British Journal of Pharmacology (2004) 142, 1255–1264 © 2004 Nature Publishing Group All rights reserved 0007–1188/04 $30.00
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Evidence for gender differences in electrophysiological properties of canine Purkinje fibres

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1 Women are more prone to develop torsades de pointes, a rare life-threatening polymorphic ventricular tachycardia, than are men during administration of medicines that have the potential to block I_{Kr} (rapid delayed rectifier cardiac K^+ current) and to prolong the QT interval. Blockade of I_{Kr}, hypokalaemia and extreme bradycardia were used to evaluate whether there are gender differences in cardiac repolarisation in canine Purkinje fibres (PFs). Microelectrode techniques were employed to measure action potential (AP) parameters in PFs from adult female and male dogs.

2 Under control conditions, fibres from female animals in normal or low K^+ conditions exhibited significantly longer AP durations at 50% (APD_{50}) and 90% (APD_{90}) of repolarisation as compared with APDs of fibres from male animals.

3 Gender-related difference to rate adaptation was also present in APD_{90} of fibres from female animals compared to males.

4 At a stimulation rate of 0.2 Hz, but not at 1.0 Hz, dofetilide elicited a significantly higher increase in APD_{90}, incidence of early afterdepolarisations, triggered and sustained-triggered activities (TAs) in fibres from female animals compared to males in either normal or low K^+ conditions. The sustained TAs were reversed by raising the concentration of $[K^+]_0$ in Purkinje preparations from both male (one out of one) and female (12 out of 12) dogs.

5 In conclusion, our data provide experimental evidence pointing to gender differences in canine AP repolarisation. PFs from female dogs can be used in safety pharmacology studies as a sensitive model for evaluating the potential proarrhythmic events *in vitro* of a new medicinal product.
British Journal of Pharmacology (2004) **142,** 1255–1264. doi:10.1038/sj.bjp.0705880

Keywords: Gender; Purkinje fibre; action potential duration; early afterdepolarisation; rapid delayed rectifier cardiac K^+ current

Abbreviations: AP, action potential; APA, amplitude of the action potential; APD, action potential duration; APD_{50} and APD_{90}, duration of the action potential at 50 and 90% repolarisation; dV/dt_{max}, maximum rate of depolarisation during the upstroke; EADs, early afterdepolarisations; I_{Kr}, rapid delayed rectifier cardiac K^+ current; JTc, rate-corrected JT interval (QT interval−QRS duration); LQTS, long QT syndrome; PFs, Purkinje fibres; QTc, rate-corrected QT interval; RMP, resting membrane potential; STA, EAD-induced sustained triggered activities; TA, EAD-induced triggered activities; TdP, torsades de pointes

Introduction

A higher incidence of QT interval prolongation has been observed in women treated with a wide range of drugs that prolong the QT interval compared with men (Makkar *et al*., 1993; Ebert *et al*., 1998; Drici & Clement, 2001). Most of these drugs have been shown to block the rapid component of the cardiac delayed rectifier potassium current (I_{Kr}), leading to prolongation of action potential duration (APD) and delayed ventricular repolarisation, which can evolve into *torsades de pointes (TdP)*, a rare polymorphic ventricular tachycardia (Drici & Clement, 2001; Redfern *et al*., 2003).

It is now well accepted that differences in cardiac electrophysiology exist between women and men, especially in the electrocardiographic pattern of ventricular repolarisation (Rautaharju *et al*., 1992; Lehmann & Yang, 2001; Pham & Rosen, 2002; Yang *et al*., 2002). Evidence from clinical studies suggest that the gender difference in the rate-corrected QT interval (QTc) may be primarily due to the influence of male sex steroid hormones. Whereas neonates and children below the age of 10 years of both sexes showed no difference in QT interval, adult women (from puberty to adulthood) had a longer QTc interval as compared with adult men (Stramba-Badiale *et al*., 1995). However, it has been proposed that the gender-related differences in repolarisation after puberty reflect shortening of the QT interval in males rather than its prolongation in females (Rautaharju *et al*., 1992; Surawicz & Parikh, 2002). A recent study (Bidoggia *et al*., 2000), comparing JTc (rate-corrected JT interval) intervals (an index for myocardial repolarisation) in castrated men to those found in intact men and women, demonstrated that JTc intervals in the castrated male group were longer than those found in normal males and were similar to those found in normal females. Moreover, the same study

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Advance online publication: 19 July 2004

reported that the changes observed in castrated men were reversed by testosterone and that women with virilisation exhibited shorter JTc intervals than did castrated men and normal women. These results suggest that testosterone can play an important role in modulating cardiac repolarisation, and may thus be responsible for the shorter JTc intervals observed in men compared with those found in women.

There is also good evidence for sex-related cardiac electrophysiology differences in animals. A high number of publications have validated the use of the rabbit heart as a model that manifests gender-related differences in cardiac repolarisation similar to those in humans (Pham & Rosen, 2002). Recently, the mouse heart has become an increasingly important model for the study of sex differences in cardiac repolarisation (Trepanier-Boulay *et al*., 2001; Wu & Anderson, 2002; Brouillette *et al*., 2003). The findings published from these two models clearly showed how the expression, properties and/or drug sensitivity of cardiac K^+ channels in animals untreated (Liu *et al*., 1998; Trepanier-Boulay *et al*., 2001; Wu & Anderson, 2002) or treated with male or female sex hormones (Drici *et al*., 1996; Pham *et al*., 2001; 2002a, b; Brouillette *et al*., 2003; Liu *et al*., 2003) modulate cardiac repolarisation and how the presence of androgens helps to suppress rate- and drug-induced delays in cardiac repolarisation.

Despite the importance of understanding gender differences in cardiac repolarisation in rabbit and mice models, there are no published studies with regard to whether or not there are sex-related differences in canine repolarisation. In a recent study, it has been demonstrated that canine Purkinje fibres (PFs) can be used as an *in vitro* model system for drug-induced long QT syndrome (LQTS) and arrhythmogenesis (Gintant *et al*., 2001). Moreover, the beagle dog is a commonly used preclinical species to test the effects of new chemical entities on cardiac repolarisation *in vivo* (Gralinski, 2003). Accordingly, the main motivation for the present study was to evaluate whether there are gender differences in the repolarisation of the cardiac action potential (AP) in canine PFs under normal conditions and those in which risk factors for TdP are combined (i.e. I_{Kr} block (Redfern *et al*., 2003), hypokalaemia (Yan & Antzelevitch, 1998; Vos *et al*., 2001) and extreme bradycardia (Lu *et al*., 2002)).

Methods

PF preparation

Alderley Park beagle dogs of either sex were used. The females weighed 7.55–11.81 kg and were 20–37 months old. Except for two dogs, the males weighed 8.85–14.93 kg and were 9–14 months old. The two male dogs that fell outside this age range were 100 and 102 months of age but AP parameters in PFs from these two animals fell within the range of values seen in PFs from the other males. All animals were maintained in accordance with the Guide for the Home Office Code and Practice for the Housing and Care of Animals used in Scientific Procedures. The procedures were authorised under a project licence granted under the animals (Scientific Procedures) act 1986.

Anaesthesia was induced with approximately 45 mg kg^{-1} pentobarbitone and the anaesthetic was topped up until there was no pedal or pupil reflexes. Once the desirable anaesthesia level was reached, the chest was opened *via* a left thoracotomy and the heart was excised and placed in a Krebs buffer, maintained at 4°C and previously saturated with a 95% O_2/5% CO_2 mixture. The composition of the Krebs buffer was as follows (mM): NaCl 118, KCl 4, $MgCl_2$ 1, $CaCl_2$ 1.8, $NaHCO_3$ 31, NaH_2PO_4 1.8, glucose 11. This Krebs buffer will be referred to as normal K^+ solution (containing 4 mM K^+ to mimic normal K^+ levels). Another Krebs buffer was also used that contained 2.25 mM K^+ (to mimic hypokalaemia; Yan & Antzelevitch, 1998; Vos *et al*., 2001); this will be referred to as low K^+ solution. PFs (with attached segments of ventricular muscle) were dissected from both ventricles of either female or male dogs and each one was placed in a separate, custom-made glass-recording chamber (Aztek Scientific Glassblowing Co., Stoke-On-Trent, U.K.). Any additional fibres were stored in the normal K^+ solution at room temperature and gassed with a 95% O_2/5% CO_2 mixture until use (within 6 h; Gintant *et al*., 2001).

The recording chamber was continuously perfused with normal K^+ solution at a rate of 5 ml min^{-1} *via* a helical glass tube passing through the water jacket through which water at 37.5±0.2°C was recycled using a heating-circulating water bath (Circulator DC50, Thermo Haake Instruments Inc., Karlsruhe, Germany); perfusion was achieved using a peristaltic pump (Gilson Minipuls 3, Gilson Inc., Villiers-le-Bel, France). Each end of the PF was fixed to the floor of the recording chamber. The fixing process was achieved *via* a single pin at each end of the fibre. As well as holding the tissue in place, the pins were also used to connect the tissue to a DS2A stimulator (Digitimer Limited, Welwyn Garden City, U.K.) that was controlled by a frequency pulse generator (TG1010 Thurlby Thandar Instruments Ltd, Huntingdon, U.K.). The following stimulation parameters were used: stimulation voltage of twice the threshold for AP generation, pulse width 2 ms, stimulation rate 1 Hz (to mimic normal human resting heart rate = 60 pulses min^{-1}) or 0.2 Hz (to mimic extreme bradycardia; Lu *et al*., 2002).

Electrophysiology

A MultiClamp 700A amplifier (Axon Instruments Inc., Union City, CA, U.S.A.) was used to record APs. The MultiClamp 700A was controlled using MultiClamp Commander software. Glass micropipettes (resistance, 12–19 MΩ), made using a micropipette puller (Sutter P97, Sutter Instrument Company, Novato, CA, U.S.A.), were filled with 3 M KCl and used to impale a PF and measure the voltage across the cell membrane. Following impalement, a threshold stimulation voltage for AP generation was defined and a stimulation voltage of twice this threshold was applied for the rest of the experiment. After an AP was evoked, no recordings were made for at least 45 min to allow the fibre to stabilise using a stimulation rate of 1.0 Hz.

The voltage signal from the scaled output of the MultiClamp 700A amplifier (amplified at 10 V V^{-1} and filtered at 1 kHz; Bessel) was then acquired, on-line, by digitising this analogue signal with an analogue to digital converter (DT 3010, Notocord Systems S.A., Croissy Sur Seine, France). The digitised signal was then captured on a computer running Notocord HEM software version 3.4. This software was set up to acquire, on-line, the following AP parameters: (APD_{40}, APD_{50}, APD_{70} and APD_{90}), the duration of the AP at 40, 50, 70 and 90% repolarisation, respectively. RMP (resting membrane potential), APA (amplitude of the AP) and

dV/dt_{max} (the maximum rate of depolarisation during the upstroke) were also measured.

Experimental protocol

After a stabilisation period of at least 45 min, APs were recorded from PFs. Normal or low K^+ solutions without and with dofetilide (3 μM) were only applied if impalements exhibited APs typical for PFs. Following impalement, stability of AP parameters was assessed for 5–10 min in a fibre incubated with the normal K^+ solution and stimulated at 1.0 Hz. The stimulation rate was then changed to 0.2 Hz to investigate the rate adaptation of APs. After a return to a stimulation rate of 1.0 Hz, the same sequence was repeated following perfusion of the same fibre with the low K^+ solution. After the assessment of the rate adaptation in low K^+ solution and before the fibre was first incubated with 3 μM of dofetilide prepared in normal K^+ solution, a washout of a short period with the normal K^+ solution was allowed to elapse until AP parameters returned to baseline levels. After the increase in APDs by dofetilide reached a steady state, the fibre was superfused with the same concentration of dofetilide prepared in low K^+ solution. After the assessment of the effect of dofetilide on AP parameters in low K^+ solution, a washout of a short period with the dofetilide prepared in the normal K^+ solution was allowed to elapse before the stimulation rate was then changed to 0.2 Hz to investigate under extreme bradycardia the rate adaptation of APDs and the incidence of abnormal APs in terms of triggered activities (TAs). An early afterdepolarisation (EAD) was identified as an afterpotential that interrupts the normal repolarisation of AP. In addition to local, nonpropagated EADs, we observed EAD-induced triggered (TA: more than one EAD) and EAD-induced sustained triggered (STA: TA evoked a nonrepolarisation of AP) activities.

Drugs

Dofetilide was purchased from Apin Chemicals Ltd (Abingdon, U.K.) dissolved in dimethylsulphoxide (DMSO) at a concentration of 3 mM and kept at –20°C until use. The 3 mM DMSO stock was diluted 1000-fold using normal or low K^+ solutions on the day of the experiment to give a final concentration of 3 μM. All the other chemicals were purchased from Sigma-Aldrich Company Ltd (Poole, U.K.).

Statistics

Results are expressed as mean ± s.e.m. Differences were tested for statistical significance using the paired (two sample for means; same sex) and unpaired (two sample assuming unequal variances; gender differences) Student's *t*-test. A value of $P<0.05$ was considered significant.

Results

Gender differences in APDs in normal and hypokalaemic conditions

When stimulated at 1 Hz, APs in fibres from female animals were longer than those from males (Table 1) and this was apparent at all measures of duration. There were no significant differences in the other AP parameters. As expected, in hypokalaemic conditions, APD was prolonged in fibres from both sexes. In these low K^+ conditions, APs recorded in fibres from female animals remained longer than those from male animals (Table 1). Hypokalaemic conditions also tended to hyperpolarise RMP of fibres from both sexes, but this was only statistically significant in males. Other parameters were unchanged in hypokalaemic conditions.

Table 1 Action potential parameters in isolated canine PFs incubated with Tyrode solutions containing either 4 (mimicking normal K^+ levels) or 2.25 mM (mimicking hypokalaemia) $[K^+]_0$ and stimulated at 1.0 Hz (mimicking normal human resting heart rate)

Parameters	*Sex*	$[K^+]_o$ (4.0 mM)	n	$[K^+]_o$ (2.25 mM)	n
APD_{90} (ms)	M	246.5 ± 9.8	14	273.5 ± 12.0$^{\$}$	14
	F	282.0 ± 9.9*	13	312.0 ± 10.4*,†	13
APD_{70} (ms)	M	210.3 ± 9.8	14	232.9 ± 11.4	14
	F	241.5 ± 8.9*	13	266.3 ± 9.1*,†	13
APD_{50} (ms)	M	170.8 ± 11.1	14	194.1 ± 11.0	14
	F	204.9 ± 9.5*	13	231.2 ± 9.4*,†	13
APD_{40} (ms)	M	134.8 ± 13.1	14	155.6 ± 13.4	14
	F	170.1 ± 10.5*	13	200.7 ± 10.1*,†	13
RMP (mV)	M	−85.4 ± 1.3	14	−89.6 ± 1.8$^{\$}$	14
	F	−86.8 ± 2.6	13	−92.8 ± 2.8	13
APA (mV)	M	119.5 ± 5.0	14	124.2 ± 4.5	14
	F	118.0 ± 2.4	13	121.9 ± 2.8	13
dV/dt_{max} ($V\,s^{-1}$)	M	214.4 ± 10.9	14	219.5 ± 12.0	14
	F	239.1 ± 15.0	13	228.9 ± 11.4	13

In all, 10 animals were used in each gender to obtain $n=13$ in female (F) and 14 in male (M) animals. APD_{40}, APD_{50}, APD_{70} and APD_{90}: the duration of AP at 40, 50, 70 and 90% repolarisation, respectively. RMP: resting membrane potential; APA: amplitude of AP; dV/dt_{max}: the maximum rate of depolarisation during the upstroke. Data are expressed as mean ± s.e.m. $^{*}P<0.05$ *versus* values from male animals, $^{\dagger}P<0.05$ and $^{\$}P<0.05$ *versus* values from female and male animals at 4 mM $[K^+]_0$, respectively.

Gender differences in reverse-rate dependence

A fundamental property of cardiac myocytes is the ability to adapt to a decrease in the heart rate with an increase in APD, the so called 'reverse-rate dependence'. Therefore, we sought to determine whether there was a gender difference in this property in both normal and low K^+ conditions. A decrease in stimulation rate from 1 to 0.2 Hz resulted in an increase in APD_{50} and APD_{90} in fibres from both female and male animals in either normal or low K^+ conditions (Figure 1a and b). No significant differences were found in the increase in APD_{50} between females and males in response to low-frequency stimulation (data not shown). However, the increase in APD_{90} (Figure 1c) was significantly greater in fibres from female animals in both normal and low K^+ conditions. On the other hand, a decrease in the stimulation rate did not significantly change RMP in fibres from female and male animals in either normal or low K^+ conditions (data not shown) and did not alter dV/dt_{max} or APA. In addition, there were no differences between females and males in dV/dt_{max}, APA and RMP.

Gender differences in the effect of an I_{Kr} blocker – dofetilide

I_{Kr} current plays a major role in the repolarisation of the cardiac AP (Tseng, 2001). Dofetilide, a highly selective blocker of I_{Kr} (Kiehn *et al*., 1994), has been shown to produce a significant prolongation of APD in the heart of different species including the dog (Gintant *et al*., 2001; Pham *et al*., 2001; Biliczki *et al*., 2002; Lu *et al*., 2002; Pham *et al*., 2002b; Obreztchikova *et al*., 2003). In both fibres from female and

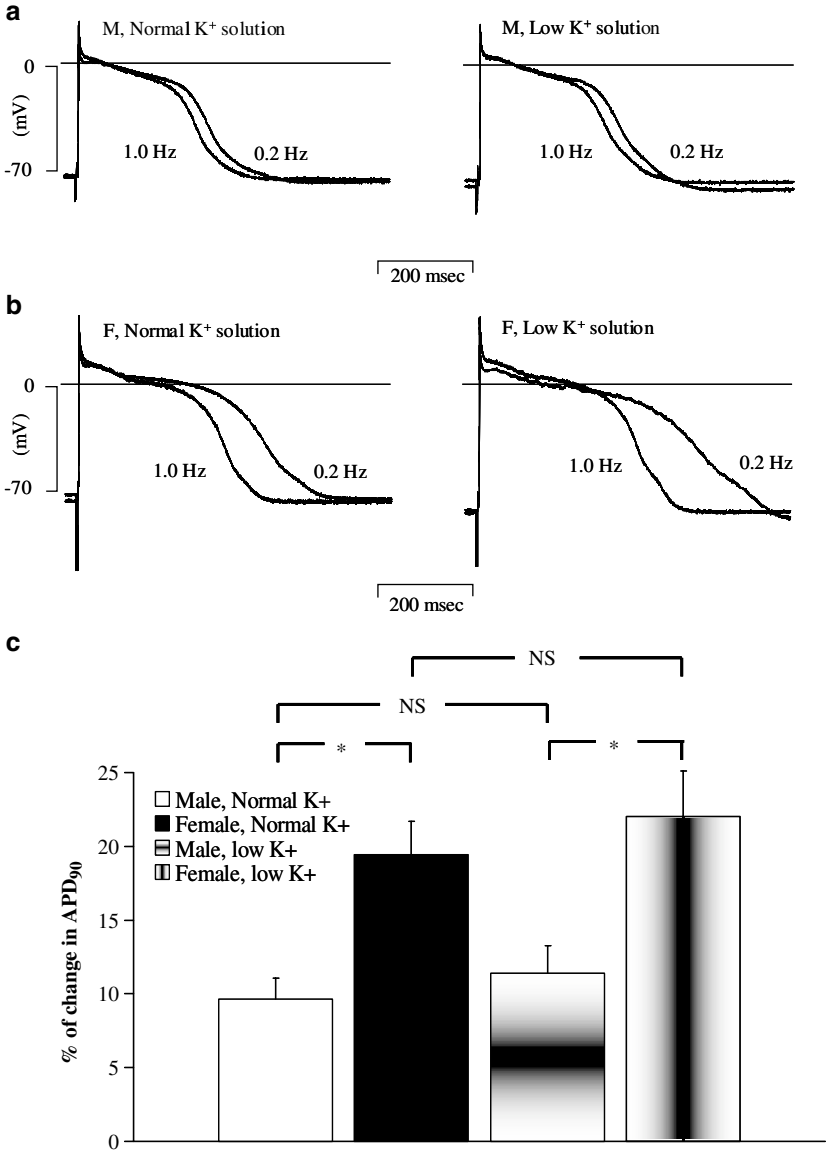

Figure 1 Effect of stimulation rate on APD_{90}. (a) and (b) show representative APs recorded in fibres from male (M) and female (F) animals in either normal or low K^+ conditions and stimulated at a stimulation rate of 1.0 or 0.2 Hz. (c) The mean % change in APD_{90} following a change in stimulation rate from 1.0 to 0.2 Hz in both M and F animals (10 dogs were used in each gender to obtain $n=13$ in F and 14 in M animals). $^*P<0.01$ *versus* values in M animals. $^{NS}P>0.05$ *versus* values in low K^+ conditions.

male animals in either normal or low K^+ conditions, dofetilide (3 μM) produced a significant prolongation of APD_{50-90} at stimulation rate of 1 and 0.2 Hz (Figures 2–4), but did not change dV/dt_{max}, RPM or APA (data not shown). At a stimulation rate of 1 Hz, the degree of prolongation of APD_{50} (data not shown) and APD_{90} (Figure 2c) evoked by dofetilide shows a trend for gender difference in both normal and low K^+ solutions, although this is not statistically significant. However, at low stimulation rate (0.2 Hz), changes in APD_{90}, but not in APD_{50} (data not shown), were greater in fibres from females (Figure 3). On the other hand, at both 1 and 0.2 Hz stimulation rate, dofetilide did not alter the other parameters of AP in fibres from female and male animals in either normal or low K^+ conditions and there were no differences between females and males for these other parameters.

Gender differences in the incidence of EADs

It is well known that excessive prolongation of the repolarisation phase of AP in both inherited (mutations in cardiac ion channels) and acquired (administration of drugs that block cardiac I_{Kr}) LQTS generally favour the generation of EADs, cellular proarrhythmic events that with appropriate dispersion of repolarisation may initiate TdP (Keating & Sanguinetti, 2001; Marban, 2002). It is now well accepted that drugs that prolong repolarisation induce TdP more frequently in women than men (Ebert *et al*., 1998; Drici & Clement, 2001). Moreover, factors such as extreme bradycardia and hypokalaemia play an important role in the development of EADs and explain the susceptibility in LQTS patients to TdP (Engelstein, 2003). We sought to determine if the incidence

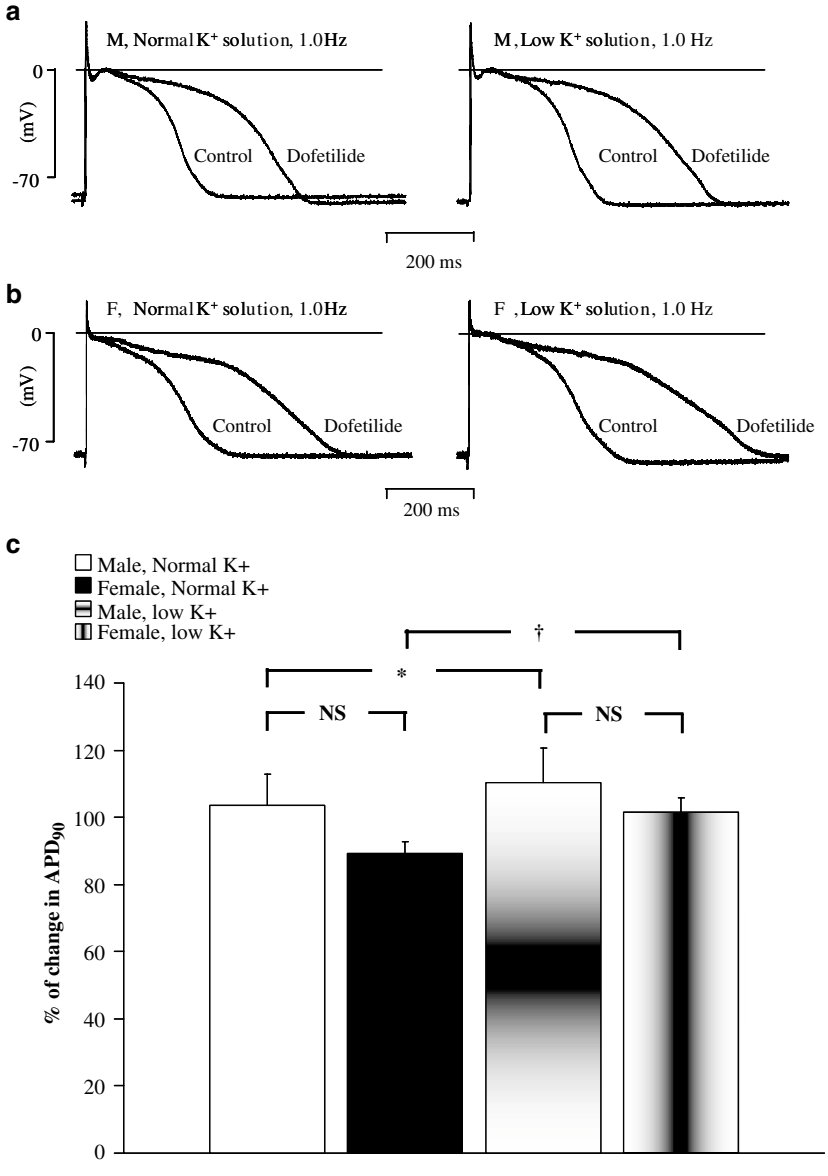

Figure 2 Effect of dofetilide (an I_{Kr} blocker) on APD_{90} in canine PFs at a stimulation rate of 1.0 Hz. (a) and (b) show representative APs recorded in fibres from male (M) and female (F) animals in either normal or low K^+ conditions in the presence and the absence of dofetilide (3 μM). (c) The mean % change in APD_{90} induced by dofetilide with a stimulation rate of 1.0 Hz in both M and F animals (10 dogs were used in each gender to obtain $n=13$ in F and 14 in M animals). $^{NS}P>0.05$ *versus* values in M animals. $^{*}P>0.05$ and $^{\dagger}P<0.01$ *versus* values in M and F animals in normal K^+ condition, respectively.

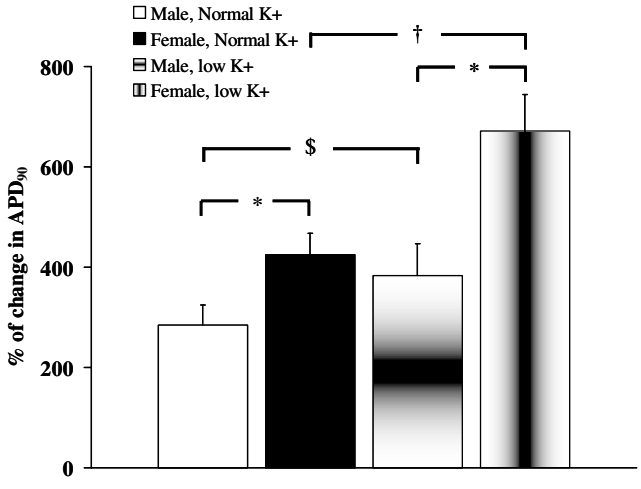

Figure 3 Mean % change in APD_{90} induced by dofetilide in canine PFs. A stimulation rate of 0.2 Hz was used in both male (M) and female (F) animals (10 dogs were used in each gender to obtain $n=13$ in F and 14 in M animals). $^{*}P<0.05$ *versus* values in M animals. $^{\dagger}P<0.05$ and $^{\$}P<0.01$ *versus* values in F and M animals in normal K^+ conditions, respectively.

of EADs, TAs and STAs in PFs differed between genders using the different experimental conditions already described. During superfusion of dofetilide (3 μM), EADs did not occur in fibres from either female or male animals in normal or low K^+ conditions at a stimulation rate of 1 Hz (Table 2). However, at a stimulation rate of 0.2 Hz, dofetilide did elicit EADs. In normal K^+ conditions, the incidence of EADs was higher in fibres from female animals as compared to males (Table 2, Figure 4). EADs were initiated from a take-off potential (the most negative plateau voltage reached before the depolarisation of an EAD) of −34 mV (range −48 to −22 mV) in fibres from female animals and −32 mV (range −45 to −22 mV) in fibres from males. The peak voltage of these EADs reached a voltage of −10 mV (range −38 to 0 mV) in fibres from females and −8 mV (range −16 to −7 mV) in those from male animals (Table 2). Only in fibres from female animals, TA was seen in 13 out of 20 preparations (65%); STA was seen in one out of 20 preparations (5%) (Table 2). In addition, at low stimulation rate and in low K^+ conditions, dofetilide evoked a higher incidence of EADs, TA and STA in both fibres from females (100, 100 and 72.2%, respectively) and male animals (57.9, 42.1 and 5.3%, respectively) as compared to normal K^+

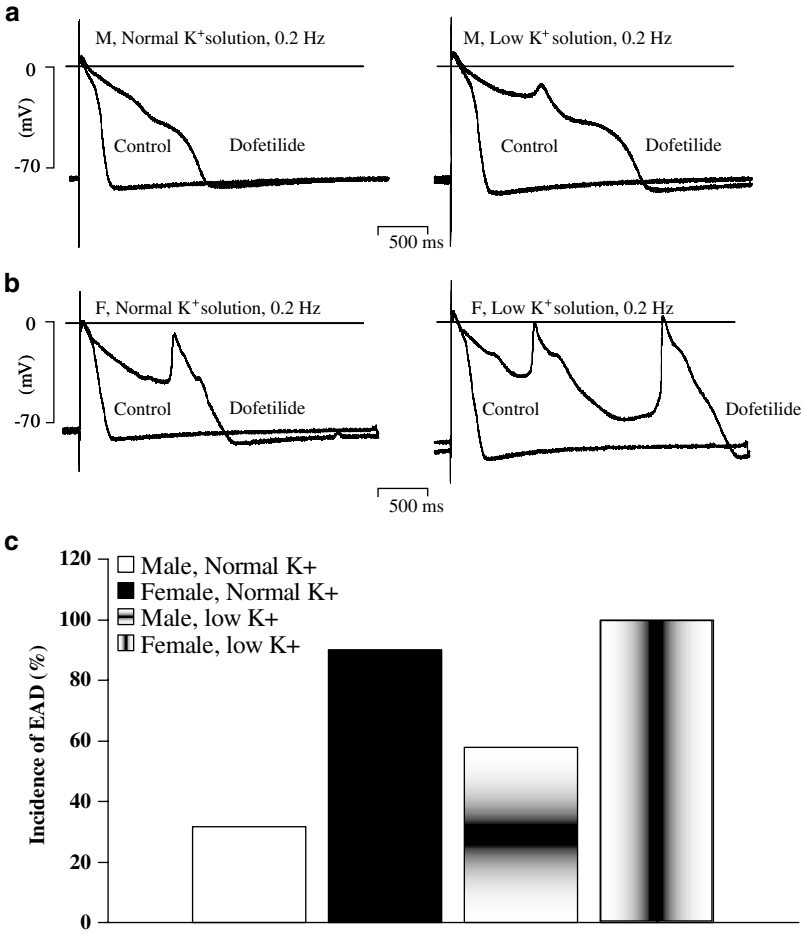

Figure 4 EAD activity in canine PFs following I_{Kr} block at a stimulation rate of 0.2 Hz. (a) and (b) show representative APs recorded in fibres from male (M) and female (F) animals in either normal or low K^+ conditions in the presence and the absence of dofetilide (3 μM). (c) The mean EAD activity in fibres from both M and F animals after exposure to dofetilide (3 μM) at a stimulation rate of 0.2 Hz (see also Table 2).

Table 2 Incidence of EADs, TA and STA elicited by dofetilide (3 μM) in canine PFs incubated with Tyrode solutions containing either 4.0 (mimicking normal K^+ levels) or 2.25 mM (mimicking hypokalaemia) $[K^+]_0$ and stimulated at 1.0 (mimicking normal human resting heart rate) or 0.2 Hz (mimicking extreme bradycardia)

Treatment	*Sex*	$[K^+]_0$ (mM)	*Stimulation rate* (Hz)	*Incidence of EADs* (n n^{-1} (%))	*EAD take-off potential* (mV)	*Peak voltage of EADs* (mV)	*Incidence of TAs* (n n^{-1} (%))	*Incidence of STAs* (n n^{-1} (%))
Dofetilide	M	4.0	1.0	0/17 (0%)	/	/	0/17 (0%)	0/17 (0%)
	F	4.0	1.0	0/20 (0%)	/	/	0/20 (0%)	0/20 (0%)
	M	2.25	1.0	0/18 (0%)	/	/	0/18 (0%)	0/18 (0%)
	F	2.25	1.0	0/16 (0%)	/	/	0/16 (0%)	0/16 (0%)
	M	4.0	0.2	6/19 (31.6%)	−32 (−45/−22)	−8 (−16/−7)	0/19 (0%)	0/19 (0%)
	F	4.0	0.2	18/20 (90%)	−34 (−48/−22)	−10 (−38/0)	13/20 (65%)	1/20 (5%)
	M	2.25	0.2	11/19 (57.9%)	−35 (−58/−22)	−7 (−22/+2)	8/19 (42.1%)	1/19 (5.3%)
	F	2.25	0.2	17/17 (100%)	−25 (−57/−24)	−7 (−32/+8)	18/18 (100%)	13/18 (72.2%)

In all, 17 female (F) and 14 male (M) animals were used to obtain the number of PFs shown in this table. The values of the onset of EAD take-off potential and peak voltage of EADs are median (minimal/maximal). TAs: EAD-induced triggered activities (more than two successive EADs); STAs: EAD-induced sustained triggered activities (nonrepolarisation of AP).

conditions (Table 2). Thus, in spite of the increase in the incidence of EADs, TA and STA in fibres from male animals at low stimulation rate and in low K^+ conditions, canine PFs from females showed a higher incidence of EADs, TA and STA under the conditions of I_{Kr} block.

STAs can be reversed by increasing the concentration of $[K^+]_0$

Recent studies have suggested that potassium therapy might be effective in the treatment of LQTS. It has been shown that an increase in serum potassium or the use of a potassium channel opener, nicorandil, reduce the repolarisation abnormalities in LQT2 (mutation in the α-subunit of cardiac I_{Kr}) and LQT1 (mutation in the α-subunit of cardiac I_{Ks}) patients (Sato *et al*., 1995; Compton *et al*., 1996; Shimizu *et al*., 1998). Potassium therapy was also demonstrated in LQT1, LQT2 and LQT3 (mutation in the α-subunit of cardiac sodium current, I_{Na}) models in which nicorandil was shown to reduce the transmural dispersion of repolarisation and prevent TdP (Shimizu & Antzelevitch, 2000). We thus sought to determine if dofetilide-induced STA in fibres from female animals in low K^+ conditions and at a stimulation rate of 0.2 Hz could be reversed by raising the level of $[K^+]_0$. As shown in Figure 5a, dofetilide (3 μM) induced STA in a fibre from a female animal in low K^+ conditions when stimulated at 0.2 Hz. Note that the repolarisation of the AP no longer took place for many seconds in this experiment (Figure 5a). STA activity, however, was reversed and repolarisation of AP recovered when the same fibre (Figure 5b) or all the other fibres with STA were incubated with dofetilide (3 μM) prepared in normal K^+ solution.

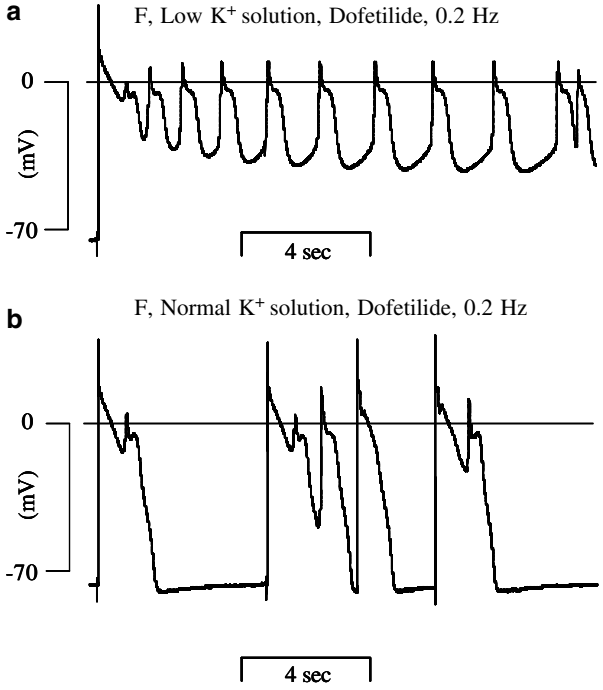

Figure 5 STA in canine PFs. (a) Shows a representative STA recorded in a fibre from a female (F) animal in the presence of dofetilide (3 μM) with low K^+ solution and stimulated at a stimulation rate of 0.2 Hz. (b) Reversal of STA and repolarisation of AP when the same fibre as in (a) was incubated with dofetilide (3 μM) prepared in normal K^+ solution.

Discussion

In this study, we report on four major findings in canine PFs in either normal or low K^+ conditions: (i) in a condition mimicking normal human resting heart rate (a stimulation rate of 1.0 Hz), fibres from female animals exhibit significantly longer APD_{50} and APD_{90} as compared with fibres from male animals, (ii) at low stimulation rate (0.2 Hz), gender differences to rate adaptation and I_{Kr} block exist in APD_{90}, (iii) the incidence of EADs, TA and STA was higher in fibres from female animals as compared to those from male animals and

(iv) STA was reversed by raising $[K^+]_0$ from 2.25 to 4 mM and repolarisation of the AP recovered.

Adult rabbit (Hara *et al*., 1998; Lu *et al*., 2000; Pham *et al*., 2001; Pham & Rosen, 2002; Valverde *et al*., 2003) and mice (Trepanier-Boulay *et al*., 2001; Wu & Anderson, 2002) hearts exhibit clear gender-related differences in the repolarisation of AP as evidenced by longer APDs in females. Our APD data from adult beagle dog hearts are in agreement with those reported in the rabbit and mice hearts. In a condition mimicking normal human resting heart rate (a stimulation rate of 1.0 Hz), our data clearly show that fibres from female animals in either normal or low K^+ conditions exhibit significantly longer APD_{50} and APD_{90} as compared to fibres from male animals (Table 1). Moreover, the investigation of the effects of female and male sex hormones on AP repolarisation in oophorectomised and orchiectomised rabbits (Liu *et al*., 1998; Pham *et al*., 2001; 2002b; Valverde *et al*., 2003), as well as in orchiectomised male mice (Brouillette *et al*., 2003) and guinea-pig myocytes (Tanabe *et al*., 1999) show similar data to those obtained from normal animals. The lack of testosterone and oestrogen plasma level determinations in our study cannot confirm that the male sex hormones contribute to the gender difference in dog hearts as previously reported for AP repolarisation in rabbit and mice hearts. The modulation of APD occurs largely as a consequence of a fine balance between depolarising and repolarising currents that are responsible for the generation of AP. A growing body of experimental evidence, suggesting possible difference in the expression of repolarising K^+ currents and their modulation by gonadal steroids, has been reported in guinea-pig (Tanabe *et al*., 1999), rabbit (Drici *et al*., 1996; Liu *et al*., 1998) and mouse (Trepanier-Boulay *et al*., 2001; Wu & Anderson, 2002; Brouillette *et al*., 2003) myocytes. A recent study demonstrated that developmental change in the expression of I_{Kr} and I_{Ks} contribute to age-related expression of dofetilide effects on cardiac repolarisation (Obreztchikova *et al*., 2003). To our knowledge, no attempts, however, have been made in dog hearts to determine whether there are sex-related differences in the expression of repolarising K^+ currents and their modulation by the sex hormones, a question which still needs to be elucidated.

Clinical data suggest that sex-related differences in the QT interval–cycle length relationship (rate adaptation) may contribute to the longer QTc interval in women. Women exhibit a greater lengthening of the QT interval as the heart rate slows, such that the sex-related differences in QT become more pronounced as cycle length increases (Kligfield *et al*., 1996). Similarly, female rabbit hearts have a steeper APD and QT interval–cycle length relationship than male hearts, which results in a significantly longer APD and QT interval at a long cycle length in female rabbit hearts (Liu *et al*., 1998; Lu *et al*., 2000; Valverde *et al*., 2003). We observed that at long cycle length (a stimulation rate of 0.2 Hz) there was a clear gender-related difference in APD to rate adaptation (Figure 1) in a condition mimicking normal K^+ levels. In addition, the gender-related difference to rate adaptation was also present in conditions mimicking hypokalaemia in humans (Figure 1). As shown in Figure 1, APD–cycle length relationship was not significantly altered by perfusion of low K^+ solution in fibres either from male or female animals. This finding is consistent with that reported by Liu *et al*. (1998), who showed that the QT interval–cycle length relationship was not significantly altered by perfusion of low K^+/Mg^{2+} in hearts either from male or female rabbits. It is more likely that the gender difference in the QT interval–cycle length relationship in rabbit hearts result from the gender difference in I_{Kr} current density (Liu *et al*., 1998). Therefore, it can be postulated that the gender difference in APD–cycle length relationship in canine PFs would be a consequence of a lower I_{Kr} current density in fibres from female animals compared to those from males, a suggestion that remains to be elucidated.

A much higher percentage of women than men develop TdP after taking a variety of medicines (Drici *et al*., 1998; Locati *et al*., 1998; Drici & Clement, 2001). All these drugs have the ability to prolong cardiac repolarisation, the QT interval and to block cardiac I_{Kr} (Redfern *et al*., 2003). The increase in QT interval was higher in orchietomised male rabbits treated with a placebo than that of a dihydrotestosterone-treated group (Liu *et al*., 2003). The incidence of EADs and the prolongation of APD by I_{Kr} blockers were higher in oophorectomised rabbit group treated with estradiol than that of the dihydrotestosterone group (Hara *et al*., 1998) or in control females than that of the dihydrotestosterone group (Pham *et al*., 2002b) or in either normal and castrated female or male rabbits (Lu *et al*., 2000; Pham *et al*., 2001). Our data in PFs are consistent with those reported by different authors in the rabbit model. In a condition mimicking extreme bradycardia (stimulation rate of 0.2 Hz; Figure 3 and Table 2), but not in a condition mimicking normal human resting heart rate (stimulation rate of 1.0 Hz; Figure 2 and Table 2), the increase in APD_{90} and the incidence of EADs induced by dofetilide were significantly higher in fibres from female animals in either normal or low K^+ conditions compared to males. Thus, PFs from rabbit (Lu *et al*., 2000) and dog (this study) hearts of male animals are less sensitive than those of females to dofetilide-induced abnormal repolarisation and proarrhythmic events. In contrast, Lu *et al*. (2001) showed in canine PFs that dofetilide tested at a concentration of 10 nM did not elicit any EADs at a stimulation rate of 0.2 Hz. Our results, however, show that dofetilide tested at a higher concentration (3 μM) induces EADs (Figures 4 and 5). Although it could be argued that the incidence of EADs in the presence of dofetilide (3 μM) could be explained by the inhibition of other cardiac currents in addition to I_{Kr}, this explanation seems unlikely given the selectivity of dofetilide reported by Kiehn *et al*. (1994) in guinea-pig ventricular myocytes. In this report, dofetilide did not inhibit or stimulate cardiac currents (i.e. I_{Ks}; inward rectifier potassium current: I_{K1}; I_{Na}; L-type calcium current: $I_{Ca,L}$) other than I_{Kr}. A more likely explanation for the appearance of EADs at 3 μM is that with this supramaximal concentration in terms of I_{Kr} block, there is unlikely to be any small, residual I_{Kr} to drive the repolarisation of AP; this may not be true for 10 nM dofetilide. Thus, rabbit (Lu *et al*., 2001) and canine (this study) PFs can be used as a model for detecting drug-induced, potential proarrhythmic events *in vitro*. However, the concentration needed to induce EADs is higher in dogs as compared to rabbit hearts. The exact role of the male sex hormones in the incidence of EADs in our study remains to be elucidated. Although a gender difference in the expression of repolarising K^+ currents in dogs may favour a reduced repolarisation reserve (Biliczki *et al*., 2002) and account for the markedly increased proarrhythmic events induced by I_{Kr} blockade in female fibres compared to males, this is a possibility that needs to be examined.

Recent studies have identified five forms of congenital LQTS caused by mutations in ion channel genes located on chromosomes 3, 7, 11 and 21 (El Sherif & Turitto, 2003). Mutations in HERG and KCNQ1 genes that encodes the α-subunits that form the human I_{Kr} and I_{Ks}, are responsible for defects in I_{Kr} and I_{Ks}, which underlies the LQT2 and LQT1 forms of the LQTS, respectively. An increase in serum potassium was shown to correct the abnormalities of repolarisation duration, T wave morphology, QT interval/cycle length slope and QT dispersion in LQT2 patients (Compton *et al*., 1996; Etheridge *et al*., 2003). In addition, recent clinical studies that used monophasic AP recordings have reported the effect of a K^+ channel opener (nicorandil) to reduce repolarisation abnormalities and abolish EADs in LQT1 patients (Sato *et al*., 1995; Shimizu *et al*., 1998). These studies therefore suggested that potassium therapy might be effective in the treatment of LQTS patients. A paper from Antzelevitch's laboratory showed in an arterially perfused wedge of canine left ventricle that nicorandil is capable of abbreviating the long QT interval, reducing transmural dispersion, and preventing spontaneous and stimulated TdP when congenital or acquired LQTS is secondary to reduced I_{Kr} or I_{Ks} (Shimizu & Antzelevitch, 2000). In the present study, dofetilide elicited a STA in one out of 20 preparations and in 13 out of 18 preparations of PFs from female animals in either normal or low K^+ conditions and stimulated at 0.2 Hz, respectively (Figure 5, Table 2). A dofetilide-induced STA, however, was not observed at all in 19 preparations and in one out of 19 preparations of fibres from male animals in either normal or low K^+ conditions and stimulated at 0.2 Hz, respectively (Table 2). These data clearly show gender differences in dofetilide-induced STA in canine PFs. Many recent *in vivo* studies present evidence supporting the fact that EAD-induced TAs initiate TdP, but that the arrhythmia is maintained by a re-entrant mechanism (Shimizu *et al*., 1995; 1998; Antzelevitch *et al*., 1996; El Sherif *et al*., 1996; Vos *et al*., 1998; Viswanathan & Rudy, 2000; El Sherif & Turitto, 2003). Figure 5a shows that under conditions of bradycardia and hypokalaemia, dofetilide induced an STA in a PF from female animals of many seconds duration; an activity we believe can initiate TdP *in vivo*. In agreement with the clinical observations (Sato *et al*., 1995; Compton *et al*., 1996; Shimizu *et al*., 1998), our data confirm that in this *in vitro* model system an increase in the level of extracellular $[K^+]_0$ abolishes STA and initiates repolarisation of the AP (Figure 5b). However, the exact mechanism for the abolition of STA in PFs by extracellular $[K^+]_0$ remains to be elucidated.

The main limitation of this study is that the female and male animals used were neither age nor weight matched (see Methods). Moreover, no quantitative measurement was performed to control for whether or not female dogs were in similar or different states of oestrus. Although our results are consistent with the existence of gender differences in cardiac AP repolarisation, we cannot rule out factors other than gender that might contribute to the differences observed.

In summary, in the present study we provide experimental evidence suggesting gender differences in canine AP repolarisation. PFs from female dogs can be used in safety pharmacology studies as a sensitive model for evaluating the potential proarrhythmic events *in vitro* of a new medicinal product. Our findings may thus help, furthermore, to gain insight into the intricate mechanisms implicated in the effect of gender on cardiac AP repolarisation.

We express our gratitude to Jackie A. Moors, Katherine Lawts, Sue Egerton, Derek Hunter, Kathryn Gracie, John McKellar and Ann J. Woods for their technical assistance.

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(Received March 8, 2004
Revised April 22, 2004
Accepted May 17, 2004)