

# Effects of seven drugs of abuse on action potential repolarisation in sheep cardiac Purkinje fibres

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## Abstract

Seven drugs of abuse have been examined for effects on the action potential in sheep isolated cardiac Purkinje fibres. Phencyclidine (5  $\mu\text{M}$ ) induced a significant increase (30.7%) in action potential duration at 90% repolarisation ( $\text{APD}_{90}$ ). Similarly, 10  $\mu\text{M}$  3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy') induced a significant increase in  $\text{APD}_{90}$  of 12.1%. Although  $\Delta^9$ -tetrahydrocannabinol (0.1  $\mu\text{M}$ ) induced a small, but statistically significant, 4.8% increase in  $\text{APD}_{90}$ , no effects were observed at 0.01 or 1  $\mu\text{M}$ . Cocaethylene (10  $\mu\text{M}$ ) induced a significant shortening of  $\text{APD}_{90}$  (–23.8%). Cocaine (up to 1  $\mu\text{M}$ ), (+)-methamphetamine ('Speed'; up to 5  $\mu\text{M}$ ), and the heroin metabolite, morphine (up to 5  $\mu\text{M}$ ), had no statistically significant effects. The possible significance of these findings is discussed in the context of other recognised cardiac effects of the tested drugs.

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## 1. Introduction

Torsades de pointes arrhythmia is a potentially lethal polymorphic ventricular tachycardia that can arise as a side-effect of treatment with certain conventional prescription medications (Darpö, 2001; De Ponti et al., 2001; Redfern et al., 2003). Sudden death can occur if the torsades de pointes arrhythmia deteriorates into ventricular fibrillation (Viskin, 1999). Drug-induced torsades de pointes is associated with prolongation of the QT interval of the electrocardiogram (ECG) secondary to an increase in the duration of the cardiac action potential at the level of the individual heart cell (Pearlstein et al., 2003). Although the cardiac action potential may be prolonged by modulation of several different ionic currents (Viskin, 1999; Belardinelli et al., 2003; Fenichel et al., 2004), the molecular mechanism underlying drug-induced action potential prolongation appears to derive overwhelmingly

from inhibition of the rapid component of the delayed rectifier  $\text{K}^+$  current,  $I_{\text{Kr}}$  (Pearlstein et al., 2003). In humans, the human ether-à-go-go related gene (hERG) encodes the pore-forming subunit of the channel that conducts  $I_{\text{Kr}}$ , and the resulting current is referred to as hERG current (Martin et al., 2004).

Such has been the concern among drug regulatory authorities and the pharmaceutical industry over potential problems with drug-induced QT interval prolongation and torsades de pointes arrhythmia that, in addition to the traditional in vivo cardiovascular safety evaluation of novel therapeutic drugs, most compounds are now also routinely tested in one or more in vitro cardiac electrophysiology assays for their potential to impair ventricular repolarisation. The two in vitro assays most commonly used to assess this pro-arrhythmic risk factor are inhibition of the  $\text{K}^+$  current conducted by hERG channels transfected into a heterologous expression system (e.g., CHO or HEK-293 cells) and prolongation of the cardiac action potential in non-human (e.g., dog, guinea pig, rabbit or sheep) ventricular tissue (e.g., Purkinje fibres, papillary muscles, or ventricular myocytes) (Martin et al., 2004).

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In terms of requirements for block, the hERG channel appears to be remarkably promiscuous, as evidenced by the structurally diverse range of molecules that inhibit the hERG current, prolong the cardiac action potential, and increase QT interval (Redfern et al., 2003; see also [www.torsades.org](http://www.torsades.org)). While the major focus of attention has been on developmental stage candidate drugs intended for the prescription pharmaceuticals market, much less is known about the potential of drugs of abuse to prolong the QT interval and precipitate torsades de pointes arrhythmia. Such an adverse effect may conceivably contribute to the morbidity and mortality not infrequently associated with illicit drug use.

The purpose of the present study, therefore, was to examine the effects of a number of common drugs of abuse on cardiac action potential repolarisation in sheep isolated Purkinje fibres. The following drugs have been examined: cocaine, cocaethylene (a psychoactive metabolite formed when cocaine and ethanol are abused concurrently), (+)-methamphetamine ('Speed'), 3,4-methylenedioxymethamphetamine (MDMA; 'Ecstasy'),  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC), morphine (a metabolite of heroin), and phencyclidine ('Angel Dust'). With the exception of phencyclidine, the drugs were selected on the basis of a review of drug use in England and Wales in 2002/2003 (Condon and Smith, 2003). Phencyclidine was studied because of evidence that recreational use of this drug in the United States, prevalent during the 1980s, may be re-emerging (DEA, 2003).

Our findings indicate that both phencyclidine and MDMA prolong the cardiac action potential in sheep isolated cardiac Purkinje fibres. Moreover, cocaethylene had profound depressant effects on the action potential, which could potentially predispose users to other types of arrhythmia.

## 2. Materials and methods

### 2.1. Preparation of sheep cardiac Purkinje fibres

Cardiac Purkinje fibres were isolated from the left ventricles of male sheep (offspring of Charollais ram × mule ewe) bred on Dstl Porton Down Farm. The weight range of the 34 sheep used in the study was  $51.5 \pm 2.3$  kg (mean ± S.E.M.). Sheep were killed by captive bolt stunning followed immediately by exsanguination. The chest was opened and the heart rapidly removed and submerged in chilled physiological salt solution, pre-gassed with 95% O<sub>2</sub>/5% CO<sub>2</sub>, to expel residual blood. The left ventricle was opened and free-running Purkinje fibres were isolated and transferred to screw-capped glass vials containing chilled pre-gassed physiological salt solution. The time interval between captive bolt administration and isolation and transfer of fibres to chilled PSS was  $7.3 \pm 0.2$  min (mean ± S.E.M.).

Isolated fibres were transported to the study laboratory where they were transferred to a holding chamber containing gassed physiological salt solution at ambient temperature (approximately 21 °C). The composition of the physiological salt solution was (in mM): NaCl 129, KCl 4, CaCl<sub>2</sub> 1.8, MgCl<sub>2</sub> 1.1, NaH<sub>2</sub>PO<sub>4</sub> 1, NaHCO<sub>3</sub> 20, D-glucose 11. The buffer was gassed with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub>.

### 2.2. Recording of cardiac action potentials

After at least 30 min equilibration at room temperature, Purkinje fibres were transferred to a two-channel recording chamber (one fibre per channel). Each fibre was pinned to the Sylgard base of the chamber using entomological pins and positioned over a pair of silver stimulation electrodes, which were used to pace the fibres at a frequency of 1 Hz. The recording chambers were perfused at approximately 10 ml/min with gassed physiological salt solution at approximately 36 °C. Electrical pacing (using a Grass S88 stimulator) was started at least 15 min after transfer of the fibres to the recording chamber using square-wave constant voltage pulses of approximately 0.05 ms duration and intensity approximately 20% above the threshold for eliciting contraction of the fibre (visible under the dissection microscope). Fibres were impaled using glass micropipettes filled with 3 M KCl (typically 15–25 M $\Omega$ ). Cardiac action potentials were then monitored over the course of the next 15–30 min and, if the measured parameters remained stable over this time, the experiment was continued.

### 2.3. Experimental design

Once the action potentials had stabilised, control fibres were exposed to drug or dimethylsulphoxide (DMSO) vehicle at three cumulatively applied concentrations (30 min exposure at each concentration). The study design consisted of seven drug-treated groups and one time- and vehicle-matched control group (four Purkinje fibres per group).

### 2.4. Drug concentrations

Drugs were tested over a two-order-of-magnitude range of concentrations designed to bracket total plasma concentrations reported in users (see Table 1).

### 2.5. Drug formulation and administration

Each drug was initially formulated in DMSO to produce a stock solution (Stock A) having a concentration 1000-fold greater than the highest concentration required for testing. A 10-fold dilution of this stock solution (also in DMSO) was also prepared (Stock B). To achieve the final desired concentrations in the recording chamber, appro-

Table 1  
Test drug concentrations and total plasma levels reported in humans

Drug	Concentrations tested ( $\mu\text{M}$ )			Total plasma concentration ( $\mu\text{M}$ )	Plasma protein binding (%)	References
Cocaine	0.01	0.1	1	0.053–0.43 <sup>a</sup>	92 <sup>b</sup>	Williams et al., 2000 <sup>a</sup> ; Edwards and Bowles, 1988 <sup>b</sup>
Cocaethylene	0.1	1	10	0.78 <sup>c</sup>	36 <sup>d</sup>	Bailey, 1996 <sup>c</sup> ; Bailey, 1997 <sup>d</sup>
MDMA	0.1	1	10	0.12–1.65 <sup>c</sup>	34–40 <sup>f</sup>	Samyn et al., 2002 <sup>c</sup> ; Garrett et al., 1991 <sup>f</sup>
(+)-Methamphetamine	0.05	0.5	5	0.09–2.01 <sup>g</sup>	10–20 <sup>h</sup>	Schepers et al., 2003 <sup>g</sup> ; Bailey, 1997 <sup>h</sup>
Morphine	0.05	0.5	5	0.12–2.17 <sup>i</sup>	35 <sup>j</sup>	Darke et al., 1997 <sup>i</sup> ; Goodman and Gilman, 1996 <sup>j</sup>
Phencyclidine	0.05	0.5	5	0.04–3.34 <sup>k</sup>	78 <sup>l</sup>	Bailey, 1979 <sup>k</sup> ; Giles et al., 1982 <sup>l</sup>
$\Delta^9$ -THC	0.01	0.1	1	0.02–0.09 <sup>m</sup>	97 <sup>n</sup>	Cone and Huestis, 1993 <sup>m</sup> ; Harvey, 1999 <sup>n</sup>

appropriate volumes of the stock solutions were cumulatively added to the 1 l perfusate reservoir (100  $\mu\text{l}$  of Stock B followed by 90  $\mu\text{l}$  of Stock A followed by 900  $\mu\text{l}$  of Stock A). These volumes of DMSO alone were added to the reservoir for the time- and vehicle-matched control group. Tissues were exposed for 30 min at each concentration. The final concentrations of DMSO at the low, medium, and high concentrations of drug were 0.01%, 0.019%, and 0.109% vol/vol.

## 2.6. Verification of test system sensitivity

In experiments where a test drug had little or no effect on the action potential, the fibres were exposed for 30 min to the well-characterised hERG/ $I_{Kr}$  blocker, *dl*-sotalol (30  $\mu\text{M}$ ), in order to confirm that this treatment induced the expected action potential prolongation.

## 2.7. Drugs and reagents

Cocaine hydrochloride, cocaethylene base, MDMA hydrochloride, morphine sulphate pentahydrate, (+)-methamphetamine hydrochloride, phencyclidine hydrochloride, and  $\Delta^9$ -THC base were sourced from Sigma-Aldrich (UK). *dl*-Sotalol hydrochloride was obtained from Tocris Cookson (UK). DMSO and the physiological salt solution reagents were sourced from BDH Laboratory Supplies (UK).

## 2.8. Data acquisition and analysis

Action potentials were digitised at 25 kHz using *eDacq* version 1.1.17 (Electro-Medical Measurement Systems, Unit 12 Woolmer Way, Bordon, Hampshire GU35 9QF, UK). Digitised action potentials were analysed by the data acquisition software to derive the following parameters: action potential duration at 50% repolarisation (APD<sub>50</sub>), action potential duration at 90% repolarisation (APD<sub>90</sub>), and maximum rate of rise in the action potential upstroke ( $V_{\text{max}}$ ), upstroke amplitude, and diastolic membrane potential. Values for the parameters were obtained from the average of 29–30 action potentials at each of the relevant time points: 0 min (control), 30 min (low

concentration), 60 min (medium concentration), and 90 min (high concentration). For each parameter, the change from the respective control value for each fibre in each treatment group was derived. For APD<sub>50</sub>, APD<sub>90</sub>, and  $V_{\text{max}}$ , the change from control was calculated as a percentage, while changes from control for upstroke amplitude and diastolic membrane potential were calculated in absolute units (mV).

## 2.9. Statistical analysis

For each of the parameters, the data from the seven test drug groups and the DMSO vehicle group (changes from control at the 30, 60, and 90 min time points) were initially analysed by a multivariate analysis of variance procedure (MANOVA), followed by, where an overall statistically significant interaction was detected, a one-way analysis of variance (to determine at which of the three concentrations the significant interaction occurred). Finally, the Tukey–Kramer Multiple Comparison test was performed to determine which of the test drug treatments differed significantly from DMSO vehicle. For each of the procedures used, statistical significance was accepted at  $P < 0.05$ . Statistical analyses were performed using NCSS software (NCSS, Kaysville, Utah).

Summarised data are presented in the text either as mean  $\pm$  S.E.M. when referring to changes from control, or as mean  $\pm$  S.E.M.D. (standard error of the mean difference) when referring to changes relative to the DMSO vehicle group.

## 3. Results

### 3.1. Baseline action potential parameters

The pre-treatment values (at a pacing frequency of 1 Hz) for APD<sub>50</sub>, APD<sub>90</sub>,  $V_{\text{max}}$ , diastolic membrane potential, and action potential upstroke amplitude in the eight treatment groups are summarised in Table 2. None of the pre-treatment parameters in the drug treatment groups was significantly different from the DMSO treatment group with one exception: diastolic membrane potential in the cocaine

Table 2

Pre-treatment action potential parameters in the eight treatment groups (1 Hz pacing frequency)

Treatment group	APD <sub>50</sub> (ms)	APD <sub>90</sub> (ms)	V <sub>max</sub> (V/s)	DMP (mV)	APA (mV)
DMSO vehicle	185 ± 11	284 ± 17	472 ± 31	-90 ± 1	122 ± 2
Cocaine	189 ± 19	261 ± 22	485 ± 28	-83 ± 2 <sup>a</sup>	120 ± 3
Cocaethylene	211 ± 4	325 ± 10	484 ± 20	-87 ± 1	122 ± 2
MDMA	166 ± 25	244 ± 30	451 ± 9	-85 ± 2	117 ± 2
(+)-Methamphetamine	214 ± 12	305 ± 18	469 ± 17	-89 ± 1	123 ± 1
Morphine	215 ± 24	307 ± 21	487 ± 25	-87 ± 1	125 ± 1
Phencyclidine	205 ± 15	287 ± 12	461 ± 26	-89 ± 1	119 ± 3
Δ <sup>9</sup> -THC	215 ± 23	308 ± 22	470 ± 14	-87 ± 1	125 ± 1

Values are mean ± S.E.M. (four fibres per treatment group). Pre-treatment values for APD<sub>50</sub>, APD<sub>90</sub>, V<sub>max</sub>, and action potential upstroke amplitude (APA) in the drug treatment groups were not significantly different from those in the DMSO vehicle group (one-way ANOVA;  $P > 0.05$ ).

<sup>a</sup> Pre-treatment diastolic membrane potential (DMP) in the cocaine group was significantly lower than that in the DMSO vehicle group (one-way ANOVA followed by Tukey–Kramer Multiple Comparison test;  $P < 0.05$ ).

group was significantly lower than that in the DMSO group ( $P < 0.05$ ).

### 3.2. Effect of drug treatments on action potential duration at 1 Hz pacing frequency

The actions of cocaine, cocaethylene, MDMA, (+)-methamphetamine, morphine, phencyclidine, Δ<sup>9</sup>-THC, and DMSO vehicle on APD<sub>90</sub> are summarised in Figs. 1 and 4. The effects of each treatment on all cardiac action potential parameters are summarised in Table 3.

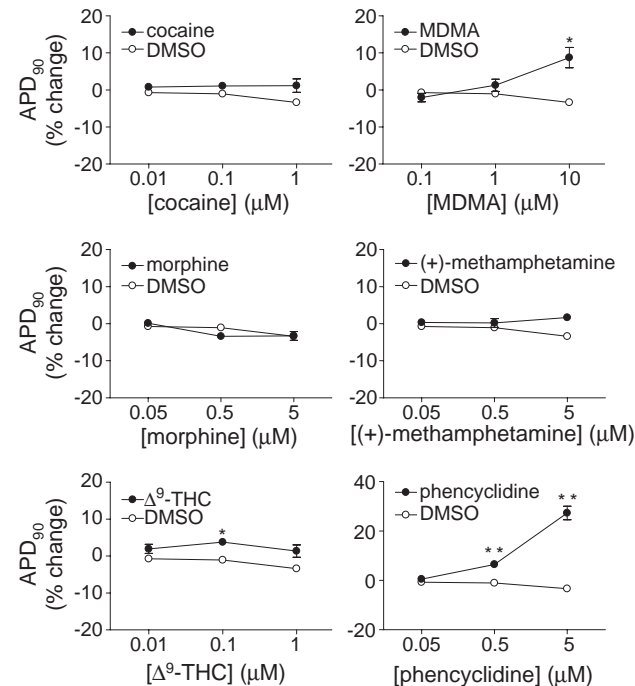


Fig. 1. Effect of cocaine, MDMA, morphine, (+)-methamphetamine, Δ<sup>9</sup>-THC, and phencyclidine on APD<sub>90</sub> in sheep isolated Purkinje fibres paced at 1 Hz. Each drug is compared with the effects of DMSO applied at 0.01%, 0.019%, and 0.109% vol/vol. Abscissa: Drug concentration in micromolars. Ordinate: Percentage change in APD<sub>90</sub> from pre-treatment control. Significant differences from DMSO vehicle are indicated (\* $P < 0.05$ ; \*\* $P < 0.001$ ). Each point represents the mean (± S.E.M.) change in APD<sub>90</sub> induced in four fibres.

### 3.3. Phencyclidine and MDMA prolonged the action potential

Phencyclidine exerted the most potent prolonging effect on APD<sub>90</sub>, inducing significant lengthening at 0.5 μM (+7.5 ± 1.5% relative to 0.019% vol/vol DMSO;  $P < 0.001$ ) and 5 μM (+30.7 ± 2.8% relative to 0.109% vol/vol DMSO;  $P < 0.001$ ). Phencyclidine also induced a prolongation of APD<sub>50</sub> at the highest concentration tested (+22.8 ± 3.0% relative to 0.109% vol/vol DMSO;  $P < 0.01$ ).

MDMA induced a significant prolongation of APD<sub>90</sub> when applied at 10 μM (+12.1 ± 2.8% relative to 0.109% vol/vol DMSO;  $P < 0.05$ ) but had no statistically significant effect on APD<sub>90</sub> in fibres exposed to 1 μM (+2.3 ± 1.8% relative to 0.019% vol/vol DMSO;  $P > 0.05$ ). MDMA had no statistically significant effects on APD<sub>50</sub>, V<sub>max</sub>, diastolic membrane potential, or action potential upstroke amplitude at any of the concentrations tested.

Examples of the effects on the action potential induced by phencyclidine and MDMA in two individual Purkinje fibres paced at 1 Hz are illustrated in Fig. 2.

Because of limitations on the availability of animals, the reverse rate dependency of the effects of phencyclidine and MDMA was examined in only two Purkinje fibres (one fibre per drug). When applied at a concentration of 5 μM for 30 min, phencyclidine induced a 21% increase in APD<sub>90</sub> at a pacing frequency of 1 Hz and a 5% increase in APD<sub>90</sub> at a pacing rate of 3 Hz. Similarly, MDMA applied at 10 μM for 30 min induced a 10% prolongation of APD<sub>90</sub> at a pacing rate of 1 Hz and a 6% prolongation at 3 Hz.

### 3.4. Cocaethylene produced marked depressant effects on the cardiac action potential

Although 0.1 μM and 1 μM cocaethylene had no effects on the action potential, when applied at 10 μM it induced profound decreases in APD<sub>50</sub> (-39.0 ± 7.6% relative to 0.109% vol/vol DMSO;  $P < 0.001$ ) and APD<sub>90</sub> (-23.8 ± 5.1% relative to DMSO;  $P < 0.001$ ) and a decrease in the amplitude of the action potential upstroke (-7.2 ± 1.9 mV relative to DMSO;  $P < 0.01$ ). Cocaethylene (10 μM) also

Table 3  
Change from pre-treatment control induced by each treatment (four fibres per treatment group)

Treatment	Concentration	Change in action potential parameter relative to control				
		APD <sub>50</sub> (%)	APD <sub>90</sub> (%)	V <sub>max</sub> (%)	DMP (mV)	APA (mV)
DMSO vehicle	0.01%	-2.1 ± 2.5	-0.7 ± 0.2	-0.6 ± 3.1	+0.2 ± 0.4	-0.7 ± 0.7
	0.019%	-5.4 ± 2.8	-1.1 ± 0.8	-0.7 ± 2.1	+0.3 ± 0.4	-0.9 ± 0.5
	0.109%	-10.2 ± 2.3	-3.4 ± 0.7	-1.1 ± 1.6	0.0 ± 0.5	-1.0 ± 0.7
Cocaine	0.01 μM	+1.2 ± 0.7	+0.8 ± 0.5	-1.7 ± 2.3	-0.4 ± 0.3	+0.7 ± 0.7
	0.1 μM	+1.3 ± 2.3	+1.1 ± 0.7	-3.6 ± 5.0	+0.4 ± 0.2	+0.2 ± 1.2
	1 μM	0.0 ± 3.6	+1.2 ± 1.8	-1.4 ± 3.5	+0.6 ± 0.3	-0.4 ± 1.1
Cocaethylene	0.1 μM	-1.5 ± 0.4	-0.6 ± 0.6	-0.3 ± 0.5	-0.4 ± 0.2	+0.2 ± 0.5
	1 μM	-7.6 ± 1.5	-0.4 ± 1.2	-1.0 ± 1.5	+0.2 ± 0.5	-0.9 ± 0.6
	10 μM	-49.2 ± 7.3***	-27.2 ± 5.1***	-10.4 ± 3.1	+3.5 ± 1.4	-8.2 ± 1.8**
MDMA	0.1 μM	-4.9 ± 3.1	-2.0 ± 1.1	-0.5 ± 1.1	+0.2 ± 0.2	-0.6 ± 0.7
	1 μM	-4.2 ± 4.6	+1.3 ± 1.6	-3.1 ± 2.1	-0.5 ± 0.1	-1.5 ± 1.2
	10 μM	-0.7 ± 3.8	+8.7 ± 2.7*	-7.5 ± 3.1	-0.1 ± 0.1	-3.6 ± 0.9
(+)–Methamphetamine	0.05 μM	-0.8 ± 1.8	+0.3 ± 0.9	+1.7 ± 1.4	-0.1 ± 0.5	0.0 ± 0.3
	0.5 μM	-4.2 ± 2.6	+0.2 ± 1.2	+2.2 ± 0.9	-0.4 ± 0.3	-1.1 ± 0.3
	5 μM	-7.3 ± 3.3	+1.7 ± 0.8	+1.5 ± 0.9	-0.4 ± 0.5	-2.1 ± 0.3
Morphine	0.05 μM	-3.2 ± 1.5	+0.1 ± 0.8	-0.1 ± 0.8	+1.0 ± 0.4	+0.2 ± 0.3
	0.5 μM	-10.3 ± 2.0	-3.4 ± 0.3	-1.0 ± 2.2	-0.1 ± 0.2	-1.0 ± 0.4
	5 μM	-13.3 ± 2.3	-3.3 ± 1.2	-0.8 ± 2.2	+0.1 ± 0.2	-1.6 ± 0.4
Phencyclidine	0.05 μM	-0.7 ± 0.8	+0.5 ± 0.6	-1.7 ± 2.3	0.0 ± 0.2	-0.5 ± 1.0
	0.5 μM	+3.8 ± 1.2	+6.5 ± 1.3***	-0.9 ± 2.7	+0.1 ± 0.3	-1.2 ± 1.2
	5 μM	+12.6 ± 1.9**	+27.3 ± 2.7***	+1.6 ± 2.9	-0.1 ± 0.1	-1.6 ± 1.3
Δ <sup>9</sup> -THC	0.01 μM	+1.9 ± 1.5	+1.9 ± 1.2	-6.7 ± 4.0	-0.2 ± 0.2	-0.7 ± 0.2
	0.1 μM	+3.8 ± 0.6	+3.8 ± 0.6**	-7.9 ± 5.8	-0.4 ± 0.4	-0.9 ± 0.6
	1 μM	-2.0 ± 2.5	+1.4 ± 1.7	-8.9 ± 5.2	-1.1 ± 1.8	-1.9 ± 0.9

DMSO or drug was applied cumulatively (30 min at each concentration). Data are mean changes from pre-treatment control (±S.E.M.).

\* Significantly different from DMSO vehicle:  $P < 0.05$  (see Materials and methods).

\*\* Significantly different from DMSO vehicle:  $P < 0.01$  (see Materials and methods).

\*\*\* Significantly different from DMSO vehicle:  $P < 0.001$  (see Materials and methods).

produced an apparent trend towards depolarisation of the diastolic membrane potential and a decrease in  $V_{max}$ , though neither achieved statistical significance ( $P > 0.05$ ). An

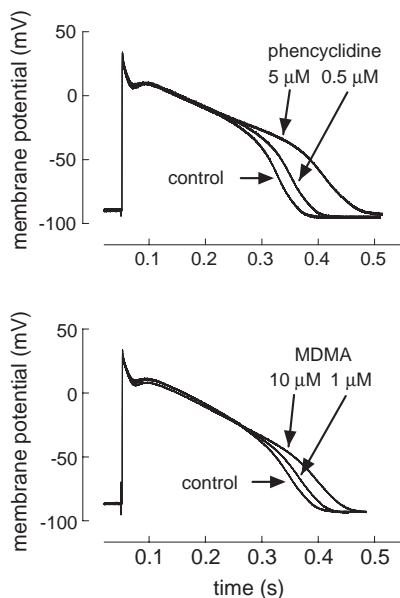


Fig. 2. Action potential prolongation induced by phencyclidine (upper traces) and MDMA (lower traces) in two Purkinje fibres electrically paced at 1 Hz. In these examples, phencyclidine induced an increase in APD<sub>90</sub> of 8.8% at 0.5 μM and 31.6% at 5 μM, while MDMA induced an increase of 5.8% at 1 μM and 15.2% at 10 μM.

example of these actions of cocaethylene in a single Purkinje fibre is illustrated in Fig. 3 and its effects in all fibres studied are summarised in Fig. 4.

### 3.5. Effect of cocaine, (+)-methamphetamine, morphine, and Δ<sup>9</sup>-THC

Cocaine (up to 1 μM), (+)-methamphetamine (up to 5 μM), and morphine (up to 5 μM) had no statistically significant effects on APD<sub>90</sub> (Fig. 1), APD<sub>50</sub>,  $V_{max}$ , diastolic membrane potential, or the action potential upstroke when

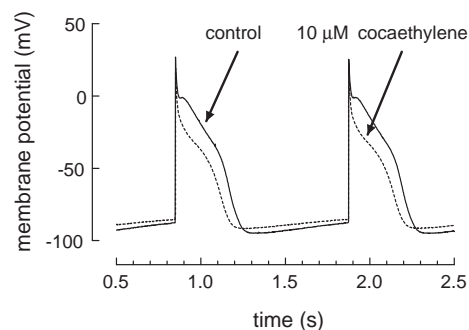


Fig. 3. Example of the effect of cocaethylene (10 μM, 30 min) on the action potential in a single Purkinje fibre paced at 1 Hz. Cocaethylene induced shortening of APD<sub>50</sub> and APD<sub>90</sub>, a small depolarisation of the diastolic membrane potential, and a reduction of upstroke amplitude (observed by superimposition of the traces).



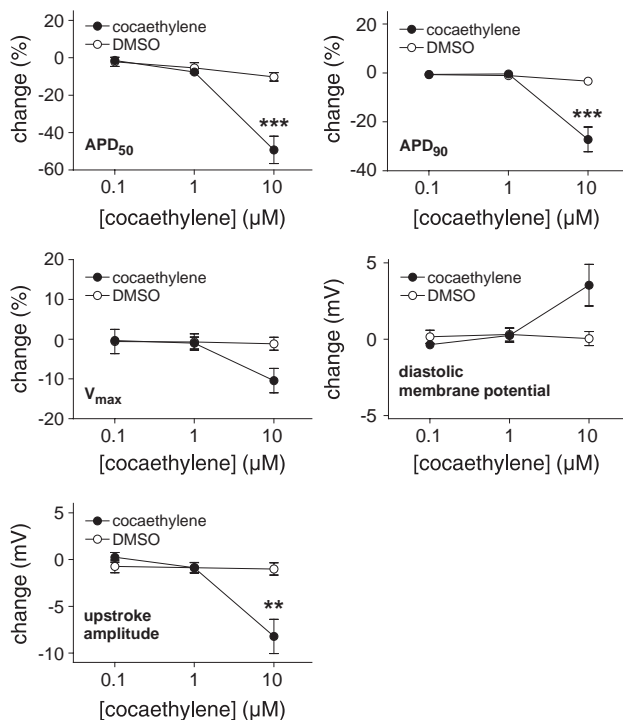


Fig. 4. Effect of cocaethylene on action potential parameters in sheep isolated Purkinje fibres paced at 1 Hz. Each drug is compared with the effects of DMSO applied at 0.01%, 0.019%, and 0.109% vol/vol. Abscissa: Cocaethylene concentration in micromolar. Ordinate: Change (% or mV) from pre-treatment control. Significant differences from DMSO vehicle are indicated (\*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ). Each point represents the mean ( $\pm$  S.E.M.) change induced in four fibres.

applied to fibres paced at 1 Hz.  $\Delta^9$ -THC (up to 1  $\mu$ M) exerted no effects on the five action potential parameters with one exception: 0.1  $\mu$ M  $\Delta^9$ -THC induced an increase in APD<sub>90</sub> ( $+4.8 \pm 0.9\%$  relative to 0.019% vol/vol DMSO;  $P < 0.05$ ) (see Fig. 1).

### 3.6. Effect of *dl*-sotalol on the action potential: verification of test system sensitivity

In experiments in which the test drug appeared to exert little or no effect on the action potential configuration, *dl*-sotalol (30  $\mu$ M, 30 min) was applied immediately after the effects of the highest concentration of test drug had been recorded. In this way, *dl*-sotalol induced the following increases in APD<sub>90</sub> (mean  $\pm$  S.E.M.; four fibres per group):  $+13.5 \pm 4.9\%$  (cocaine group),  $+29.1 \pm 3.5\%$  (morphine group),  $+26.6 \pm 4.5\%$  ( $\Delta^9$ -THC group), and  $+28.3 \pm 2.1\%$  ((+)-methamphetamine group). None of these increases was significantly different from that induced by *dl*-sotalol in the DMSO vehicle group ( $+17.3 \pm 2.1\%$ ) ( $P > 0.05$ ; one-way analysis of variance followed by Dunnett's Multiple Comparison test). *dl*-Sotalol had no effect when applied to the four fibres that had been previously treated with cocaethylene (data not shown). *dl*-Sotalol was not applied to fibres treated with phencyclidine or MDMA.

## 4. Discussion

The main findings of this study were: (1) phencyclidine and MDMA prolonged the action potential in sheep isolated Purkinje fibres; (2) cocaethylene profoundly depressed the cardiac action potential; and (3) cocaine, (+)-methamphetamine, morphine, and  $\Delta^9$ -THC were without substantive effects.

### 4.1. Phencyclidine ('Angel Dust')

Phencyclidine induced significant prolongation of APD<sub>90</sub> (7.5% and 31% increases at 0.5 and 5  $\mu$ M, respectively). Phencyclidine has been reported to prolong the action potential in frog ventricular muscle (D'Amico et al., 1983) and guinea-pig atrial muscle (Temma et al., 1985). Although an action potential-prolonging effect of phencyclidine in guinea-pig ventricular myocytes has been observed (Hadley and Hume, 1986), interpretation of this finding is complicated by the fact that recordings were made at room temperature. The present study represents the first demonstration of action potential prolongation by phencyclidine in mammalian ventricular tissue at physiological temperatures.

There are no published reports of phencyclidine block of hERG currents. Similarly, there are no reports of QT prolongation or torsades de pointes associated with phencyclidine in animal or human studies. This could mean that the in vitro data are not representative of effects in vivo or that some other effect of phencyclidine masks any actions on QT interval. In this regard, the well-established tachycardia produced by phencyclidine in humans (Barton et al., 1981) may functionally antagonise QT prolongation by virtue of the apparent reverse rate dependence of hERG/ $I_{Kr}$  block. Of potential interest are two reports of fatalities associated with neck hold restraint during police arrest of users intoxicated with phencyclidine (Lerner and Burns, 1986; Mercy et al., 1990). Neck hold restraint produces carotid sinus stimulation and reflex bradycardia. Although speculative, if hERG/ $I_{Kr}$  is blocked by phencyclidine, the reverse rate dependence of block could mean that any QT prolongation would be exaggerated at slower heart rates.

### 4.2. MDMA ('Ecstasy')

At the highest concentration tested (10  $\mu$ M), MDMA prolonged APD<sub>90</sub> by about 12%. This action displayed the apparent reverse rate dependency characteristic of  $I_{Kr}$  blockers (Hondeghe and Snyders, 1990). This is the first report of cardiac action potential prolongation by MDMA and indicates that the drug may induce QT prolongation in vivo. There are no published reports of an effect of MDMA on hERG/ $I_{Kr}$  current.

There is a single case report in which QT prolongation has been attributed to MDMA (Drake and Broadhurst, 1996). The authors describe a 25-year-old male admitted to a hospital after taking an MDMA tablet (dose not stated).

The electrocardiogram showed a rate corrected QT interval of 640 ms, which returned to normal (<430 ms) after wash-out of the drug. Plasma concentrations of MDMA were not determined. The authors speculated that the QT prolongation may have resulted from a direct action of MDMA on the ventricular myocardium. The present data lend support to this hypothesis.

Although there are no reports linking torsades de pointes arrhythmia to MDMA, ventricular fibrillation (a potential sequel to torsades de pointes) has been reported. Dowling et al. (1987) describe an 18-year-old female who ingested 150 mg of MDMA together with an undetermined amount of alcohol. She died from ventricular fibrillation shortly afterwards.

In humans, recreational doses of MDMA (75 or 125 mg orally) induce marked increases in blood pressure and heart rate (Mas et al., 1999). The peak plasma concentration of MDMA was achieved within about 2.5 h after dosing and averaged 0.8  $\mu\text{M}$  after the 75 mg dose and 1.4  $\mu\text{M}$  after the 125 mg dose (Mas et al., 1999). No electrocardiographic data were reported.

MDMA is metabolised primarily by the cytochrome P450 enzyme, CYP2D6 (De La Torre et al., 2000). This has two potentially important implications. Firstly, there are polymorphisms in the CYP2D6 enzyme which divide the population into ultrarapid, extensive, intermediate, and poor metabolisers (Meyer, 2000). Thus, poor metabolisers may experience a higher plasma concentration of MDMA than, say, the extensive metaboliser, with the consequence that toxicity may be more likely in the former group. Secondly, a large number of therapeutic drugs are also metabolised by CYP2D6 (e.g., many antidepressant and antipsychotic drugs) and, if used concurrently with MDMA, would compete for the same enzyme (with the result that plasma levels of both may be elevated). A further complicating factor in the behaviour of MDMA is its non-linear pharmacokinetics, the consequence of which is that a small increase in dose has the potential to induce a disproportionate increase in plasma concentration (De La Torre et al., 2000).

On the assumption that the present animal data are predictive of effects in humans, it is possible that QT interval prolongation may occur during recreational use of MDMA. Whether MDMA is torsadogenic remains, at present, unknown.

#### 4.3. Cocaethylene

Cocaethylene produced profound shortening of the action potential at the highest concentration tested (10  $\mu\text{M}$ ). This finding is consistent with in vivo studies in dog in which cocaethylene induced marked dose-dependent decreases in myocardial function at plasma concentrations ranging from about 5 to 20  $\mu\text{M}$  (Wilson et al., 1995).

Although cocaethylene blocks hERG currents ( $\text{IC}_{50}$ =1.2  $\mu\text{M}$ ; Ferreira et al., 2001), no action potential prolongation

was observed in the present study. We suspect that the action potential shortening was due to cocaethylene block of  $\text{Na}^+$  and/or  $\text{Ca}^{2+}$  channels, which would be consistent with the significant decreases in action potential duration and amplitude, and the trend towards a decreased  $V_{\text{max}}$ . The failure of *dl*-sotalol to increase the action potential duration in fibres treated with cocaethylene may imply that  $I_{\text{Kr}}$  may have already been blocked by cocaethylene.

Cocaethylene is a psychoactive metabolite of cocaine formed by hepatic transesterification of cocaine in the presence of ethanol (Smith, 1984). It has been speculated that the production of cocaethylene is responsible for the 25-fold increase in risk of sudden death in individuals who co-abuse cocaine and ethanol (Bunn and Giannini, 1992).

If the effects in the Purkinje fibre translate into humans, then it is possible that cocaethylene may exert adverse effects on cardiac electrophysiology that may predispose the heart, if not to torsades de pointes, then to other potentially lethal arrhythmias associated with facilitated reentry (Roden, 1998).

#### 4.4. Cocaine

The lack of effect of cocaine in the present study contrasts with reports of action potential prolongation in canine Purkinje fibres (prolongation at 10  $\mu\text{M}$ ; Boutjdir et al., 1995), cat ventricular myocytes (prolongation at 10  $\mu\text{M}$ ; Kimura et al., 1992), and guinea-pig ventricular myocytes (prolongation at 3  $\mu\text{M}$ ; Clarkson et al., 1996). However, we set out deliberately to constrain the top end of the concentration range tested to a value marginally above the top end of the range of plasma concentrations in cocaine users (Table 1). Cocaine blocks hERG currents with  $\text{IC}_{50}$  values ranging from 4.4 to 7.2  $\mu\text{M}$  (Ferreira et al., 2001; O'Leary, 2001; Zhang et al., 2001) and there are reports of QT prolongation in humans (Perera et al., 1997; Singh et al., 2001) and in anaesthetised dogs (Clarkson et al., 1993). However, on balance, we surmise that any torsadogenic potential of cocaine may be overshadowed by the well-documented sympathomimetic effects on the heart. The resulting increase in myocardial oxygen demand in the face of coronary artery constriction may account for the estimated 25-fold increased risk of myocardial infarction after cocaine ingestion (Mittleman et al., 1999).

#### 4.5. (+)-Methamphetamine ('Speed')

The lack of action potential prolongation by (+)-methamphetamine is consistent with the absence of any reports of QT prolongation or torsades de pointes in humans. The main cardiotoxic effect of (+)-methamphetamine appears to be cardiomyopathy (Hong et al., 1991).

#### 4.6. Morphine

Heroin (diacetylmorphine) is rapidly converted to 6-monoacetylmorphine and then to morphine (Rentsch et al., 2001). For this reason, morphine was selected for investigation in the present study. Morphine had no effects on the Purkinje fibre action potential. Marsch et al. (2001) investigated the effects of morphine in male subjects aged 18–45 years. When morphine was injected to achieve a peak plasma concentration of 0.7  $\mu\text{M}$ , blood pressure and heart rate were not significantly altered. There are no published clinical reports of morphine- or heroin-induced QT prolongation or torsades de pointes arrhythmia.

#### 4.7. $\Delta^9$ -THC

With the exception of a +4.8% increase in  $\text{APD}_{90}$  at 0.1  $\mu\text{M}$ ,  $\Delta^9$ -THC was without effect on the Purkinje fibre action potential. The increase in  $\text{APD}_{90}$  at the middle concentration is either a statistical anomaly (it did not carry through to the highest concentration tested) or  $\Delta^9$ -THC exerted a biphasic effect on  $\text{APD}_{90}$ . Neither of these interpretations can be excluded without further experimentation. There are no reports concerning the effects of  $\Delta^9$ -THC on hERG currents. No incidences of  $\Delta^9$ -THC-related QT prolongation or torsades de pointes have been published. In terms of adverse cardiovascular events, both marijuana and its principal psychoactive component,  $\Delta^9$ -THC, appear to be relatively benign (Frishman et al., 2003).

In summary, phencyclidine and MDMA produced cardiac action potential prolongation in the present study. Additional studies looking at the effect of these drugs of abuse on hERG currents would provide useful insight into the mechanism underlying the observed action potential prolongation. Although cocaethylene, cocaine, and (+)-methamphetamine did not induce prolongation, a review of the literature revealed that these drugs may still compromise cardiac function in a way that could precipitate a life-threatening cardiac event. Morphine (the principal metabolite of heroin) and  $\Delta^9$ -THC (the principal component of cannabis), both relatively benign in terms of cardiotoxicity, exerted little effect in the present study.

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