobarbital reduced the incidence of the drug-induced torsades de pointes in arterially perfused canine left ventricular wedge preparations (Shimizu *et al*., 1999) and in dogs *in vivo* (Weissenburger *et al*., 2000; Yamamoto *et al*., 2001; Voss *et al*., 2002). Interestingly, pentobarbital did not reduce the almokalant-induced increase in transmural dispersion of the effective refractory period, whereas it did prevent the repolarization-prolonging almokalant from evoking torsades de pointes in dogs *in vivo* (Voss *et al*., 2002). Thus, it is still not fully verified whether the anti-arrhythmic activity of pentobarbital against drug-induced torsades de pointes is related to a reduction in the transmural dispersion of the repolarization.

The temporal dispersion of repolarization defined as the beat-to-beat variability of the monophasic action potential duration has been shown to predict the pro-arrhythmic activity of repolarization-prolonging drugs (Hondeghem *et al*., 2001; Thomsen *et al*., 2004). In an earlier study with isolated rabbit hearts, pentobarbital prolonged the duration of the action potential without increasing the beat-to-beat variability of the duration of the action potential in the presence or in the absence of the repolarization-prolonging sea anemone toxin ATX-II (Wu *et al*., 2006), which can also explain the preventive effect of pentobarbital in the present study.

As found in our experiments, the incidence of dofetilide-induced torsades de pointes in $\alpha_1$-adrenergically stimulated rabbits was only 50% when pentobarbital anaesthesia was applied (Bril *et al*., 1996). In contrast, the incidence of torsades de pointes induced by the selective $I_{Kr}$ blocker E-4031 was as high as 90% in $\alpha_1$-adrenergically stimulated rabbits despite the fact that the animals were anaesthetized with pentobarbital (Bril *et al*., 1996). Accordingly, the preventive effect of pentobarbital against torsades de pointes may be specifically related to dofetilide, but this needs further investigations to clarify.

### *Electrophysiology of propofol: the lack of effect on the dofetilide-induced pro-arrhythmia*

Therapeutic concentrations of propofol inhibit $I_{Na}$, the L-type $Ca^{2+}$ current ($I_{Ca,L}$) and the transient outward $K^+$ current ($I_{to}$) and delay ventricular conduction in the rabbit (Wu *et al*., 1997). This combined ion channel-blocking property should provide propofol with an anti-arrhythmic effect against drug-induced torsades de pointes, comparable to that of pentobarbital (Antzelevitch *et al*., 1999) or

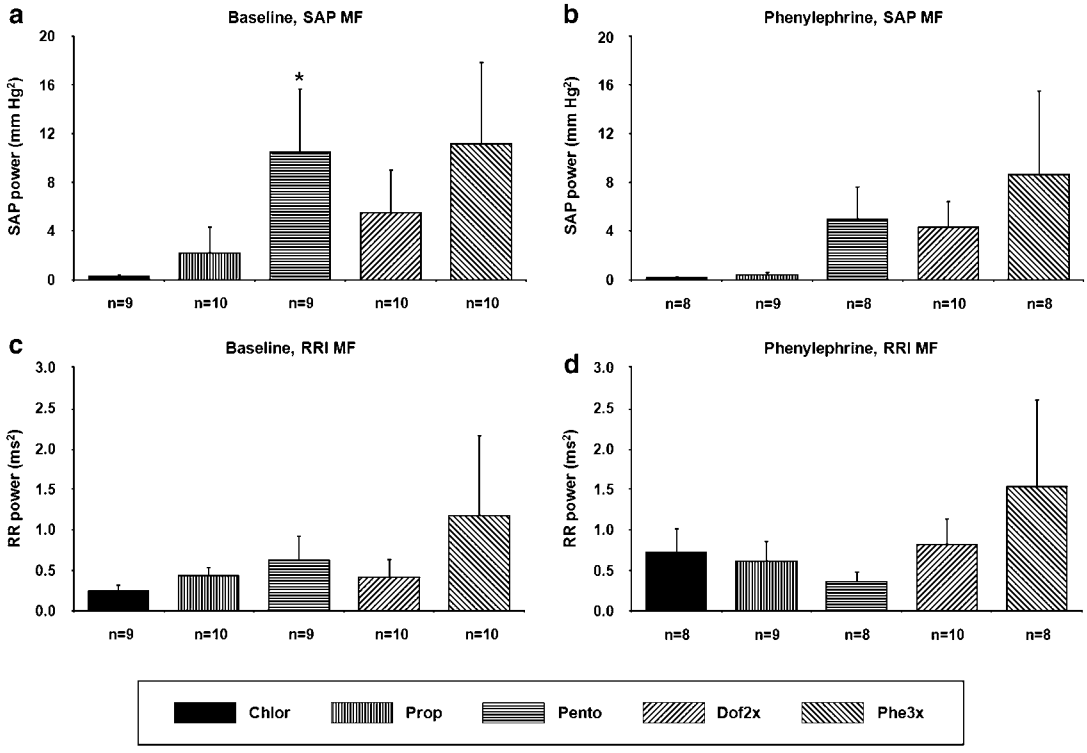


**Figure 3** The spectral power of the systolic arterial pressure and the RR interval calculated in the mid-frequency range (0.15–0.5 Hz) in anaesthetized rabbits. (**a** and **b**) The spectral power of the systolic arterial blood pressure at baseline and during phenylephrine infusion, respectively. (**c** and **d**) The spectral power of the RR interval at baseline and during phenylephrine infusion, respectively. Chlor, group of animals anaesthetized with α-chloralose ('chloralose' group); Prop, group of animals anaesthetized with propofol; Pento, group of animals anaesthetized with pentobarbital ('pentobarbital' group); Dof2x, group of animals anaesthetized with pentobarbital, and dofetilide is infused at a rate of 0.08 mg kg$^{-1}$ min$^{-1}$ ('double dofetilide' group; see Figure 1 for protocol); Phe3x, group of animals anaesthetized with pentobarbital and the maximum phenylephrine infusion rate is 45 μg kg$^{-1}$ min$^{-1}$ ('triple phenylephrine' group); Baseline, values measured before drug administration; Phenylephrine, values measured during phenylephrine infusion at a rate of 9 μg kg$^{-1}$ min$^{-1}$; SAP MF, the spectral power of the systolic arterial pressure integrated in the mid-frequency band; RRI MF, the spectral power of the RR interval integrated in the mid-frequency band; *n*, number of animals. *$P<0.05$ vs 'chloralose' and 'propofol' groups.

amiodarone (Sicouri *et al*., 1997). In contrast, propofol anaesthesia did not prevent the dofetilide-induced torsades de pointes in the present study. A possible explanation for this lack of an anti-arrhythmic effect is that propofol delayed ventricular conduction (Wu *et al*., 1997), which might increase the number of functioning re-entry circles in the presence of dofetilide, thereby counteracting the beneficial effect of the multiple ion channel-blocking property of the drug. The lack of an anti-arrhythmic effect of the multiple ion channel-blocking propofol against the dofetilide-induced torsades de pointes implies that *in vitro* studies, which examine only ion channel effects of drugs, can not properly predict the anti-arrhythmic or pro-arrhythmic activity of drugs; drug effects related to torsades de pointes can be examined best in integrated pro-arrhythmia models (Thomsen *et al*., 2006).

*Electrophysiology of α-chloralose: different effects of pentobarbital and α-chloralose on the dofetilide-induced pro-arrhythmia*

Similarly to pentobarbital, α-chloralose at a concentration of 500 mg l$^{-1}$, which is approximately five times higher than the anaesthetic concentration of the drug, decreased the transmural dispersion of the repolarization in the canine ventricular muscle *in vitro* (Antzelevitch *et al*., 1999). This was attributed to a possible blocking effect of the drug on the late $I_{Na}$, and it was therefore suggested that caution should be exercised in the interpretation and the extrapolation of arrhythmia data obtained from studies involving α-chloralose or pentobarbital anaesthesia (Antzelevitch *et al*., 1999). In contrast, when the anaesthetic concentrations of pentobarbital and α-chloralose were examined in dogs *in vivo* and in guinea pig papillary muscles *in vitro*, marked differences were found between the electrophysiological effects of the two anaesthetics, that is pentobarbital prolonged the action potential duration and the effective refractory period and decreased the maximum rate of voltage change ($V_{max}$) in phase 0 of the action potential, whereas α-chloralose (at a concentration of $\leqslant$100 mg l$^{-1}$) was devoid of direct electrophysiological effects in both species (Nattel *et al*., 1990). This may explain our results showing that, when anaesthetic (and equianaesthetic) concentrations of pentobarbital and α-chloralose are applied, there is a substantial difference between the effects of these anaesthetics on drug-induced pro-arrhythmia.

*No relationship between the repolarization-related ECG parameters and the dofetilide-induced pro-arrhythmia*

In the present study, the QT interval did not correlate with the pro-arrhythmic activity of dofetilide in the various

anaesthesia groups. This is in line with the findings of others and our own earlier results with the anaesthetized rabbit model (Carlsson *et al*., 1993; Farkas and Coker, 2002; Farkas *et al*., 2002) and supports the widely accepted notion that use of the QT interval as a predictor of drug-induced pro-arrhythmia can be grossly misleading (Hondeghem, 2006; Thomsen *et al*., 2006).

The electrocardiographic $T_{peak}$–$T_{end}$ interval has been shown to provide an index of the transmural dispersion of the repolarization (Antzelevitch and Oliva, 2006). In our study, dofetilide did not prolong the $T_{peak}$–$T_{end}$ interval before the induction of frequent arrhythmias in any anaesthesia group. Furthermore, the $T_{peak}$–$T_{end}$ interval of the pentobarbital-anaesthetized animals did not differ from those in the other anaesthesia groups, which indicates that the $T_{peak}$–$T_{end}$ interval measured in the standard limb Lead II of the ECG does not predict drug-induced pro-arrhythmia susceptibility in anaesthetized rabbits. Further investigations are needed to clarify whether this low predictive value of the $T_{peak}$–$T_{end}$ interval in anaesthetized rabbits is a result of the lack of a drug-induced increase in the transmural dispersion of the repolarization or of the lack of any relationship between the transmural dispersion of the repolarization and the $T_{peak}$–$T_{end}$ interval measured in the standard limb Lead II of the rabbit ECG.

It has been suggested that the variability of the QT interval may reflect the beat-to-beat variability of the duration of the action potential of the ventricular myocytes and, thus, may predict the pro-arrhythmic liability of the repolarization-prolonging drugs (Hondeghem, 2006). In accordance with this, the QT interval variability increased in dogs after the administration of pro-arrhythmic repolarization-prolonging drugs (Schneider *et al*., 2005). Surprisingly, the QT variability parameters in the different anaesthesia groups did not differ in the present study, and hence, did not predict the pro-arrhythmic activity of dofetilide. On the other hand, the significant increases in PNN8 and the short-term variability of the QT interval during phenylephrine and dofetilide infusion may predict a higher arrhythmia susceptibility of the α-chloralose-anaesthetized rabbits in the present study. However, the roles of the different parameters of the QT variability as predictors of drug-induced torsades de pointes in rabbits demand further investigations.

*The effects of the anaesthetics on the sympathetic activity*

The applied rabbit model of pro-arrhythmia depends critically on the stimulation of the $\alpha_1$-adrenoceptors (Carlsson *et al*., 1990). Since pentobarbital does not affect the $\alpha_1$-adrenoceptors, it is unlikely that the anti-arrhythmic activity of the drug was related to a reduction of the sensitizing $\alpha_1$-adrenoceptor stimulation in the present study. Interestingly, tripling of the rate of the background phenylephrine infusion did not affect the protective effect of pentobarbital against the dofetilide-induced torsades de pointes, while it elevated the blood pressure markedly. This is in accordance with the results of earlier investigations showing that neither an increased blood pressure (Coker and Farkas, 2004) nor the corresponding elevated ventricular stretch (Farkas *et al*., 2006) is of importance in the generation of drug-induced torsades de pointes in rabbits.

An increased sympathetic activity may contribute to the genesis of torsades de pointes in either the acquired or the inherited form of the long QT syndrome (Verrier and Antzelevitch, 2004; Schwartz, 2006). The highest baseline blood pressure, the largest number of arrhythmias during phenylephrine infusion and the highest baseline spectral power of the systolic arterial pressure in the mid-frequency range (SAP MF) were found in the animals anaesthetized with pentobarbital in the present study. These data indicate that the pentobarbital anaesthesia allowed a higher sympathetic activity as compared with those in the animals anaesthetized with the equianaesthetic dose of propofol or α-chloralose. Pentobarbital (Morita *et al*., 1987) and propofol (Xu *et al*., 2000) are known to reduce sympathetic nerve activity. On the other hand, an earlier study revealed elevation in the heart rate, blood pressure and serum noradrenaline level in α-chloralose-anaesthetized rabbits, and accordingly it was concluded that α-chloralose anaesthesia elevated the sympathetic activity (Pettersson *et al*., 1990). However, the dose of α-chloralose applied was very low (75 mg kg$^{-1}$) and the non-depolarizing muscle relaxant pancuronium was administered to the animals after anaesthesia, which was followed by the surgical preparation of the left femoral artery (Pettersson *et al*., 1990). Thus, it could well have been the pain that elevated the sympathetic activity of the rabbits rather than the α-chloralose anaesthesia in that study. Our SAP MF data indicate that the sympathetic activity of the α-chloralose-anaesthetized animals was low when the anaesthetic was administered in a high dose needed for a sufficiently deep anaesthesia. Accordingly, the sympathetic activity of the animals was suppressed by all the anaesthetics, but pentobarbital caused a small suppression in the sympathetic activity relative to the other two anaesthetics in the present study. Since the incidence of torsades de pointes was the lowest in the pentobarbital group (with the 'single dofetilide' dose), it is concluded that there is no positive correlation between the incidence of dofetilide-induced torsades de pointes and the activity of the sympathetic nervous system in the animal model applied.

*The effects of the anaesthetics on the vagal nerve activity*

A decreased heart rate variability and a decreased baroreflex sensitivity are predictors of arrhythmic and total cardiac mortality after myocardial infarction and also in patients with heart failure (Lombardi, 2002; Verrier and Antzelevitch, 2004). In the present study, the pronounced SAP MF did not coincide with an outstanding spectral power of the RR interval in the mid-frequency range in the pentobarbital-anaesthetized groups, which indicates that the baroreflex sensitivity of the pentobarbital-anaesthetized animals was suppressed (Cevese *et al*., 2001). Furthermore, the α-chloralose- and propofol-anaesthetized rabbits displayed lower variability of the RR interval and lower baroreflex sensitivity values, especially during the phenylephrine infusion, which indicates that the vagal nerve activity of these animals was significantly lower before the dofetilide administration than in the pentobarbital-anaesthetized animals. Accordingly, the

vagal nerve activity of the animals was suppressed by all the anaesthetics, but pentobarbital suppressed it least in the present study. This implies that a markedly decreased parasympathetic activity might predispose the animals to dofetilide-induced torsades de pointes, since the incidence of this arrhythmia was high in the α-chloralose- and propofol-anaesthetized groups. This is in accordance with earlier results demonstrating that a decreased baroreflex sensitivity predicted the occurrence of polymorphic ventricular tachycardia induced by the repolarization-prolonging caesium ions in rabbits (Ou *et al*., 1999). In contrast, isolated rabbit hearts, which lack vagal stimulation, have a very low susceptibility without atrioventricular blockade to dofetilide-induced torsades de pointes, even when perfused with a high concentration of the $\alpha_1$-adrenergic stimulant methoxamine (Farkas *et al*., 2006). Thus, further investigations are needed to verify whether there is a correlation between decreased vagal nerve activity and the occurrence of drug-induced torsades de pointes.

## Conclusions

Pentobarbital anaesthesia reduces, whereas propofol anaesthesia does not affect the incidence of dofetilide-induced torsades de pointes as compared with that seen with the standard α-chloralose anaesthesia in rabbits. The strong protective effect of pentobarbital against dofetilide-induced torsades de pointes may be related to its complex cardiac electrophysiological effects rather than its autonomic effects. Furthermore, in rabbits, anaesthetics may affect the genesis of drug-induced torsades de pointes differently, which must be considered when results of different studies are compared.

## Acknowledgements

The generous gift of dofetilide by EGIS Pharmaceuticals PLC (Budapest, Hungary) is gratefully acknowledged. This work was supported by the Hungarian Ministry of Health (ETT 203/2003 and 353/2006), the Hungarian National Research Fund (OTKA F-046776 OTKA K-69018 and NI-61902), the National Research and Development Programmes (NKFP 1A/046/2004), the Hungarian Academy of Sciences and the EU FPG grant LSHM-CT-2005-018833, EUGeneHeart.

## Conflict of interest

The authors state no conflict of interest.

## References

Aeschbacher G, Webb AI (1993a). Propofol in rabbits. 1. Determination of an induction dose. *Lab Anim Sci* **43**: 324–327.

Aeschbacher G, Webb AI (1993b). Propofol in rabbits. 2. Long-term anesthesia. *Lab Anim Sci* **43**: 328–335.

Antzelevitch C, Oliva A (2006). Amplification of spatial dispersion of repolarization underlies sudden cardiac death associated with catecholaminergic polymorphic VT, long QT, short QT and Brugada syndromes. *J Intern Med* **259**: 48–58.

Antzelevitch C, Shimizu W, Yan GX, Sicouri S, Weissenburger J, Nesterenko VV *et al*. (1999). The M cell: its contribution to the ECG and to normal and abnormal electrical function of the heart. *J Cardiovasc Electrophysiol* **10**: 1124–1152.

Bachmann A, Mueller S, Kopp K, Brueggemann A, Suessbrich H, Gerlach U *et al*. (2002). Inhibition of cardiac potassium currents by pentobarbital. *Naunyn Schmiedebergs Arch Pharmacol* **365**: 29–37.

Batey AJ, Coker SJ (2002). Proarrhythmic potential of halofantrine, terfenadine and clofilium in a modified *in vivo* model of torsade de pointes. *Br J Pharmacol* **135**: 1003–1012.

Berger RD, Kasper EK, Baughman KL, Marban E, Calkins H, Tomaselli GF (1997). Beat-to-beat QT interval variability: novel evidence for repolarization lability in ischemic and nonischemic dilated cardiomyopathy. *Circulation* **96**: 1557–1565.

Bertinieri G, Di Rienzo M, Cavallazzi A, Ferrari AU, Pedotti A, Mancia G (1988). Evaluation of baroreceptor reflex by blood pressure monitoring in unanesthetized cats. *Am J Physiol* **254**: H377–H383.

Booker PD, Whyte SD, Ladusans EJ (2003). Long QT syndrome and anaesthesia. *Br J Anaesth* **90**: 349–366.

Brennan M, Palaniswami M, Kamen P (2001). Do existing measures of Poincare plot geometry reflect nonlinear features of heart rate variability? *IEEE Trans Biomed Eng* **48**: 1342–1347.

Bril A, Gout B, Bonhomme M, Landais L, Faivre JF, Linee P *et al*. (1996). Combined potassium and calcium channel blocking activities as a basis for antiarrhythmic efficacy with low proarrhythmic risk: experimental profile of BRL-32872. *J Pharmacol Exp Ther* **276**: 637–646.

Buchanan LV, Kabell G, Brunden MN, Gibson JK (1993). Comparative assessment of ibutilide, D-sotalol, clofilium, E-4031, and UK-68,798 in a rabbit model of proarrhythmia. *J Cardiovasc Pharmacol* **22**: 540–549.

Burjorjee JE, Milne B (2002). Propofol for electrical storm; a case report of cardioversion and suppression of ventricular tachycardia by propofol. *Can J Anaesth* **49**: 973–977.

Carlsson L, Abrahamsson C, Andersson B, Duker G, Schiller-Linhardt G (1993). Proarrhythmic effects of the class III agent almokalant: importance of infusion rate, QT dispersion, and early afterdepolarisations. *Cardiovasc Res* **27**: 2186–2193.

Carlsson L, Almgren O, Duker G (1990). QTU-prolongation and torsades de pointes induced by putative class III antiarrhythmic agents in the rabbit: etiology and interventions. *J Cardiovasc Pharmacol* **16**: 276–285.

Cevese A, Gulli G, Polati E, Gottin L, Grasso R (2001). Baroreflex and oscillation of heart period at 0.1 Hz studied by alpha-blockade and cross-spectral analysis in healthy humans. *J Physiol* **531**: 235–244.

Coker SJ, Farkas A (2004). Influence of bradycardia on the induction of torsade de pointes with clofilium. *J Mol Cell Cardiol* **36**: 722.

Copie X, Le Heuzey JY, Iliou MC, Khouri R, Lavergne T, Pousset F *et al*. (1996). Correlation between time-domain measures of heart rate variability and scatterplots in postinfarction patients. *Pacing Clin Electrophysiol* **19**: 342–347.

Farkas A, Batey AJ, Coker SJ (2004). How to measure electrocardiographic QT interval in the anaesthetized rabbit. *J Pharmacol Toxicol Methods* **50**: 175–185.

Farkas A, Coker SJ (2002). Limited induction of torsade de pointes by terikalant and erythromycin in an *in vivo* model. *Eur J Pharmacol* **449**: 143–153.

Farkas A, Leprán I, Papp JG (1998). Comparison of the antiarrhythmic and the proarrhythmic effect of almokalant in anaesthetised rabbits. *Eur J Pharmacol* **346**: 245–253.

Farkas A, Leprán I, Papp JG (2002). Proarrhythmic effects of intravenous quinidine, amiodarone, D-sotalol, and almokalant in the anesthetized rabbit model of torsade de pointes. *J Cardiovasc Pharmacol* **39**: 287–297.

Farkas AS, Acsai K, Tóth A, Dézsi L, Orosz S, Forster T *et al*. (2006). Importance of extracardiac alpha1-adrenoceptor stimulation in assisting dofetilide to induce torsade de pointes in rabbit hearts. *Eur J Pharmacol* **537**: 118–125.

Hondeghem LM (2006). Thorough QT/QTc not so thorough: removes torsadogenic predictors from the T-wave, incriminates safe drugs, and misses profibrillatory drugs. *J Cardiovasc Electrophysiol* **17**: 337–340.

Hondeghem LM, Carlsson L, Duker G (2001). Instability and triangulation of the action potential predict serious proarrhythmia, but action potential duration prolongation is antiarrhythmic. *Circulation* **103**: 2004–2013.

Iwao T, Yonemochi H, Nakagawa M, Takahashi N, Saikawa T, Ito M (2000). Effect of constant and intermittent vagal stimulation on the heart rate and heart rate variability in rabbits. *Jpn J Physiol* **50**: 33–39.

Lombardi F (2002). Clinical implications of present physiological understanding of HRV components. *Card Electrophysiol Rev* **6**: 245–249.

Lu HR, Remeysen P, Somers K, Saels A, De Clerck F (2001). Female gender is a risk factor for drug-induced long QT and cardiac arrhythmias in an *in vivo* rabbit model. *J Cardiovasc Electrophysiol* **12**: 538–545.

Malpas SC, Burgess DE (2000). Renal SNA as the primary mediator of slow oscillations in blood pressure during hemorrhage. *Am J Physiol Heart Circ Physiol* **279**: H1299–H1306.

Mazur A, Roden DM, Anderson ME (1999). Systemic administration of calmodulin antagonist W-7 or protein kinase A inhibitor H-8 prevents torsade de pointes in rabbits. *Circulation* **100**: 2437–2442.

Moguilevski VA, Shiel L, Oliver J, McGrath BP (1996). Power spectral analysis of heart-rate variability reflects the level of cardiac autonomic activity in rabbits. *J Auton Nerv Syst* **58**: 18–24.

Morita H, Nishida Y, Uemura N, Hosomi H (1987). Effect of pentobarbital anesthesia on renal sympathetic nerve activity in the rabbit. *J Auton Nerv Syst* **20**: 57–64.

Nattel S, Wang ZG, Matthews C (1990). Direct electrophysiological actions of pentobarbital at concentrations achieved during general anesthesia. *Am J Physiol* **259**: H1743–H1751.

Ou B, Nakagawa M, Yonemochi H, Ri C, Ishida S, Takahashi N *et al*. (1999). Baroreflex sensitivity predicts the induction of ventricular arrhythmias by cesium chloride in rabbits. *Jpn Circ J* **63**: 783–788.

Pettersson K, Bejne B, Bjork H, Strawn WB, Bondjers G (1990). Experimental sympathetic activation causes endothelial injury in the rabbit thoracic aorta via beta 1-adrenoceptor activation. *Circ Res* **67**: 1027–1034.

Schneider J, Hauser R, Andreas JO, Linz K, Jahnel U (2005). Differential effects of human ether-a-go-go-related gene (HERG) blocking agents on QT duration variability in conscious dogs. *Eur J Pharmacol* **512**: 53–60.

Schwartz PJ (2006). The congenital long QT syndromes from genotype to phenotype: clinical implications. *J Intern Med* **259**: 39–47.

Shimizu W, McMahon B, Antzelevitch C (1999). Sodium pentobarbital reduces transmural dispersion of repolarization and prevents torsades de pointes in models of acquired and congenital long QT syndrome. *J Cardiovasc Electrophysiol* **10**: 154–164.

Sicouri S, Moro S, Litovsky S, Elizari MV, Antzelevitch C (1997). Chronic amiodarone reduces transmural dispersion of repolarization in the canine heart. *J Cardiovasc Electrophysiol* **8**: 1269–1279.

Thomsen MB, Matz J, Volders PG, Vos MA (2006). Assessing the proarrhythmic potential of drugs: current status of models and surrogate parameters of torsades de pointes arrhythmias. *Pharmacol Ther* **112**: 150–170.

Thomsen MB, Verduyn SC, Stengl M, Beekman JD, de Pater G, van Opstal J *et al*. (2004). Increased short-term variability of repolarization predicts d-sotalol-induced torsades de pointes in dogs. *Circulation* **110**: 2453–2459.

Verrier RL, Antzelevitch C (2004). Autonomic aspects of arrhythmogenesis: the enduring and the new. *Curr Opin Cardiol* **19**: 2–11.

Voss F, Schreiner KD, Senges JC, Becker R, Bauer A, Kuebler W *et al*. (2002). Refractory patterns and susceptibility to drug-induced polymorphic ventricular tachycardias in dogs with chronic atrioventricular block: relation to the type of anesthesia. *J Cardiovasc Pharmacol* **40**: 651–659.

Walker MJA, Curtis MJ, Hearse DJ, Campbell RWF, Janse MJ, Yellon DM *et al*. (1988). The Lambeth conventions: guidelines for the study of arrhythmias in ischaemia infarction, and reperfusion. *Cardiovasc Res* **22**: 447–455.

Wartenberg HC, Wartenberg JP, Urban BW (2001). Human cardiac sodium channels are affected by pentobarbital. *Eur J Anaesthesiol* **18**: 306–313.

Weissenburger J, Nesterenko VV, Antzelevitch C (2000). Transmural heterogeneity of ventricular repolarization under baseline and long QT conditions in the canine heart *in vivo*: torsades de pointes develops with halothane but not pentobarbital anesthesia. *J Cardiovasc Electrophysiol* **11**: 290–304.

Wu L, Shryock JC, Song Y, Belardinelli L (2006). An increase in late sodium current potentiates the proarrhythmic activities of low-risk QT-prolonging drugs in female rabbit hearts. *J Pharmacol Exp Ther* **316**: 718–726.

Wu MH, Su MJ, Sun SS (1997). Comparative direct electrophysiological effects of propofol on the conduction system and ionic channels of rabbit hearts. *Br J Pharmacol* **121**: 617–624.

Wu Y, Carlsson L, Liu T, Kowey PR, Yan GX (2005). Assessment of the proarrhythmic potential of the novel antiarrhythmic agent AZD7009 and dofetilide in experimental models of torsades de pointes. *J Cardiovasc Electrophysiol* **16**: 898–904.

Xu H, Aibiki M, Yokono S, Ogli K (2000). Dose-dependent effects of propofol on renal sympathetic nerve activity, blood pressure and heart rate in urethane-anesthetized rabbits. *Eur J Pharmacol* **387**: 79–85.

Yamamoto K, Tamura T, Imai R, Yamamoto M (2001). Acute canine model for drug-induced torsades de pointes in drug safety evaluation-influences of anesthesia and validation with quinidine and astemizole. *Toxicol Sci* **60**: 165–176.