Rate and concentration-dependent effects of UK-68,798, a potent new class III antiarrhythmic, on canine Purkinje fibre action potential duration and $\dot{V}_{\max}$

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1 The frequency-dependent electrophysiological effects of UK-68,798 in concentrations of 1, 3, 10 and 30 nM were examined in isolated cardiac Purkinje fibres of the dog at both a number of constant rates of stimulation and following abrupt changes in pacing cycle length.

2 In all concentrations evaluated, UK-68,798 lengthened action potential duration in a concentration- and rate-dependent manner (e.g., at a cycle length = 500 ms, control APD₉₀ = 234.0 ± 3.3 ms, while after 10 nM UK-68,798, APD₉₀ = 315.0 ± 5.9 ms).

3 The duration of action potentials evoked following abrupt changes in pacing rate were also increased in a concentration-dependent manner at all diastolic intervals tested.

4 The fast and slow time constants for restitution of APD were not altered by UK-68,798. However, the amplitude terms for this relation were increased.

5 In addition, the maximum upstroke velocity ($\dot{V}_{\max}$) was not significantly affected by exposure to UK-68,798 at any concentration or diastolic interval. The kinetics for recovery of $\dot{V}_{\max}$ were thus unaffected.

6 These findings are similar to those previously reported for recognized class III antiarrhythmic agents (e.g., bretylium, clofilium, and sotalol); however, UK-68,798 was 1,000 to 10,000 times more potent.

7 The combined potency and selectivity of this agent seem to make it an ideal tool for the investigation of cardiac potassium channels believed responsible for controlling the duration of the action potential.

8 This potent and highly selective compound may prove extremely useful in the control of cardiac arrhythmias.

Keywords: UK-68,798; antiarrhythmic drugs; APD restitution; potassium channel blockers; recovery of $\dot{V}_{\max}$; electrophysiology; Purkinje fibres

Introduction

Antiarrhythmic agents which are categorized as Class III (Vaughan Williams, 1975) are widely recognized as being effective in the clinical treatment of potentially lethal cardiac arrhythmias (Singh & Nademanee, 1985). Vaughan Williams (1975) noted that the only recognized drugs that produced acute Class III actions also demonstrated Class II effects (as do sotalol and bretylium). He commented that, 'in the future, it is probable that drugs with an acute (solely) Class III action will become available'.

UK-68,798 (see Figure 1) is a recently described novel, potent and selective Class III antiarrhythmic which is believed to act by blocking a time-dependent potassium current (Gwilt *et al.*, 1989a; 1990). This compound has been reported (Gwilt *et al.*, 1989a; 1990) to prolong action potential duration and effective refractory period in nanomolar concentrations in canine ventricular muscle and Purkinje fibres *in vitro* (paced at 1 Hz) without slowing final repolarization or significantly affecting diastolic potential, action potential amplitude or maximum upstroke velocity. In guinea-pig isolated papillary

muscle, the increase in effective refractory period was demonstrated to be rate-dependent (Gwilt *et al.*, 1989a; 1990). In addition, this newly synthesized compound has also been demonstrated to increase ventricular fibrillation threshold in dog heart *in situ* without decreasing blood pressure (Gwilt *et al.*, 1989b).

Some antiarrhythmic drugs exert their effects in a frequency-dependent manner (Strauss *et al.*, 1970; Lathrop, 1985; Varró *et al.*, 1986). We have, therefore, examined the frequency-dependent characteristics of UK-68,798 in greater detail. Specifically, we have investigated the effects of UK-68,798 at various constant pacing cycle lengths and described its influence on restitution of action potential duration and recovery of maximum rising velocity in isolated cardiac Purkinje fibres and ventricular muscle of the dog.

Methods

Hearts from adult mongrel dogs of either sex were used for these studies. The dogs were first anaesthetized with sodium pentobarbitone (30 mg kg⁻¹) and the hearts were removed rapidly through a right lateral thoracotomy. The hearts were placed in cooled (0°C) oxygenated Tyrode solution containing (in mM): Na⁺ 153.0, K⁺ 5.4, Cl⁻ 162.9, Ca²⁺ 2.7, Mg²⁺ 1.05, HEPES buffer 5.04 and glucose 11.0. The solution had a pH of 7.35 to 7.45 when gassed with 100% oxygen at 37°C. Free running Purkinje strands and trabecular muscle fibres from either ventricle were dissected and mounted in a small volume (0.5 ml) tissue superfusion chamber. Each preparation was continuously superfused with oxygenated Tyrode solution at a rate of 5 ml min⁻¹ at 37°C. The preparations were stimulated at a constant basic cycle length of 500 ms. Rectangular

Figure 1 Chemical structure of UK-68,798.

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constant current stimuli were delivered from a MECA (Indianapolis, IN, USA) model STM-2 stimulator while isolated from ground by a WP-Instruments (New Haven, CT, USA) model 830 stimulus isolator. Stimuli were 1 ms in duration and were delivered to two platinum wire electrodes placed in the superfusion chamber at either end of the fibre. Each preparation was allowed to equilibrate in the bath for a period of 1 h.

Transmembrane potentials were recorded with conventional microelectrode techniques. Microelectrodes were filled with 3 M KCl and had tip resistances of 10–25 M Ω . These electrodes were connected to the input of a high impedance $10^{12} \Omega$ electrometer (WP-Instruments, Model 750) having capacitance neutralization. The first derivative of transmembrane voltage with respect to time ($\dot{V}_{\max}$) was electronically derived by use of an amplifier (MECA, Model EP 0.3) which was linear over a range of 20 to 1000 V s $^{-1}$.

The outputs of each of these amplifiers were displayed on a single beam storage oscilloscope (Tektronix, Beaverton, OR, USA; model R-5115) with dual sweep speed capability (Tektronix, model 5B12N). Oscilloscope displays were photographed on Polaroid film (type 667) with a Tektronix camera (model C-59).

Protocol for determining response to pacing at different basic cycle lengths

In these experiments, both canine Purkinje strands and ventricular muscle preparations were continuously stimulated at four different cycle lengths (500, 700, 1000, 3000 ms). After equilibration to the new pacing frequency, action potential amplitude, maximum diastolic potential, APD $_{50}$, APD $_{90}$ and $\dot{V}_{\max}$ were determined. Following this procedure and after superfusing the preparation for 30 min in successively higher concentrations of UK-68,798 (1, 3, 10 and 30 nM), the procedure was repeated. Cumulative concentration-response relations were thus developed. Exposure to UK-68,798 was followed by a 30 min period of washout in drug-free normal Tyrode solution and the measurements were repeated.

Protocol for establishing concentration-response relationship: restitution of APD and recovery of $\dot{V}_{\max}$

In another group of Purkinje strand preparations, following a 60 min equilibration period while each preparation was stimu-

lated at constant frequency (2 Hz), control action potential parameters were again measured. To determine the characteristics of restitution of APD and recovery of $\dot{V}_{\max}$ a single extra stimulus was introduced at diastolic intervals varying from 0 to 4 s following a train of 30 stimuli delivered at a cycle length of 500 ms. Diastolic intervals were measured from the point corresponding to 90% repolarization of the preceding basic action potential to the delivery of the extra stimulus. The effective refractory period, defined as the shortest inter-stimulus interval at which an extra stimulus could be introduced while still evoking an action potential, was also determined. Following this procedure the preparations were sequentially exposed to increasing concentrations of UK-68,798 (1, 3, 10 and 30 nM) for 30 min and the measurements were repeated at each concentration.

Drug preparation

UK-68,798 (Pfizer Central Laboratories, Sandwich, England) was prepared daily from a 10 mM stock solution by dilution in normal Tyrode solution.

Data analysis

The concentration-response relations were fit to the equation (DeLean *et al.*, 1978):

$$y = (a - d)/(1 + (x/c)^b) + d$$

where x was the concentration, y the estimated response, a the estimated minimum response, b the estimated slope factor, c the estimated 50% effective concentration and d the estimated maximum response.

The relationship between the diastolic interval and APD $_{90}$ of the action potential evoked by the extra stimulus defined the relation for restitution of APD. This relation was resolved into two exponential components by normalizing APD $_{90}$ at each diastolic interval (APD $_i$) to a duration 5% greater than the APD measured at 4 s (APD $_{pl}$). The fast and slow time constants and amplitude terms for this relation were estimated using standard exponential curve fitting procedures. The curve was thus fit to the equation (Elharrar & Surawicz, 1983):

$$\text{APD}_i = \text{APD}_{pl} \cdot \{1 - [A_f \cdot e^{(-t/\tau_f)}] - [A_s \cdot e^{(-t/\tau_s)}]\} \quad (2)$$

Figure 2 Concentration-dependent effects of UK-68,798 on the action potential characteristics of dog isolated Purkinje fibres stimulated at a constant basic cycle length of 500 ms. Calibration pulses (100 mV in amplitude and 50 ms in duration) follow each action potential. Voltage scale and time scale for action potentials are seen to the left. Amplitude and time scales for the recording of the first derivative of transmembrane voltage with respect to the time are seen to the right. The derivative traces have been inverted in this illustration.

where t represented the diastolic interval preceding APD_{90} , and A and τ the amplitudes and the time constants of the fast (A_f and τ_f) and the slow (A_s and τ_s) exponential components.

The relations for recovery of $\dot{V}_{max}$ were similarly fit to a single exponential relation utilizing a plateau value equal to the highest measured $\dot{V}_{max}$.

Student's t tests (Sokal & Rohlf, 1969) for either paired or unpaired data were used to determine statistical significance of the results. Results of these tests were considered significant when $P < 0.05$.

Results

Effect of UK-68,798 at basic cycle length (500 ms)

The effect of increasing concentrations of UK-68,798 (1, 3, 10 and 30 nM) on the electrophysiological characteristics of dog Purkinje fibres paced at a basic cycle length of 500 ms are summarized in Table 1 and illustrated in Figure 2. At all concentrations applied, UK-68,798 did not significantly alter the MDP, APA or $\dot{V}_{max}$. The rate of the initial rapid repolarization (phase I), observed at rapid sweep speeds, was not affected by UK-68,798 (Figure 3). UK-68,798 did, however, increase APD_{90} , APD_{50} and ERP in a concentration-dependent manner. The estimated 50% effective concentration for increasing APD_{90} (EC_{50}) was 5.5 ± 0.9 nM (Figure 4).

Effect of UK-68,798 at different constant pacing cycle lengths: lack of effect at all concentrations applied, of UK-68,798 on MDP, APA or $\dot{V}_{max}$

The influence of 10 nM UK-68,798 on electrophysiological parameters of dog Purkinje fibres was evaluated at four dif-

Figure 3 Effect of UK-68,798 (10 nM) on initial action potential repolarization (phase I). The preparation was stimulated at a constant basic cycle length of 500 ms. Action potential in (a) was recorded prior to exposure to drug; (b) was recorded after 30 min exposure to 10 nM UK-68,798 and (c) after 30 min washout of the drug.

Figure 4 Concentration-response relation for the effect of UK-68,798 on the duration of the action potential at 90% of repolarization (APD_{90}) in dog Purkinje strands stimulated at a constant basic cycle length of 500 ms. Five preparations, each obtained from a different dog, were used. Error bars and dashed lines represent mean $\pm$ s.e.

Figure 5 Effect of UK-68,798 (10 nM) on the action potential characteristics of dog ventricular muscle stimulated at four different basic cycle lengths. Calibration pulses (100 mV in amplitude and 50 ms in duration) follow each action potential. Voltage and time scales for action potentials are given to the left, amplitude and time scales for the first derivative of the transmembrane voltage with respect to time are to the right. Derivatives have been inverted in this illustration.

Table 1 The effects of UK-68,798 on the electrophysiological characteristics of dog Purkinje fibres at a basic cycle length of 500 ms

	UK-68,798				
	Control	1 nM	3 nM	10 nM	30 nM
MDP (mV)	-82.8 ± 0.5 (5)	-83.6 ± 0.8 (5)	-82.4 ± 0.8 (5)	-82.8 ± 0.5 (5)	-82.4 ± 0.4 (5)
APA (mV)	120.0 ± 0.6 (5)	122.0 ± 0.6 (5)	122.0 ± 0.6 (5)	121.1 ± 0.5 (5)	120.0 ± 0.6 (5)
APD_{50} (ms)	157.0 ± 4.6 (5)	172.0 ± 4.1 (5)*	180.0 ± 2.7 (5)*	199.0 ± 2.4 (5)*	203.0 ± 4.4 (5)*
APD_{90} (ms)	234.0 ± 3.3 (5)	249.0 ± 4.3 (5)*	270.0 ± 3.2 (5)*	315.0 ± 5.9 (5)*	361.0 ± 9.5 (5)*
$\dot{V}_{max}$ ($V s^{-1}$)	598.0 ± 18.3 (5)	604.0 ± 18.6 (5)	614.0 ± 23.2 (5)	596.0 ± 25.6 (5)	574.0 ± 32.8 (5)
ERP (ms)	225.8 ± 4.3 (4)	239.5 ± 0.9 (4)*	258.5 ± 2.1 (4)*	304.5 ± 5.8 (4)*	353.0 (2)
ERP/ APD_{90}	0.96 ± 0.01 (4)	0.96 ± 0.02 (4)	0.95 ± 0.01 (4)	0.95 ± 0.02 (4)	0.97 (2)

* $P \leq 0.01$.

Mean $\pm$ standard error (number of observations).

MDP, maximum diastolic potential; APA, action potential amplitude; APD_{50} , action potential duration at 50% of repolarization; APD_{90} , action potential duration at 90% of repolarization, $\dot{V}_{max}$, maximum rising velocity of the action potential upstroke; ERP, effective refractory period.

Table 2 The effects of UK-68,798 on premature action potentials (diastolic interval = 40 ms) in dog Purkinje fibre

	Control	1 nM	UK-68,798			
			3 nM	10 nM	30 nM	
TOP (mV)	-79.6 ± 0.8	-80.4 ± 1.0	-79.2 ± 1.0	-78.8 ± 0.8	-78.0 ± 1.4	
APD ₉₀ (ms)	173.0 ± 4.1	185.0 ± 5.2*	199.0 ± 5.8*	241.0 ± 9.1*	290.0 ± 14.6*	
$\dot{V}_{\max}$ (V s ⁻¹)	576.0 ± 12.9	586.0 ± 21.6	570.0 ± 35.6	558.0 ± 25.0	498.0 ± 46.2	

* $P \leq 0.01$.Mean ± standard error, number of preparations (n) = 5.TOP, take off potential; APD₉₀, action potential duration at 90% of repolarization; $\dot{V}_{\max}$ maximum rising velocity of the action potential upstroke.

ferent constant pacing cycle lengths (500, 700, 1000 and 3000 ms). UK-68,798 did not significantly affect $\dot{V}_{\max}$ or MDP. However APD₉₀ was increased in a rate-dependent manner such that at long cycle lengths the increase was proportionately greater than at short cycle lengths. Similar

changes were observed in ventricular muscle fibres, although the absolute magnitude of the increase in APD₉₀ was less than that observed in Purkinje fibres (Figure 5).

Effects of UK-68,798 following abrupt changes in pacing cycle length

The effects of UK-68,798 on action potentials evoked at a short diastolic interval (40 ms) are summarized in Table 2. The only significant effect produced by UK-68,798 at this diastolic interval was lengthening of APD₉₀, as noted at the basic cycle length of 500 ms.

The response of action potential duration to UK-68,798 over a wide range of diastolic intervals is illustrated in Figure 6a. Analysis of the restitution curve was undertaken as described in the Methods section. Slow and fast time constants for restitution of APD were not significantly changed following exposure to any concentrations of UK-68,798 examined (Table 3) and the only significant effect was to increase the magnitude of the amplitude terms of the relation.

In addition, UK-68,798 did not affect the time constant for the recovery of $\dot{V}_{\max}$ at any concentration examined (Figure 6b, Table 4).

Figure 6 (a) Effect of UK-68,798 (10 nM) on dog Purkinje fibre restitution of action potential duration measured at 90% of repolarization (APD₉₀). (b) Effect of UK-68,798 (10 nM) on dog Purkinje fibre recovery of $\dot{V}_{\max}$: (●) represent the mean control values obtained from five separate Purkinje strand preparations, (▲) represent mean values obtained after 30 min exposure to 10 nM UK-68,798. Both of the APD restitution curves were fit to a double exponential relation (equation 2). There was no significant difference between the time constants of these relations. The curves for recovery of $\dot{V}_{\max}$ were fitted to a single exponential relation. There was no significant change in the time constant of these relations before or after UK-68,798.

Discussion and conclusions

The cardiac electrophysiological effects of UK-68,798 were studied in dog isolated Purkinje strands and ventricular muscle preparations at several constant rates of stimulation and in dog Purkinje strands following abrupt changes in the diastolic interval. The only significant effect produced was a lengthening of action potential duration and effective refractory period. These effects occurred in a frequency-dependent manner at all examined concentrations and were observed after both changing constant pacing cycle lengths and abrupt changes in diastolic intervals. There was no significant effect on $\dot{V}_{\max}$, APA or MDP. These findings are similar to those previously reported to be produced by class III antiarrhythmic agents (e.g., bretylium, clofilium, and sotalol: Strauss *et al.*, 1970; Steinberg *et al.*, 1981; Nattel & Zeng, 1984; Lathrop, 1985; Varró *et al.*, 1986). However, as a marked difference, UK-68,798 exerted its effects at concentrations which are 1,000 to 10,000 times less than those produced by these previously recognized class III antiarrhythmics.

Table 3 The effects of UK-68,798 on the kinetics of restitution of action potential duration

	Control	1 nM	UK-68,798			
			2 nM	10 nM	30 nM	
τ_f	176.5 ± 22.5	157.7 ± 16.1	167.1 ± 10.6	163.0 ± 15.4	218.0 ± 38.6	
A_f	107.1 ± 5.6	113.9 ± 6.2	131.7 ± 6.6*	151.2 ± 13.2*	188.2 ± 7.2*	
τ_s	2904 ± 237	2794 ± 188	3008 ± 134	3265 ± 611	3134 ± 564	
A_s	55.7 ± 8.5	61.8 ± 6.4	58.2 ± 3.6	104.1 ± 34.4*	132.7 ± 34.4*	
n	5	5	5	5	5	

* $P \leq 0.05$; ^b $P \leq 0.01$.Mean ± standard error, number of preparations (n) = 5. τ_f , time constant of fast exponential component; A_f , amplitude of fast exponential component; τ_s , time constant of the slow exponential component; A_s , amplitude of the slow exponential component.

Table 4 The effects of UK-68,798 on the kinetics of recovery of $\dot{V}_{\max}$

	UK-68,798				
	Control	1 nM	3 nM	10 nM	30 nM
τ	33.7 $\pm$ 7.1	38.4 $\pm$ 4.3	40.6 $\pm$ 8.1	30.0 $\pm$ 5.0	45.6 $\pm$ 5.7
A	193.2 $\pm$ 22.8	119.4 $\pm$ 10.6	156.5 $\pm$ 37.1	181.8 $\pm$ 27.3	156.8 $\pm$ 27.3
Plateau $\dot{V}_{\max}$	616.0 $\pm$ 17.2	622.0 $\pm$ 17.7	618.0 $\pm$ 25.0	594.0 $\pm$ 24.2	515.0 $\pm$ 45.0
n	5	5	5	5	4

Mean $\pm$ standard error; n , number of observations.

τ , time constant of exponential; A , amplitude of exponential; Plateau $\dot{V}_{\max}$, maximum value of $\dot{V}_{\max}$.

The effects on lengthening of APD at a basic cycle length (500 ms) confirm the findings of Gwilt *et al.* (1989a, 1990). The frequency-dependent effects extend those previously reported in guinea-pig isolated papillary muscle preparations (Gwilt *et al.*, 1989a; 1990) and further support the classification of UK-68,798 as a specific class III antiarrhythmic.

In agreement with Gwilt *et al.* (1989a; 1990), our demonstration of a lack of effect on MDP supports the conclusion that UK-68,798 does not affect the inward rectifier current. In addition, we have demonstrated that UK-68,798 (10 nM) does not alter the slope of phase I repolarization at a basic cycle length of 500 ms suggesting that UK-68,798 also does not affect the transient outward current.

The high potency of UK-68,798 (EC_{50} for lengthening of APD₉₀ = 5.5 nM) might be of benefit in clinical application as

the lower concentrations which may be necessary to achieve the desired cardiac effects will provide fewer adverse extra-cardiac effects. In addition, the apparently selective effect of UK-68,798 on inhibition of a time-dependent potassium current suggests that this compound also may have great potential as a research tool to study and isolate specific potassium channels within the cardiac cell membrane.

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