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Effect of trazodone on hERG channel current and QT-interval

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Abstract

Trazodone has been associated with prolonged QT-interval and increased risk of polymorphous ventricular tachycardias clinically and has demonstrated in vitro inhibition of hERG (human ether-á-go-go-related gene) channel current. This study attempts to put the effects of trazodone into perspective by comparing its hERG inhibition to that of three agents known to inhibit I_{Kr}, and comparing the effects of trazodone and cisapride on action potential duration and the QT-interval in the rabbit Langendorff heart preparation. Trazodone inhibited hERG channel current in a concentration-dependent manner with an IC_{50} of 0.69 μM. Like astemizole, terfenadine and cisapride, trazodone inhibits hERG channel current at clinically relevant concentrations. Like cisapride, trazodone increased both the QT-interval and APD_{90} in the Langendorff heart preparation in a reverse frequency-dependent manner at clinically relevant concentrations. These data strongly suggest that trazodone prolongs the QT-interval through inhibition of hERG channel current.

Keywords: Trazodone; QT-interval; hERG; Polymorphous ventricular tachycardia; Torsade de pointes

1. Introduction

Trazodone is a second-generation antidepressant that differs chemically from tricyclic, tetracyclic, or other known antidepressants. In addition to its antidepressant properties, it also exhibits some anxiolytic and hypnotic activity, and is increasingly prescribed off-label for the treatment of insomnia and neuralgia. Of the approximately 15.5 million trazodone prescriptions written in the United States in 2003, nearly 11.7 million were written to promote sleep.

Trazodone has antidepressant activity comparable to older tricyclic antidepressants, and although the cardiac effects of trazodone are thought to be less than those of the tricyclics, the use of trazodone has been associated with undesirable side effects such as orthostatic hypotension, priapism and arrhythmias (Hayes and Kristoff, 1986). Clinical studies have associated trazodone with a prolonged QTc interval, indicating increased risk for the development of potentially fatal arrhythmias (Burgess et al., 1982; de Meester et al., 2001; Levenson, 1999; Mazur et al., 1995; Pohl et al., 1986; Tibbles et al., 1997; Wittebole et al., 2000). Indeed, case studies of trazodone overdose have reported cardiac dysrhythmias, including premature ventricular contractions and polymorphous ventricular tachycardias such as torsades de pointes, which have sometimes resulted in death (Burgess et al., 1982; de Meester et al., 2001; Janowsky et al., 1983; Levenson, 1999; Mazur et al., 1995; Pohl et al., 1986; Tibbles et al., 1997; Van de Merwe et al., 1984; Vitullo et al., 1990; Wittebole et al., 2000). Torsades de pointes and other arrhythmias have been observed in patients who have received trazodone at the prescribed doses (Mazur et al., 1995; Pohl et al., 1986).

Few nonclinical studies have investigated the electrophysiologic effects of trazodone. A study in anesthetized dogs demonstrated that, during cumulative intravenous administration, trazodone prolonged the QTc interval by up to 19%. (Byrne and Gomoll, 1982) In rat ventricular myocytes, trazodone inhibited the transient outward potassium current (IC_{50} of 100 μM), but it did not significantly affect the L-type Ca^{2+} current at concentrations up to 10 μM

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(Park et al., 1999). A recent study demonstrated that trazodone inhibits hERG (human ether-á-go-go-related gene) channel current in a concentration dependent manner (Zitron et al., 2004).

hERG encodes the pore-forming subunit of I_{Kr}, the rapid component of the delayed rectifier potassium channel current, which plays an important role in ventricular repolarization. As inhibition of I_{Kr} is the most common cause of QT-interval prolongation by noncardiac drugs, we investigated the effect of trazodone on hERG channel current in stably transfected HEK293 cells (Brown and Rampe, 2000). In this assay system, hERG serves as a surrogate for I_{Kr}. Evaluation of the effects of noncardiac compounds on hERG channel current is currently recommended in the development of pharmaceuticals for human use, but was not recommended at the time of trazodone's development. For comparison, 3 other agents, cisapride, terfenadine, and astemizole, which are known to inhibit I_{Kr} and have been clinically associated with torsades de pointes (Ducic et al., 1997; Mohammad et al., 1997; Rampe et al., 1997; Roy et al., 1996; Zhou et al., 1999) were also evaluated. In order to determine whether the effects of trazodone on hERG channel current resulted in an effect on the QT-interval in an intact preparation, the effect of trazodone on both action potential duration and the QT-interval was evaluated in a rabbit Langendorff heart preparation. Both cisapride and E-4031 (data not shown) were included as positive controls.

2. Materials and methods

2.1. hERG experiments

2.1.1. Preparation of solutions

Trazodone, astemizole, and terfenadine were obtained from Sigma (St. Louis, MO, USA), and cisapride was obtained from Research Diagnostics, Inc. (Flanders, NJ, USA). Stock solutions were prepared in dimethyl sulfoxide (DMSO, Sigma), aliquoted, and stored frozen. Final perfusion solutions were prepared by diluting DMSO stock solutions into a HEPES-buffered physiologic saline solution (Tyrode's solution) [137 mM NaCl, 4 mM KCl; 1.8 mM $CaCl_2$; 1 mM $MgCl_2$; 10 mM HEPES; 10 mM glucose; pH adjusted to 7.4 with NaOH]. All solutions contained 0.1% DMSO. Final concentrations were: 0.01, 0.1, 0.3, 1, 3, 10, and 30 μM trazodone; 0.3, 1, 3, and 10 nM astemizole; 3, 10, 30, 100, and 300 nM cisapride; and 3, 10, 30, and 100 nM terfenadine. A solution of 0.1% DMSO in Tyrode's solution served as a vehicle control, and a solution containing 500 nM E-4031–a potent, selective hERG inhibitor–served as a reference. Full inhibition (100%) of hERG channel current was defined as the amount of inhibition of current after application of the reference solution (to eliminate any contribution of endogenous currents).

2.1.2. Preparation of cell cultures

HEK293 cells were stably transfected with hERG cDNA. Stable transfectants were selected by co-expression of the hERG cDNA and G418-resistance gene incorporated into the expression plasmid. Selection pressure was maintained by including G418 in the culture medium. Cells were cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (D-MEM/F-12) supplemented with 10% fetal bovine serum, 100U/ml penicillin G sodium, 100 μg/ml streptomycin sulfate, and 200 μg/ml G418.

2.1.3. Electrophysiology

HEK293 cells stably expressing hERG were transferred to the recording chamber and superfused with HEPES-buffered physiological saline (Tyrode's) solution. Pipette solution for whole-cell recordings was: 130 mM potassium aspartate; 5 mM $MgCl_2$; 5 mM EGTA; 4 mM ATP; 10 mM HEPES; pH adjusted to 7.2 with KOH. Onset and steady-state block of hERG tail current were measured using a pulse pattern with fixed amplitudes (conditioning prepulse: +20 mV for 1 s; repolarizing test ramp to −80 mV [−0.5 V/s] repeated at 5 s intervals). Currents were recorded with the use of a patch clamp amplifier (either from Axon Instruments, CA, USA, or Warner Instruments, CT, USA). Before digitization, records of the currents were low-pass filtered at one fifth of the sampling frequency. All experiments were performed at a temperature of 35±2°C.

2.1.4. Experimental protocols

Seven concentrations of trazodone were applied to HEK293 cells expressing hERG (one concentration per cell) for 12 min exposure time or until a new steady state was achieved. Each concentration was applied to at least 3 separate cells. The comparator drugs and vehicle control were applied in an identical manner, and each concentration of each comparator was applied to at least 2 separate cells.

Onset and steady-state block of hERG current were measured. Each recording ended with a final application of a supramaximal concentration of the reference compound (500 nM E-4031) to establish 100% block of hERG current. Any remaining unblocked current was assumed not to be hERG current and was consequently subtracted off-line from the hERG current recordings obtained during that experiment.

2.1.5. Statistical methods

Data acquisition and analyses were performed using the suite of pCLAMP (v. 8.2) programs (Axon Instruments, CA). Steady state was defined by the minimum rate of change with time (linear time dependence). The steady state before and after drug application was used to calculate the percentage of current inhibited at each concentration. Percent inhibition at each drug concentration was compared with the vehicle control using a one-way ANOVA. Data are graphed as mean±S.E.M. Concentration response data were fit into the following equation: % Block={1−1[1+([Drug]/

$IC_{50})^N]\}$*100. Where [Drug] is the concentration of the drug, IC_{50} is the concentration producing half-maximal inhibition of hERG current, N is the Hill coefficient, and % Block is the percentage of hERG current inhibited at each concentration of the drug. Nonlinear least squares fits were solved with the Solver add-in for Excel 2000, and the IC_{50} was calculated.

2.2. Langendorff experiments

2.2.1. Preparation of solutions

Trazodone was obtained from Sigma (Poole, UK) and dissolved in DMSO (Sigma, UK) to provide stock solutions of 0.3, 1, 3, 10, 30, and 100 mM trazodone; aliquots of these solutions were added to Krebs Henseleit solution (118 mM NaCl; 4.7 mM KCl; 2.5 mM $CaCl_2$; 1.2 mM $MgSO_4$; 1.2 mM KH_2PO_4; 25 mM $NaHCO_3$; 5.5 mM D-glucose, 2.0 mM Na-pyruvate, gassed at 37°C with 95% O_2:5% CO_2) for perfusion of 0.3, 1, 3, 10, 30, 100, and 300 μM trazodone. Trazodone-treated hearts were exposed to either 1, 10, 30, 100 and 300 μM trazodone (n=6), or 0.3, 1, 3, 10 and 30 μM trazodone (n=4); hearts were exposed to only 5 concentrations. Cisapride-treated hearts were exposed to 10, 30, 100, 300 and 1000 nM cisapride. The vehicle control solution for each trazodone/cisapride concentration was 0.1%, 0.1%, 0.1%, 0.1%, and 0.3% DMSO, respectively.

2.2.2. Preparation of rabbit hearts

Male New Zealand white rabbits weighing 1.4–2.5 kg (Harlan, UK) were used. The rabbits were single- or double-housed in a room with air temperature at 18–22 °C on a 12 h light/12 h dark cycle. The rabbits were administered heparin i.v. (1000 IU/kg) and euthanized under sodium pentobarbital anesthesia (i.v., 90–107 mg/kg). Hearts were excised and transferred to chilled Krebs Henseleit solution prior to transfer to the Langendorff apparatus.

2.2.3. Electrophysiology

Continuous retrograde perfusion (at approximately 30 ml/min) with Krebs Henseleit solution, at 37 °C, was begun once the isolated hearts were transferred to the Langendorff

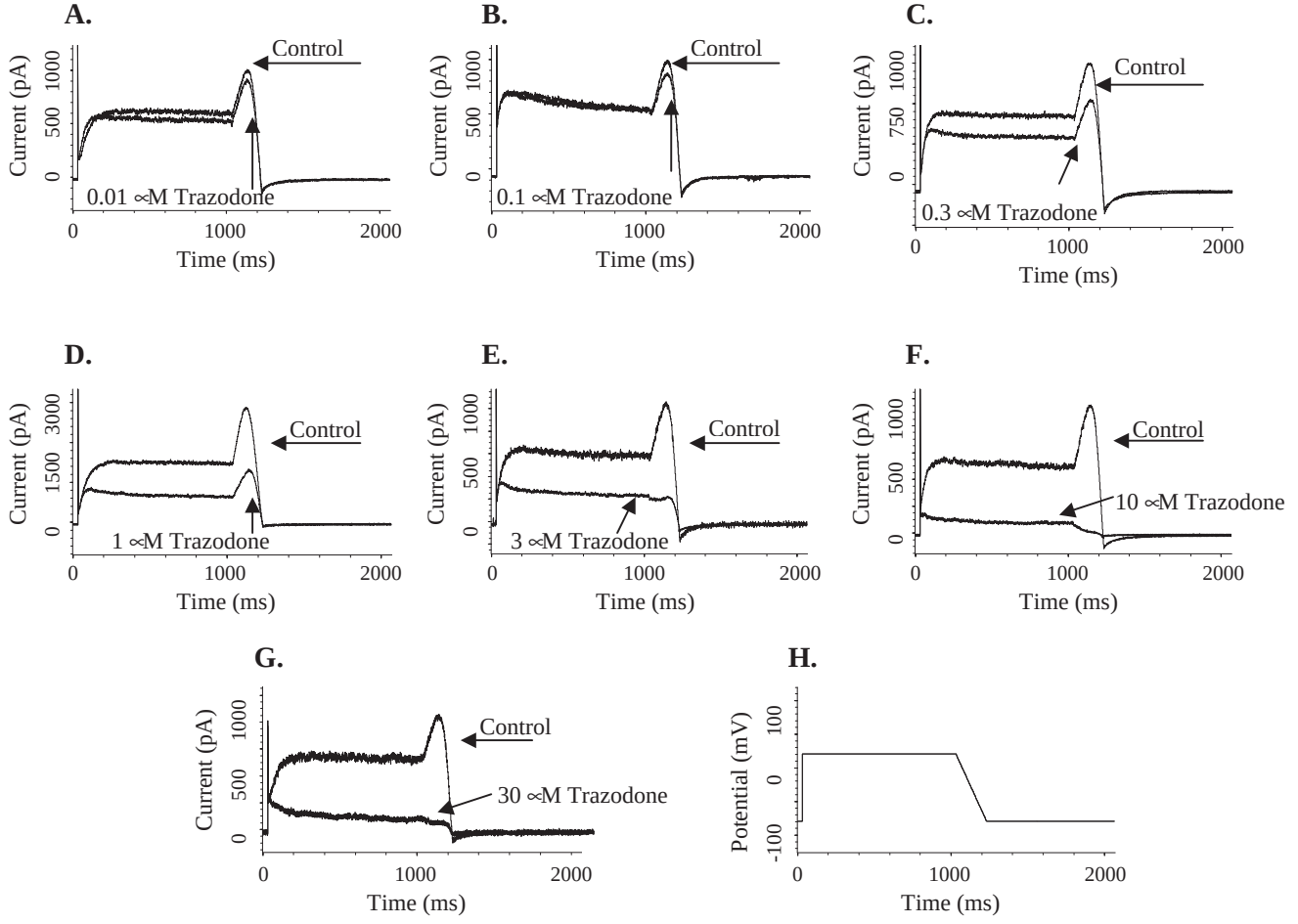

Fig. 1. Concentration-dependent effects of trazodone on peak hERG tail current. The *Y*-axis depicts current (pA), and the *X*-axis depicts time (ms). Effects of: A. 0.01 μM, B. 0.1 μM, C. 0.3 μM, D. 1 μM, E. 3 μM, F. 10 μM, G. 30 μM, H. voltage stimulus: prepulse+20 mV, repolarizing test ramp (+20 mV to −80 mV at −0.5 V/s), repeated at 5 s intervals from a holding potential of −80 mV.

Table 1
Relationship between maximum circulating plasma concentrations following therapeutic doses and inhibition of hERG channel current

Compound	Dose	C_{max} following marketed dose (nM)	Plasma protein binding (%)	hERG IC_{50} (nM)	hERG IC_{50}: C_{max} ratio	hERG IC_{50}: C_{max} (free) ratio
Trazodone	50 mg	3037 (1.07 μg/ml)[a]	93[b]	690	0.23	3.25
Astemizole	10 mg	1.61 (0.74 ng/ml)[c]	97[d]	1.3	0.81	26.9
Cisapride	10 mg	95.9 (45.0 ng/ml)[b]	97.5[e]	20.9	0.22	8.71
Terfenadine[f]	60 mg	3.60 (1.7 ng/ml)[g]	97[d]	8.7	2.42	80.6

[a] Ankier et al., 1981.
[b] Goodman et al., 2001.
[c] Lefebvre et al., 1997.
[d] ADIS International Clinical Pharmacokinetics: Drug Data Handbook 1998.
[e] PDR, 1998.
[f] Although the ratios for terfenadine are expressed in terms of dosing following a 60 mg dose, free plasma concentrations of up to 4 nM terfenadine have been reported (Redfern et al., 2003).
[g] Physicians' Desk Reference, 1997.

apparatus. The right atria and sinoatrial node were removed, the His bundles were severed, and the hearts were stimulated electrically at 1 Hz. Surface electrodes were placed on the heart to record ECG (electrocardiogram, to measure QT-interval and QRS complex duration) and monophasic action potentials (monophasic action potential duration at 90% repolarization [$MAPD_{90}$]). If the quality of the electrode placement and the recordings gained proved satisfactory, the hearts were left to equilibrate for a minimum of 30 min prior to any further experimental manipulation. If the quality of electrode recordings was judged to be poor, the heart was replaced by another and the process repeated. Once stable recordings were achieved, monophasic action potentials and ECGs from each preparation were continuously monitored and recorded using the Notocord HEM v3.3 data capture system. $MAPD_{90}$, QT-interval and QRS complex duration were continuously analyzed, monitored and recorded using this system also. The parameters developed by each heart were considered acceptable if $MAPD_{90}$ was $\geq$ 140 ms. Digital markers were

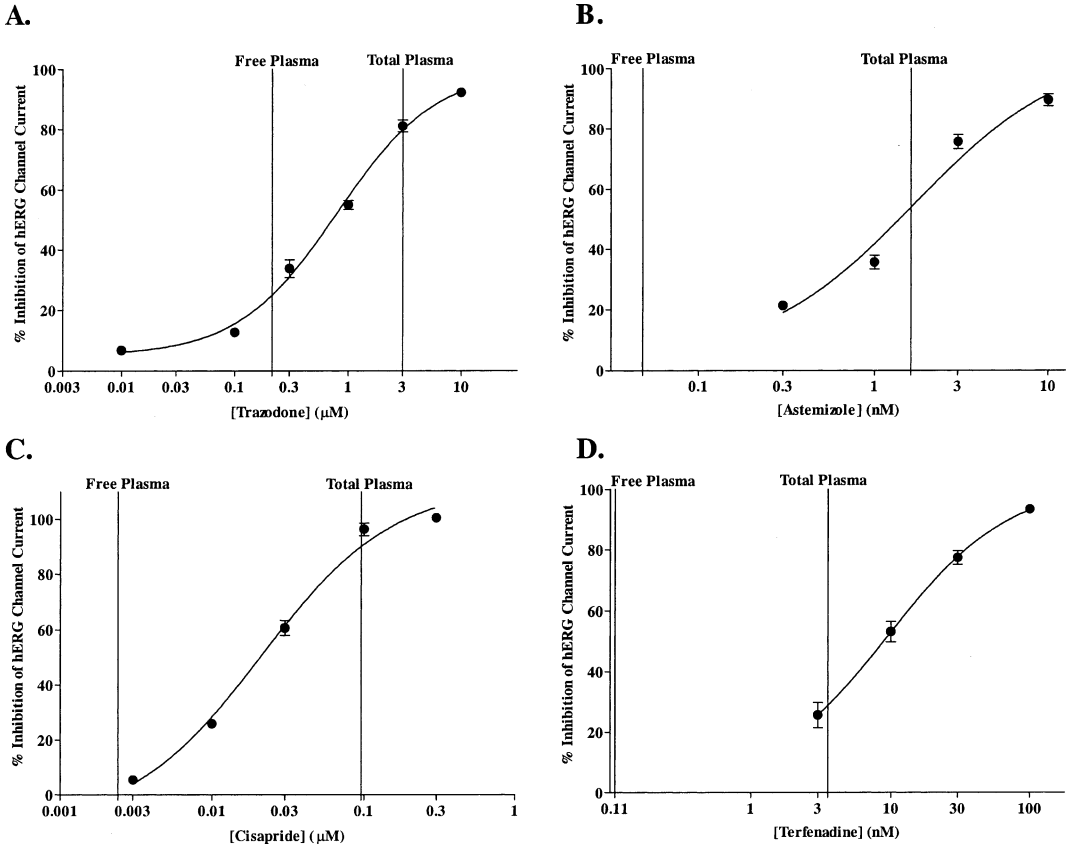

Fig. 2. Concentration–response relationships of trazodone (A), astemizole (B), cisapride (C), and terfenadine (D) (mean±S.E.M.) on hERG channel current. Total plasma concentration and free plasma concentrations following therapeutic doses [trazodone (50 mg), astemizole (10 mg), cisapride (10 mg), terfenadine (60 mg)] are indicated by vertical lines on the graphs.

placed in the data file to define the point at which the data for each condition were analyzed.

2.2.4. Experimental protocols

Having established stable recordings, the baseline values for QT-interval, QRS complex duration, and $MAPD_{90}$ parameters were evaluated at 1 and 2 Hz. The drug or vehicle control at the lowest concentration was then administered for approximately 30 min to the perfusate supplying an isolated Langendorff heart, paced at 1 Hz. Recordings of QT-interval, QRS complex duration, and $MAPD_{90}$ were then made at 1 and 2 Hz. This cycle was repeated with escalating concentrations of each drug or corresponding vehicle control concentration. Each heart was exposed to either 5 consecutive trazodone or cisapride concentrations, or 5 consecutive vehicle control changes (for a time-matched vehicle control).

These procedures were then repeated so that data were obtained from 4 to 10 hearts for each trazodone concentration, 6 hearts for each cisapride concentration and from 8 hearts for the vehicle control group.

2.2.5. Statistical methods

Statistical analysis was performed using Excel 97 and GraphPad Prism v2.01 operating within Windows NT v4.0. Data from each heart represent the average of 10 ECG or action potential waveforms. Data (incorporating all hearts at that concentration) are graphed as mean±S.E.M. As there were no effects of vehicle on QT-interval, QRS duration or $MAPD_{90}$ in a time-matched vehicle control group, each parameter at each concentration in the trazodone treatment group was compared with its respective pretreatment control value to establish whether there were any effects of trazodone.

For trazodone, at each stimulation frequency, QT-interval, QRS complex duration and $MAPD_{90}$ 'change from baseline' values were used to calculate t statistics for each parameter at each concentration of trazodone. This was then evaluated according to Student's t distribution to calculate a P value. The null hypothesis (that no difference existed between trazodone and pretreatment values) was rejected when $P<0.05$.

For cisapride, where the variances between the cisapride and vehicle treatment groups were found to be significantly different, values were compared using the non-parametric method (Kruskal–Wallis statistic followed by Dunn's test). If the variances between the treatment groups were found not to be significantly different, then the values were compared using the parametric method (ANOVA followed by Dunnett's t-test). The null hypothesis (that no difference existed between the cisapride and vehicle groups) was rejected when $P<0.05$. Choices of parametric or non-parametric tests were based on whether the groups to be compared satisfied the homogeneity of variance criterion evaluated by the Levene-Mean test (the Levene-Mean test was conducted at the significance level 0.05).

3. Results

3.1. Effect on hERG channel current

As shown in Fig. 1, with superimposition of typical hERG voltage clamp current records acquired after equili-

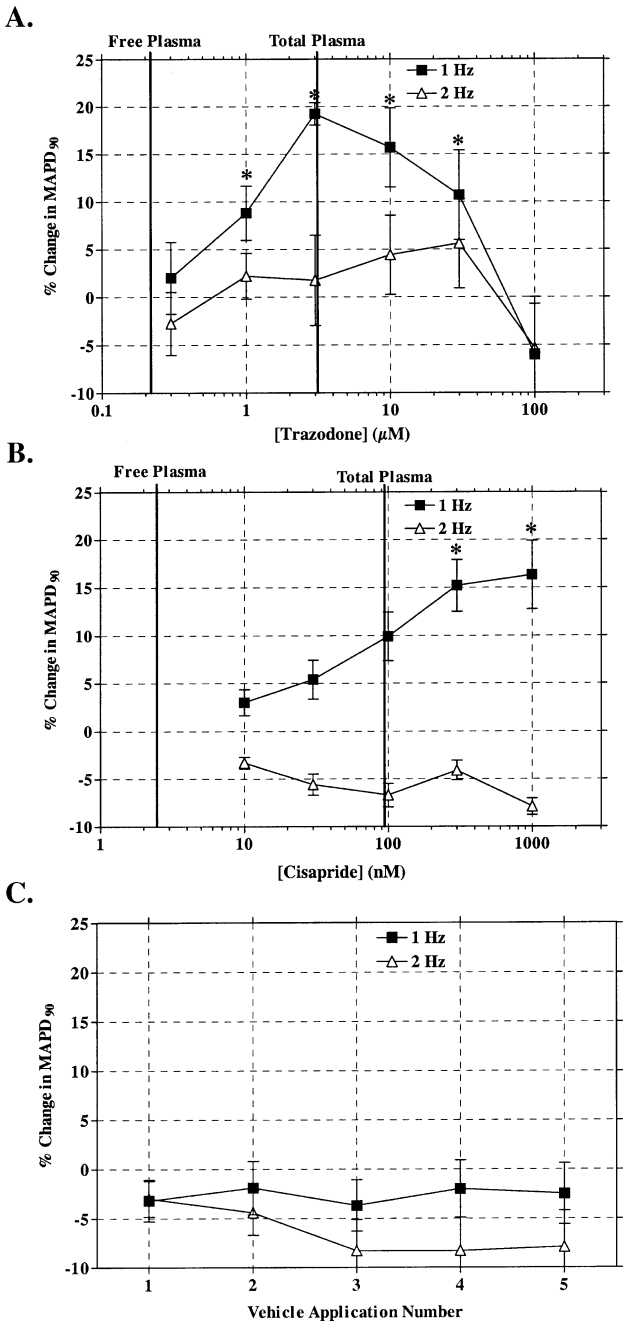

Fig. 3. The effect of trazodone (A), cisapride (B) and time-matched vehicle control (C) on $MAPD_{90}$ in a rabbit Langendorff heart preparation. All values are expressed as percent change from baseline established during the initial incubation period [mean±S.E.M. (n=4–10)]. *Significantly different from time-matched vehicle control ($P<0.05$) [above the curves corresponds to 1 Hz, below the curves 2 Hz]. Total plasma concentration and free plasma concentrations following therapeutic doses [trazodone (50 mg), cisapride (10 mg)] are indicated by vertical lines on the graphs.

bration with vehicle control and subsequent application of trazodone, trazodone inhibited peak hERG tail current in a concentration-dependent manner. Full inhibition of tail current was apparent at concentrations ≥10 μM. The potential clinical significance of the concentrations at which trazodone inhibits hERG channel current is discussed below and presented in Table 1. Trazodone inhibited hERG channel current rapidly following application, and its effects were readily reversible (as apparent following washout with vehicle). Maximum inhibitable hERG channel current was defined by the subsequent application of 500 nM E-4031. Trazodone, at 1 μM, inhibited slightly more than half of the maximal inhibitable hERG current.

Trazodone's concentration-dependent effects on hERG channel current are compared with those of astemizole, cisapride, and terfenadine in Fig. 2. All inhibit hERG channel current in a concentration-dependent manner. The IC_{50} for inhibition by trazodone (Fig. 2A) was 0.69 μM. The IC_{50} values for astemizole (Fig. 2B), cisapride (Fig. 2C), and terfenadine (Fig. 2D) were 1.3 nM, 20.9 nM, and 8.7 nM, respectively. The results with astemizole, cisapride, and terfenadine are consistent with results reported in prior literature (Crumb, 2000; Rampe et al., 1997; Zhou et al., 1999).

3.2. Effect on action potential duration, QT-interval, and QRS duration

3.2.1. Action potential duration

The concentration- and frequency-dependent effects of trazodone, cisapride and time-matched vehicle control on $MAPD_{90}$ are shown in Fig. 3. Values are presented as percent changes from baseline parameters established prior to the addition of the first incubation solution (whether vehicle, cisapride or trazodone). No significant change in $MAPD_{90}$ was seen in the time-matched vehicle control at 1

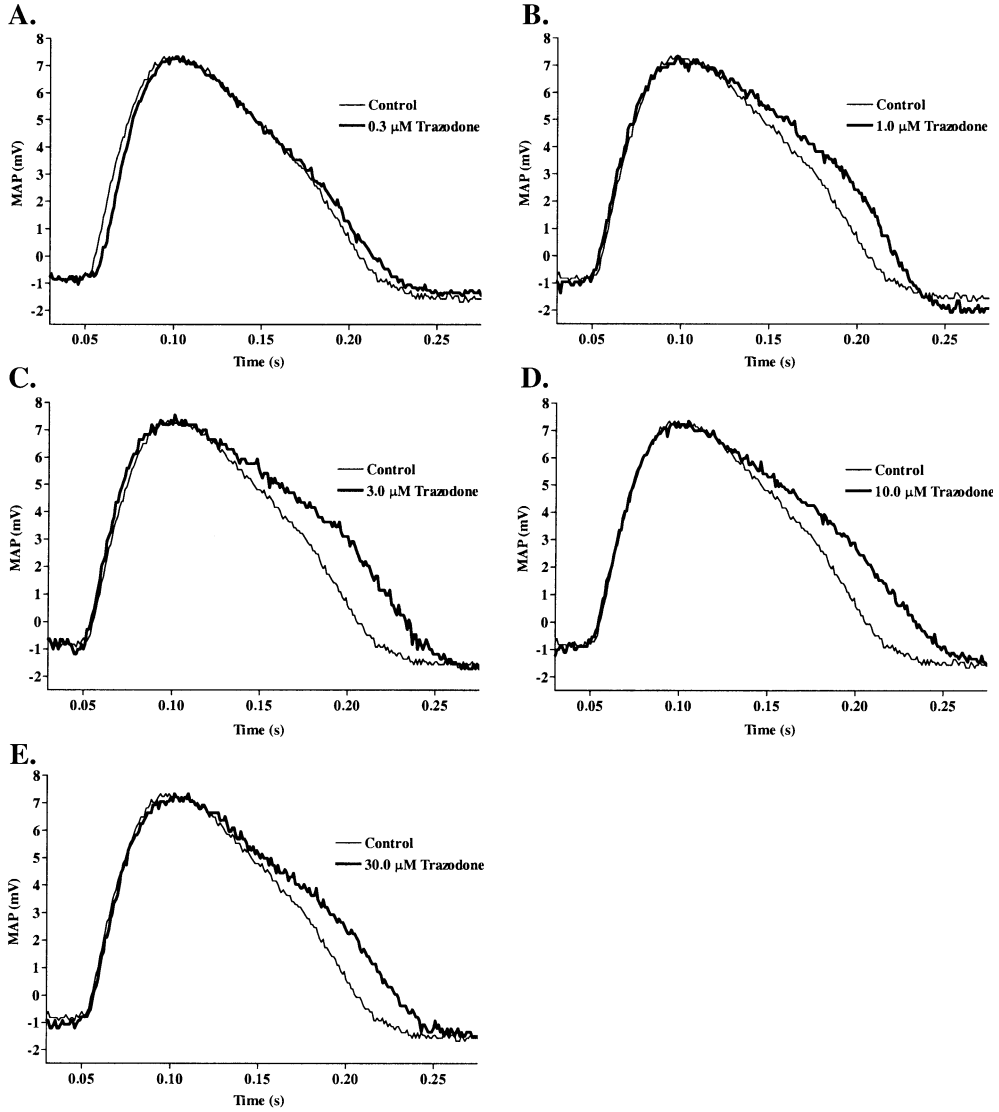

Fig. 4. Effect of trazodone on monophasic action potentials recorded from an isolated, perfused rabbit heart paced at 1 Hz at concentrations of: A. 0.3 μM, B. 1.0 μM, C. 3.0 μM, D. 10.0 μM, and E. 30.0 μM.

Hz. At 2 Hz pacing, there was a slight decrease over the first three incubation periods, after which $MAPD_{90}$ stabilized (Fig. 3C).

At a pacing rate of 1 Hz, trazodone significantly increased ($P<0.05$) $MAPD_{90}$ at concentrations of 1, 3, 10 and 30 μM (Fig. 3A). At a pacing rate of 2 Hz, trazodone had no significant effect on $MAPD_{90}$. The effects of trazodone on $MAPD_{90}$ were most pronounced at a concentration of 3 μM, with a mean increase of 19.0% at a pacing rate of 1 Hz. Trazodone displayed a bell-shaped concentration response in terms of its effects on $MAPD_{90}$, suggestive of mixed ion channel effects at higher concentrations (>3 μM). The effects at 10 μM were similar to those at 3 μM, but concentrations of trazodone of ≥30 μM had lesser effects (with a mean decrease in $MAPD_{90}$ at 100 μM). Typical effects of trazodone on monophasic action potential duration and morphology at a pacing rate of 1 Hz are shown in Fig. 4. Trazodone had little effect on the monophasic action potential at a concentration of 0.3 μM, but had significant effects at concentrations from 1 to 30 μM in this preparation, with the most pronounced effects at 3 μM.

Cisapride significantly increased $MAPD_{90}$ at concentrations of 300 nM and 1 μM at a pacing rate of 1 Hz, without a significant effect ($P>0.05$) at lower concentrations (although a concentration-dependent increase was evident). Cisapride had no significant effect on $MAPD_{90}$ at a pacing rate of 2 Hz at any concentration tested (Fig. 3B).

Reverse frequency-dependence was seen for the effects of both trazodone and cisapride on $MAPD_{90}$, as the effects at 1 Hz are more apparent than those at 2 Hz.

3.2.2. QT-interval

The effects of trazodone, cisapride and time-matched vehicle control on the QT-interval are shown in Fig. 5. No change QT-interval was seen in the time-matched vehicle control at either pacing rate (Fig. 5C).

At a pacing rate of 1 Hz, trazodone significantly increased ($P<0.05$) QT-interval at concentrations of 1, 3, 10 and 30 μM (Fig. 5A). At a pacing rate of 2 Hz, trazodone significantly increased ($P<0.05$) QT-interval at concentrations of 1, 10 and 30 μM. As with $MAPD_{90}$, the effects of trazodone on QT-interval were most pronounced at a concentration of 3 μM, with a mean increase of 27.9% at a pacing rate of 1 Hz. A bell-shaped concentration response was evident for the effects of trazodone on QT-interval.

Representative ECGs from a rabbit Langendorff heart preparation exposed to trazodone concentrations ranging from 0.3 to 30 μM are shown in Fig. 6. Trazodone affected both T-wave morphology and QT-interval in a concentration-dependent manner. In this heart, T-wave inversion was seen at concentrations as low as 0.3 μM, with the most dramatic effects on both T-wave morphology (widening and flattening of the T-wave) and QT-interval (prolongation) apparent at a concentration of 3 μM. Evidence of the bell-shaped concentration response is also apparent in this heart,

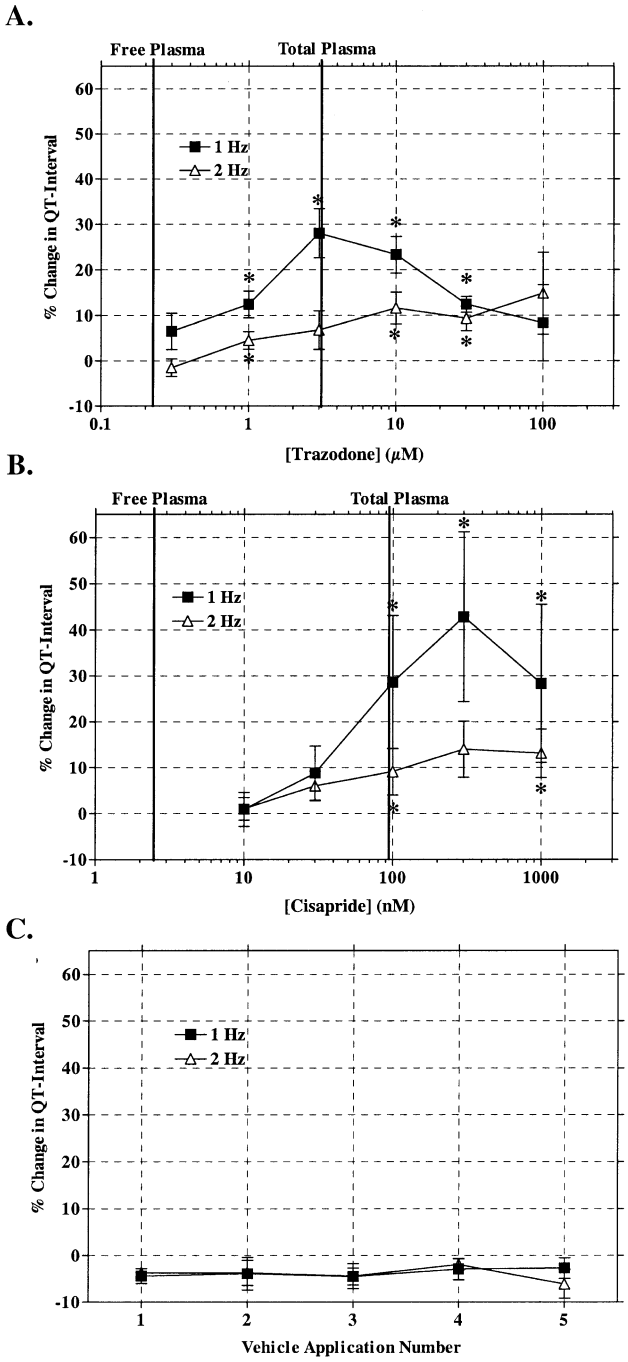

Fig. 5. The effect of trazodone (A), cisapride (B) and time-matched vehicle control (C) on QT-interval in a rabbit Langendorff heart preparation. All values are expressed as percent change from baseline established during the initial incubation period [mean±S.E.M. (n=4–10)]. *Significantly different from time-matched vehicle control ($P<0.05$) [above the curves corresponds to 1 Hz, below the curves 2 Hz]. Total plasma concentration and free plasma concentrations following therapeutic doses [trazodone (50 mg), cisapride (10 mg)] are indicated by vertical lines on the graphs.

as the effects on QT-interval at both 10 μM and 30 μM are less pronounced than those at 3 μM.

Cisapride significantly increased QT-interval at concentrations of 100 and 300 nM and 1 μM at a pacing rate of 1 Hz, and at concentrations of 100 nM and 1 μM at a pacing

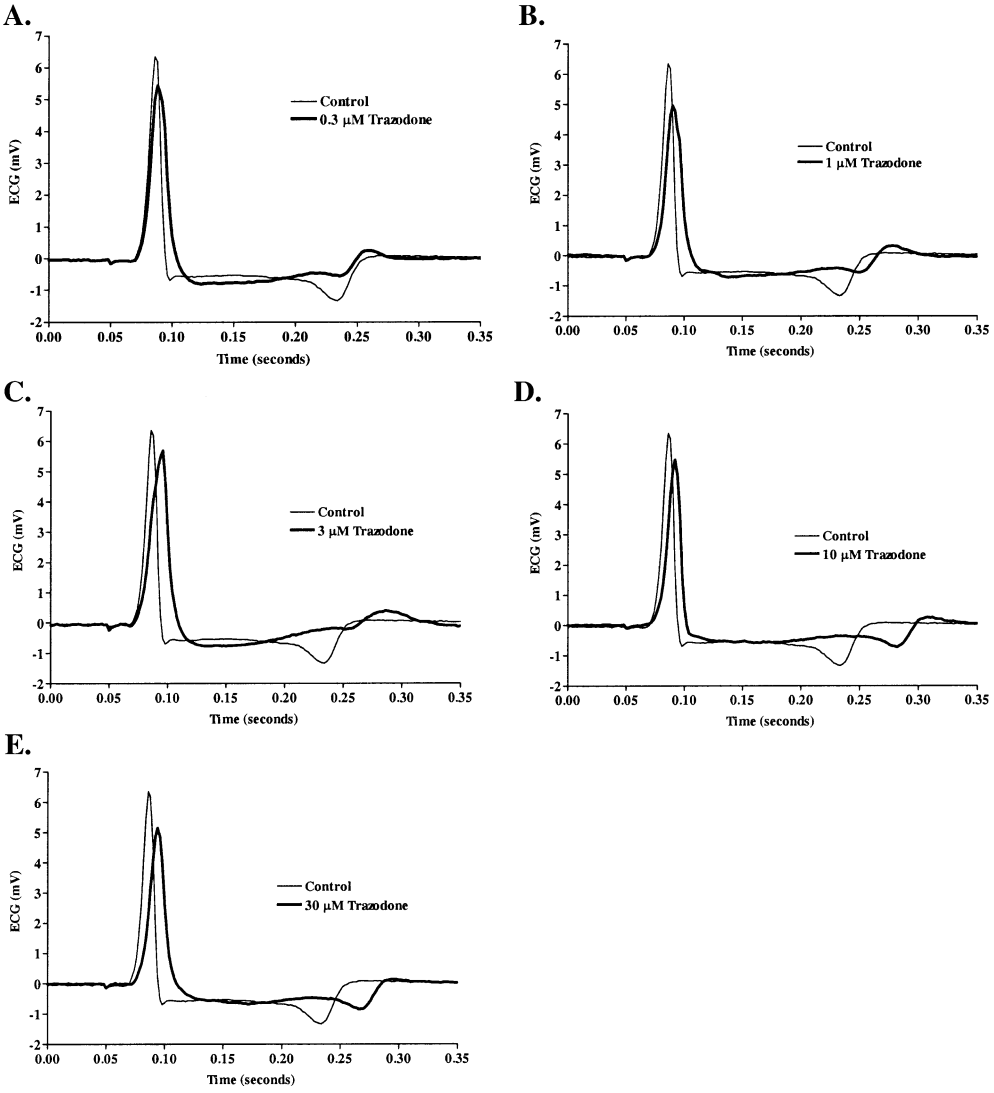

Fig. 6. Effect of trazodone on T-wave morphology and the QT-interval in an isolated, perfused rabbit heart paced at 1 Hz at concentrations of: A. 0.3 μM, B. 1.0 μM, C. 3.0 μM, D. 10.0 μM, and E. 30.0 μM.

rate of 2 Hz (Fig. 5B). Like trazodone, cisapride had a bell-shaped concentration response curve for its effects on QT-interval, with the maximal effect at a concentration of 300 nM and a pacing frequency of 1 Hz.

3.2.3. QRS duration

The effects of trazodone, cisapride and time-matched vehicle control on the QRS complex duration are shown in Fig. 7. Neither cisapride nor time-matched vehicle control significantly affected QRS duration at either pacing frequency.

Trazodone had minimal effects on QRS duration at concentrations up to and including 3 μM. At higher concentrations, trazodone caused widening of the QRS complex, with a mean increase of 88.1% seen at 100 μM, during pacing at 2 Hz. Increases during pacing at 1 Hz were less pronounced than those at 2 Hz, indicating the frequency-dependence of this effect.

Efforts were made to investigate the effects of trazodone at a concentration of 300 μM. Of the 6 hearts exposed to 300 μM trazodone, data could be obtained from only 3, and only at a pacing frequency of 1 Hz. Upon pacing at 2 Hz, ectopic beats were present and/or hearts were unable to accommodate to the stimulation rate. At 300 μM, a mean increase in QT-interval of 38.1% and QRS duration of 252% was seen in these hearts with no effect on $MAPD_{90}$.

4. Discussion

The results demonstrate that trazodone, like other agents associated with torsades de pointes (astemizole, cisapride, and terfenadine), inhibits hERG channel current in a concentration-dependent manner. The effects on hERG channel current are consistent with those recently reported

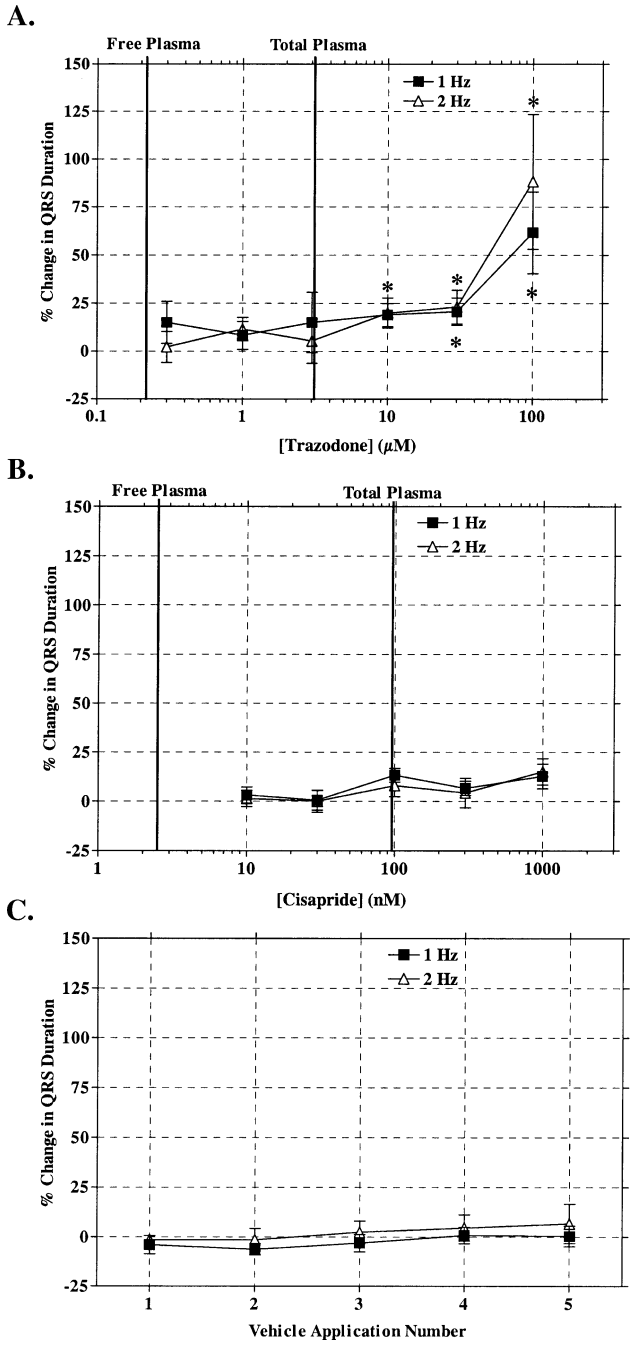

Fig. 7. The effect of trazodone (A), cisapride (B) and time-matched vehicle control (C) on QRS duration in a rabbit Langendorff heart preparation. All values are expressed as percent change from baseline established during the initial incubation period [mean±S.E.M. (n=4–10)]. *Significantly different from time-matched vehicle control (P<0.05) [above the curves corresponds to 1 Hz, below the curves 2 Hz]. Total plasma concentration and free plasma concentrations following therapeutic doses [trazodone (50 mg), cisapride (10 mg)] are indicated by vertical lines on the graphs.

(Zitron et al., 2004). The effects on hERG channel current correlated well with a prolongation of both $MAPD_{90}$ and QT-interval in this study. The effects on QT-interval in vitro are consistent with the prolongation of QTc seen following administration of trazodone in the clinic and reports of cardiac dysrhythmias and torsades de pointes associated with trazodone (Burgess et al., 1982; de Meester et al., 2001; Janowsky et al., 1983; Levenson, 1999; Mazur et al., 1995; Pohl et al., 1986; Tibbles et al., 1997; Van de Merwe et al., 1984; Vitullo et al., 1990; Wittebole et al., 2000).

Although the absolute potency of trazodone in inhibiting hERG channel current is less than that of astemizole, terfenadine, and cisapride, one must take into account the significantly higher exposure to trazodone following clinical administration. The vertical lines in Fig. 2 show the relationship between plasma concentration and hERG inhibition. When exposures to each of the agents following therapeutic doses are compared with the hERG IC_{50} values (Table 1), it is apparent that the hERG IC_{50}/C_{max} ratio of trazodone is similar to that of the comparator agents. A further comparison, using calculated free plasma concentrations (Table 1), demonstrates that trazodone's hERG liability may be greater than that of astemizole, terfenadine, and cisapride. Recent data suggest that comparisons to free plasma concentrations are a more accurate predictor of the arrhythmogenic potential of inhibitors of I_{Kr} than total plasma concentration (Redfern et al., 2003).

Trazodone had a bell-shaped concentration response curve with respect to its effects on the QT-interval and $MAPD_{90}$ in a rabbit Langendorff heart preparation. The bell-shaped concentration response is also seen with agents such as cisapride (Gintant et al., 2001, Fig. 5B) that have multiple ion channel effects (cisapride also inhibits Ca^{2+} current) at concentrations higher than those that inhibit I_{Kr} (Chiang et al., 2004). Trazodone had minimal effects on QRS duration at concentrations up to and including 3 μM, but caused widening of the QRS complex at higher concentrations. The frequency-dependence of this effect suggests that trazodone may inhibit cardiac sodium channels at concentrations greater than 10 μM.

The vertical lines in Figs. 3, 5 and 7 show the relationship between plasma concentration and the MAP and ECG effects of trazodone and cisapride. Like cisapride, trazodone has significant effects on $MAPD_{90}$ and QT-interval at clinically relevant concentrations in the Langendorff heart preparation.

The most pronounced effects of trazodone on both $MAPD_{90}$ and QT-interval in the Langendorff heart preparation were seen at a concentration of 3 μM. During a fatal overdose with trazodone in a 40-year-old patient, where torsades de pointes was recorded (de Meester et al., 2001) and plasma concentrations were measured, the patient's blood concentration of trazodone was 68.3 μM [25.4 μg/ml]. Assuming 93% plasma protein binding (Goodman et al., 2001), free plasma concentrations were approximately 4.8 μM, very close to those where maximal effects were seen on QT-interval in the isolated rabbit heart and correlating those effects seen in the in vitro model with clinical toxicity.

Other factors, such as drug metabolism, also play an important role in determining potential cardiotoxicity. For

instance, from the ratios presented in Table 1, terfenadine appears to be a relatively safe drug, even though it is known to be a potent inhibitor of I_{Kr}. Terfenadine is nearly completely transformed by the CYP3A enzyme system to fexofenadine. A variety of mechanisms which can markedly increase serum levels, such as overdose, agents known to inhibit the CYP3A4 isoenzyme (e.g., ketoconazole, clarithromycin) or reduced activity of CYP3A4 due to disease (e.g., cirrhosis) can increase free plasma concentrations of terfenadine up to 4 nM (Redfern et al., 2003). These concentrations of terfenadine result in inhibition of I_{Kr}, prolongation of the QT-interval, and greatly increased risk for torsades de pointes (De Ponti et al., 2002; Roden, 2004; Webster et al., 2001). Trazodone, cisapride, and astemizole are also metabolized by the CYP3A4 pathway, and their free plasma concentrations can likewise increase substantially if the activity of this pathway is diminished. In May 2004, the U.S. labeling for trazodone was revised to advise of potential drug interactions if trazodone is used with CYP3A4 inhibitors such as ritonavir, ketoconazole or nefazodone, due to the resultant increases in plasma concentrations of trazodone.

The trazodone C_{max} in Table 1 corresponds to that following a 50 mg clinical dose, yet trazodone is labeled to be prescribed at doses as high as 600 mg/day, resulting in increased plasma concentrations. Moreover, many patients receive other medications in addition to trazodone, increasing the risk for drug interactions with trazodone. For instance, central nervous depressants or tricyclic antidepressants may interact with trazodone to cause oversedation, and antihypertensive medications received together with trazodone may cause undesirably low blood pressure. Elderly patients and those with preexisting heart disease may be more susceptible to these effects. Indeed, the product label indicates caution in prescribing trazodone to patients with existing cardiac disease due to these associations with arrhythmias.

The data presented in this paper, together with trazodone's known interactions with other medications and recorded instances of torsades de pointes, suggest that trazodone's safety profile warrants further study.

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