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Antifibrillatory effects of clofilium in the rabbit isolated heart

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1 This study was designed to determine whether clofilium exhibits antifibrillatory activity in a pinacidil + hypoxia-induced model of ventricular fibrillation (VF) in Langendorff-perfused hearts.

2 Ten minutes after exposure to vehicle or clofilium (0.1, 1.0 and 10.0 μM), hearts were exposed to pinacidil (1.25 μM), then subjected to 12 min of hypoxia and reoxygenated. Onset to VF was recorded. Additional groups of hearts were pretreated with UK-68,798 (1.0, 3.0 and 10.0 μM), a delayed rectifier channel blocker, and 5-hydroxydecanoate (10 μM), a known ATP-dependent K^+ channel blocker, and subjected to an identical protocol.

3 Clofilium decreased the incidence of VF in a concentration-dependent manner; 7/9 control hearts developed VF *vs* 1/9 hearts ($P = 0.007$, Fisher's Exact) treated with 10.0 μM clofilium. In addition, 5-hydroxydecanoate protected hearts from VF, while UK-68,798 pretreatment did not.

4 In a separate group of hearts, electrically-induced VF was converted to sinus rhythm in 10/11 hearts after clofilium was introduced as a bolus.

5 Clofilium is capable of preventing VF in the rabbit isolated heart in a concentration-dependent manner. We have data to suggest that the ability of clofilium to attenuate the effects of pinacidil + hypoxia in our model may include blockade of metabolically active K^+ channels, i.e., K_{ATP} (glibenclamide-sensitive) channel.

Keywords: Class III antiarrhythmic drugs; clofilium; UK-68,798; 5-hydroxydecanoate; ATP-dependent K^+ channels; rabbit isolated heart; chemical defibrillation; ventricular fibrillation

Introduction

In view of the inefficacy of Class I agents to prevent sudden cardiac death, there is a renewed effort in the development of antiarrhythmic drugs with selected cardiac electrophysiological properties. Agents with demonstrated experimental efficacy against ventricular arrhythmias include: UK-68,798 (Black *et al.*, 1991), CK3579 and sematilide (Chi *et al.*, 1990a), E-4031 (Lynch *et al.*, 1989), (±)-sotalol (Lynch *et al.*, 1984), and amiodarone (Patterson *et al.*, 1983). In each of the examples cited, the incidence of ventricular fibrillation (VF), or sudden cardiac death in the conscious dog, was reduced significantly when compared to vehicle-treated controls. Another promising investigational agent is WAY-123,398, purported to prolong ventricular refractoriness and increase the ventricular fibrillation threshold without affecting impulse conduction (Spinelli *et al.*, 1992).

The antiarrhythmic compound clofilium, is a bretylium congener devoid of sympathomimetic or sympatholytic effects (Steinberg & Molley, 1975; Steinberg *et al.*, 1981; Lindstrom *et al.*, 1982). Clofilium has been shown to prolong the effective refractory period or action potential duration in a number of animal models (Steinberg & Molley, 1975; Steinberg *et al.*, 1981; Kowey *et al.*, 1985; Wu *et al.*, 1989; Li *et al.*, 1990) as well as to increase the refractory period in the human ventricular myocardium without affecting conduction time or haemodynamics (Greene *et al.*, 1983). An additional potential benefit of clofilium derives from its ability to decrease the electrical energy requirement for ventricular defibrillation (Kopia *et al.*, 1985; Dorian *et al.*, 1991). However, its reported mechanism of action varies with species and model employed. For example, clofilium is capable of inducing closed states in batrachotoxin-actived Na^+ channels from rabbit skeletal muscle (Nettleton *et al.*, 1991). Others report a decrease in outward potassium (delayed rectifier) current of guinea-pig ventricular myocytes after clofilium treatment (Snyders & Katzung, 1985), a finding repeated in ischaemic Purkinje fibres of the dog (Gough *et al.*, 1988). It has been reported that the delayed rectifier, but not the inward rectifier current, is blocked by quaternary clofilium in guinea-pig ventricular cells (Arena & Kass, 1988). Moreover, clofilium and its tertiary homologue LY 97119 have been shown to block the transient outward potassium current (I_{to}) in rat ventricular myocytes (Castle, 1991). Most recently, clofilium was shown to inhibit glibenclamide-sensitive K^+ channels in voltage-clamped *Xenopus* oocytes (Sakuta *et al.*, 1993).

It is clear the clofilium may have the capacity to affect a number of potassium channels in the normal or ischaemic myocardium. To define further its antifibrillatory actions, we studied clofilium in a Langendorff-perfused rabbit isolated heart model in which K_{ATP} channels have been implicated in the genesis of VF (Chi *et al.*, 1993). The primary objective of our study was to determine whether clofilium was capable of antagonizing the effects of pinacidil, a known K_{ATP} channel opener in ventricular myocardium, especially when applied in the presence of hypoxia and an associated decrease in tissue ATP content (Chi *et al.*, 1993). The conditions for perfusion, low extracellular K^+, pinacidil and hypoxia, favoured the induction of ventricular fibrillation through activation of the K_{ATP}-dependent or glibenclamide-sensitive K^+ channel (Chi *et al.*, 1993). We postulated that clofilium may owe its antifibrillatory effect to the blockade of the metabolically active K_{ATP} channel or glibenclamide-sensitive K^+ channel. We examined this hypothesis in the rabbit perfused isolated heart.

Methods

Guidelines for animal research

The procedures followed in this study are in accordance with the guidelines of the University of Michigan Committee on the Use and Care of Animals. Veterinary care was provided by the University of Michigan Unit for Laboratory Animal Medicine. The University of Michigan is accredited by the American Association of Accreditation of Laboratory Animal Care. The animal care and use programme conforms

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to the standards in *The Guide for the Care and Use of Laboratory Animals*, DHEW Publ. No. (NIH) 86-23.

Isolated heart preparation

New Zealand, white rabbits (1.8–2.0 kg) were rendered unconscious by cervical dislocation. A median sternotomy was performed immediately therafter and the heart removed. The aorta was cannulated (Langendorff preparation) and perfused with a buffer solution at a constant rate of 18–20 ml min^{-1}. The time taken to complete the isolation of the heart and initiate perfusion was less than 10 s. The isolated heart was allowed to stabilize for approximately 20 min during which time, flow rate (Master Flex Roller Pump, Cole Parmer, Chicago, IL, U.S.A.) was adjusted to produce a coronary artery perfusion pressure of 50 ± 5 mmHg. Once a steady state had been established, pump settings remained constant throughout the protocol. The buffered perfusion medium (pH 7.4) was composed of (in mM): NaCl 117, KCl 1.41, $CaCl_2$ 2.4, $MgCl_2$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.1, glucose 5.0, L-glutamate (Na^+ salt) 5.0 and sodium pyruvate 2.0. The final K^+ concentration in the perfusate was 2.5 mM.

The perfusion medium was passed through an 'artificial lung' that consisted of 6 m of medical grade gas permeable tubing (Silastic, Dow Corning Corp, Midland, MI, U.S.A.) placed in a double-walled, water-heated chamber. Changing the oxygen mixture in the lung chamber caused the perfusion medium to equilibrate with the introduced gas mixture within 60 s.

During normoxia, the chamber was gassed continuously with 95% O_2/5% CO_2. Hypoxia was produced by gassing the chamber with 95% N_2/5% CO_2. The temperature of the perfusion medium and isolated heart were maintained at 37°C using a temperature controlled circulating water bath. The hearts were paced electrically from the right atrium at a frequency of 20–30% above the intrinsic sinus rate. Electrical pacing was maintained for the duration of each protocol.

A latex balloon was advanced into the left ventricle through an incision in the left atrial appendage and connected *via* a rigid cannula to a pressure transducer positioned at the level of the heart. The intraventricular balloon was expanded with distilled water to establish a ventricular end-diastolic pressure of 5 mmHg. Continuous measurement of left ventricular isovolumic developed pressure and end-diastolic pressure were made with the intraventricular balloon. A polyethylene cannula was placed in the left ventricle to vent the chamber of fluid entering the left ventricle *via* the Thebesian vessels. A thermistor probe (Tele-thermometer, Yellow Springs Instrument Co., Yellow Springs, OH, U.S.A.) was inserted in the left ventricle to monitor the temperature of the heart. A pressure transducer was attached to a side arm of the aortic cannula and was zeroed at the level of the heart. The rate of coronary artery flow was maintained constant by the roller pump. Therefore, changes in perfusion pressure in the aortic cannula reflected changes in coronary artery resistance.

Oxygen tension (P_{O_2}) of the perfusion buffer was monitored continuously by an in-line Clark type oxygen electrode (Instech dual oxygen electrode amplifier model 203, Instech Laboratories, Plymouth Meeting, PA, U.S.A.) placed immediately proximal to the aortic cannula. The electrogram was recorded from leads placed on the aorta and at the cardiac apex.

The following parameters were recorded with a Model 7 Grass Polygraph (Grass Instrument Co. Quincy, MA, U.S.A.): electrogram, monophasic action potential, coronary perfusion pressure, left ventricular pressure and its first derivative ± dP/dt.

Experimental protocol

Hearts were randomized by blind selection of preassigned treatments. Ten experimental groups, consisting of Langendorff perfused hearts were used in this study: Group I: Vehicle + pinacidil (1.25 μM) + hypoxia + reoxygenation (n = 9); Group II: clofilium (0.1 μM) + pinacidil (1.25 μM) + hypoxia + reoxygenation (n = 7); Group III: clofilium (1.0 μM) + pinacidil (1.25 μM) + hypoxia + reoxygenation (n = 7); Group IV: clofilium (10.0 μM) + pinacidil (1.25 μM) + hypoxia + reoxygenation (n = 9); Group V: electrophysiological studies (n = 8); Group VI: electrically induced ventricular fibrillation, treatment with clofilium (n = 11); Group VII: UK-68,798 (1.0 μM) + pinacidil (1.25 μM) + hypoxia + reoxygenation (n = 5); Group VIII: UK-68,798 (3.0 μM) + pinacidil (1.25 μM) + hypoxia + reoxygenation (n = 4); Group IX: UK-68,798 (10.0 μM) + pinacidil (1.25 μM) + hypoxia + reoxygenation (n = 5); Group X: 5-hydroxydecanoate (10.0 μM) + pinacidil (1.25 μM) + hypoxia + reoxygenation (n = 9).

A drug-free equilibration period of 20 min elapsed before the start of each protocol (Figure 1). In groups I–IV, clofilium or drug-free buffer, perfused the hearts for 10 min. In groups VII-X either UK-68,798 or 5-hydroxydecanoate was added to the buffer and perfused the hearts for 10 min. Pinacidil was added to the buffer to achieve a final concentration of 1.25 μM. Five minutes after pinacidil was added to the perfusion medium, global hypoxic perfusion was initiated by changing the gas mixture in the 'artificial lung' to 95% N_2 5% CO_2. The state of global hypoxic perfusion was maintained for 12 min (hypoxic period). Normoxic perfusion was re-established by returning the gas mixture in the 'artificial lung' to 95% O_2, 5% CO_2 (reoxygenation) for the remaining 40 min of the experimental protocol. The period of time to onset of spontaneous, sustained ventricular fibrillation (30 s minimum) was recorded. Groups I–IV and VII–X were studied under conditions of a reduced potassium concentration (2.5 mM) in the perfusion medium. Under these conditions, during global hypoxic perfusion, myocardial ATP content decreases approximately 50%. We have shown in the past that under conditions of 2.5 mM K^+ and reduced ATP, the heart becomes susceptible to the development of ventricular fibrillation in the presence of pinacidil (Chi *et al.*, 1993).

Electrophysiological effects of clofilium

In Group V hearts, a miniature bipolar plunge electrode (INAPRES, Norwich, NY, U.S.A.) was sutured over the right ventricular outflow tract (RVOT) for delivery of the S2 ventricular stimulus from a Grass S8800 square wave stimulator and stimulus isolation unit (SIU5; Grass Instrument Co., Quincy, MA, U.S.A.). The S2 stimulus duration was 4 ms and was triggered 250 ms after the R wave of the ECG. The threshold current was defined as the minimum current required to elicit a propagated ventricular impulse. To determine effective refractory period (ERP), stimulation (S2) to the RVOT (at 1.5 times threshold current) was initiated at a 250 ms delay. The S2 delay stimulation was decreased repeatedly by 10 ms until the refractory period was reached. The refractory period determined in the region of the RVOT is defined as the longest R-wave stimulus interval that fails to elicit a propagated ventricular complex.

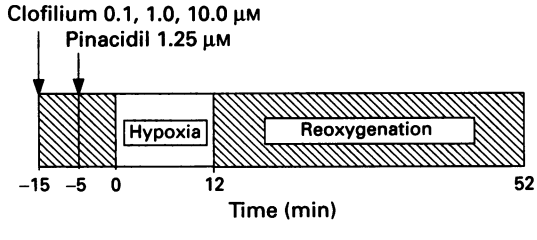

Figure 1 Experimental protocol for Groups I–IV and VII–X; hatched areas, 95% O_2/5% CO_2; open areas, 95% N_2/5% CO_2.

Determination of the ventricular effective refractory period under conditions of low K^+ (2.5 mM) invariably resulted in the induction of ventricular fibrillation. Therefore, Group IV hearts were perfused with perfusion medium containing a potassium concentration of 5.1 mM. Refractory periods were determined at 5, 15 and 30 min after clofilium was added to the perfusion medium.

A Ag-AgCl electrode was used to record the endocardial monophasic action potential duration. Action potential durations (APD) at 90% repolarization were determined at baseline, 10 min after addition of clofilium, and 5 min after pinacidil plus clofilium in groups II–IV.

Group VI hearts received a train of stimuli delivered to the region of the RVOT (S2, S3, S4-instead of a single impulse) until ventricular fibrillation occurred. After 30 s of sustained ventricular fibrillation, a volume of drug diluent (perfusion medium) was administered immediately proximal to the cannulated aorta. Time for injection to reversion of normal sinus rhythm was recorded. If 30 s had elapsed and reversion was not evident, clofilium (0.35 or 0.6 ml of 10.0 mM), dissolved in perfusion medium, was administered rapidly, and time to return of normal rhythm was recorded.

Drugs

Pinacidil and clofilium were gifts from the Eli Lilly Co. (Indianapolis, IN, U.S.A.). A stock solution of pinacidil was prepared daily, by dissolving the drug in acidified perfusate (pH 2.0–2.5). The stock solution was added to the perfusion buffer to yield a final concentration of 1.25 μM. Clofilium was dissolved in the perfusate buffer and prepared just before use in each experiment. UK-68,798 (dofetilide) was a gift from Pfizer Central Research (Sandwich, U.K.) and 5-hydroxydecanoate was a gift from Parke-Davis (Ann Arbor, MI, U.S.A.). Analytical grade chemicals used for preparation of the buffer solution were obtained from commercial sources.

Statistical analysis

The data are expressed as mean ± s.e.mean. The difference between groups (fibrillation occurrence) was analysed by Fisher's Exact test. A one-way ANOVA was used for comparisons between groups (factorial) at specific time points, as well as within groups (repeated measures). Differences were considered significant at $P<0.05$.

Results

Antifibrillatory effects of clofilium

The antifibrillatory effect of clofilium was examined in a Langendorff-perfused rabbit isolated heart model in which ventricular fibrillation was induced by the combined introduction of pinacidil (1.25 μM) to the perfusion medium with the subsequent induction of global hypoxia for 12 min followed by reoxygenation for 40 min. Exposure of the heart to pinacidil (1.25 μM) during normoxic perfusion, did not result in the development of cardiac arrhythmias. However, when pinacidil-treated hearts were subjected to 12 min of hypoxia followed by reoxygenation, there was a 78% (7 of 9 hearts) incidence of ventricular fibrillation in the control group (Group I). Most of the hearts in Group I developed ventricular fibrillation during hypoxia or within the first 20 min of reoxygenation (Figure 2). Clofilium pretreatment was associated with a concentration-related suppression in the incidence of ventricular fibrillation. At a concentration of 10.0 μM, 1 of 9 (11%) hearts developed ventricular fibrillation during the period of reoxygenation ($P<0.05$ *vs* vehicle-treated hearts; Figure 2). Clofilium concentrations of 0.1 and 1.0 μM did not confer a significant protective effect against the induction of ventricular fibrillation upon exposure to the combined effects of pinacidil and hypoxia/reoxygenation.

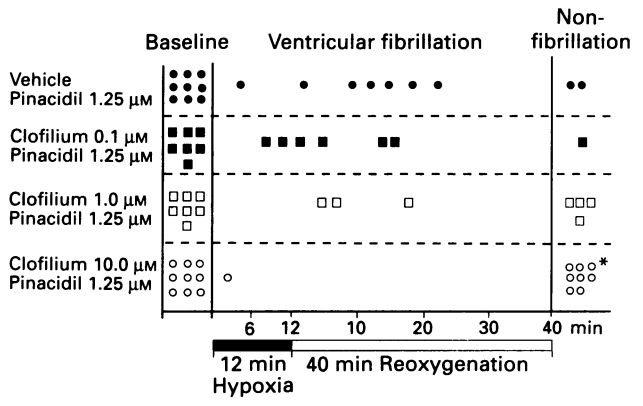

Figure 2 Incidence of ventricular fibrillation in the presence of vehicle or clofilium. Each symbol represents an individual isolated heart. *$P<0.05$ vs vehicle, Fisher's exact test.

Effects of UK-68,798 and 5-hydroxydecanoate on ventricular fibrillation

Groups VII–IX were added to our study to determine if another putative Class III antiarrhythmic agent, devoid of ATP-dependent/glibenclamide-sensitive K^+ channel blocking activity, was able to prevent the induction of ventricular fibrillation by exposing the perfused heart to the combined effects of pinacidil and hypoxia. The selective inhibitor of the rapid component of the delayed rectifier channel, UK-68,798 was used to test this hypothesis. Finally, Group X was included to examine a specific ATP-dependent/glibenclamide-sensitive K^+ channel blocker, 5-hydroxydecanoate, in the isolated heart model of ventricular fibrillation.

UK-68,798 pretreatment was not associated with a concentration-related suppression in the incidence of ventricular fibrillation. At a concentration of 10.0 μM, 3 of 5 (60%) hearts developed ventricular fibrillation during the period of reoxygenation (Figure 3). UK-68,798 concentrations of 1.0 μM (80% VF) and 3.0 μM (100% VF) did not confer a significant protective effect against the induction of ventricular fibrillation upon exposure to the combined effects of pinacidil and hypoxia-reoxygenation. However, at a concentration of 10 μM, 5-hydroxydecanoate, reduced the incidence of ventricular fibrillation to 2 of 9 (22%) hearts during the period of reoxygenation ($P<0.05$ *vs* vehicle treated hearts).

Effects of clofilium on cardiac function

Coronary perfusion pressure (Table I) did not differ between Groups I–IV at baseline. The highest concentration of

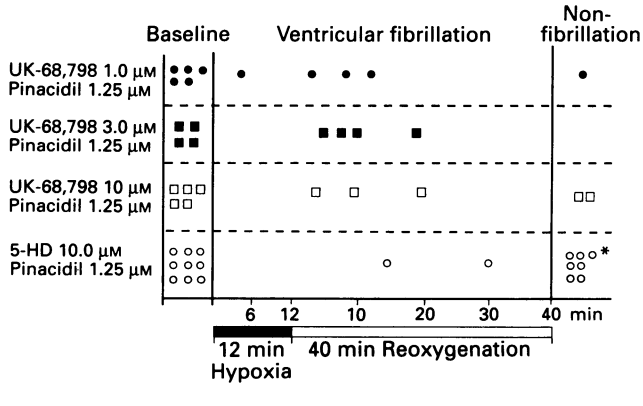

Figure 3 Incidence of ventricular fibrillation in the presence of UK-68,798 and 5-hydroxydecanoate (5-HD). Each symbol represents an individual isolated heart. *$P<0.05$ vs vehicle, Fisher's Exact test.

Table 1 Coronary perfusion pressure (mmHg) in the rabbit isolated heart treated with vehicle or clofilium

	Vehicle	0.1 μM	1.0 μM	10.0 μM
Baseline	52 ± 3 (9)	52 ± 1 (7)	61 ± 4 (7)	58 ± 3(9)
Clofilium (10 min)	-	57 ± 2 (7)[a]	66 ± 6 (7)[a]	50 ± 2 (9)[ac]
Pinacidil (5 min)	37 ± 2 (9)[a]	45 ± 2 (7)[ab]	50 ± 4 (7)[ab]	48 ± 2 (9)[ab]
Hypoxia (5 min)	31 ± 4 (7)[a]	38 ± 4 (7)[a]	42 ± 4 (7)[a]	38 ± 3 (8)[a]
Hypoxia (12 min)	40 ± 4 (7)[a]	45 ± 3 (6)[a]	55 ± 6 (7)	53 ± 4 (8)
Reoxygen (10 min)	48 ± 3 (5)	57 ± 9 (3)	59 ± 6 (5)	55 ± 4 (8)
Reoxygen (40 min)	-	-	82 ± 18 (4)	75 ± 6 (8)[a]

Data are expressed as mean ± s.e.mean, *n* in parentheses.
[a]$P<0.05$ vs. corresponding baseline; [b]$P<0.05$ vs. respective clofilium (10 min); [c]$P<0.05$ vs 1.0 μM clofilium

clofilium (10.0 μM) produced a reduction in coronary perfusion pressure from baseline, 58 ± 3 mmHg to 50 ± 2 mmHg after 10 min of exposure ($P<0.05$). More striking was the coronary perfusion pressure (CPP) response upon the subsequent administration of pinacidil (1.25 μM). For hearts treated with 0.1 and 1.0 μM clofilium, CPP decreased in the presence of pinacidil. In contrast, hearts treated with 10.0 μM clofilium exhibited a modest reduction of CPP. Clearly, at a concentration of 10.0 μM clofilium attenuates the vasodilator effects of pinacidil. Hypoxia further decreased CPP in each group and there were no differences in coronary artery perfusion pressure among groups during hypoxia or reoxygenation.

Clofilium produced minimal effects upon myocardial contractile performance when added to the perfusion medium (10.0 μM) compared to the control group of hearts. In hearts exposed to clofilium followed by pinacidil (1.25 μM), $+dP/dt$ was 475 ± 6 mmHg s^{-1}, compared to 470 ± 9 mmHg s^{-1} before pinacidil treatment. Vehicle-treated hearts typically exhibit a 10% reduction in $+dP/dt$ upon the addition of pinacidil (1.25 μM), clofilium attenuated this response. During hypoxia, ($t = 12$ min) $+dP/dt$ was 355 ± 14 mmHg s^{-1} in the clofilium-treated group. Lower concentrations of clofilium failed to show significant haemodynamic changes compared to vehicle-treated hearts throughout the experimental protocol.

Electrophysiological effects of clofilium

As shown in Table 2, a group of hearts was used for the characterization of the ventricular effective refractory period (ERP) determinations in the absence and presence of cumulative concentrations of clofilium (0.3, 1.0 and 3.0 μM). In the presence of the lowest concentration of clofilium (0.3 μM), ERP increased 17 ± 5% above baseline after 30 min of exposure ($P<0.05$). At a concentration of 1.0 μM, clofilium progressively increased ERP 25 ± 9% above baseline after 30 min of exposure to the drug in the perfusion medium. An additional prolongation to 213 ± 16 ms was found after 30 min of exposure to 3.0 μM clofilium ($P<0.05$ vs baseline). The total increase in ERP was 37 ± 18% compared to baseline values. Stepwise increases above 3.0 μM clofilium decreased atrioventricular conduction velocity and interfered with the ability to maintain atrial pacing. Therefore the electrophysiological effects of clofilium at concentrations greater than 3.0 μM were not studied.

Table 2 Ventricular effective refractory periods (ms) of paced rabbit isolated hearts in the presence of graded concentrations of clofilium

Concentration	*Predrug*	*5 min*	*15 min*	*30 min*
0.3 μM ($n = 8$)	156 ± 13	173 ± 16	175 ± 17[a]	170 ± 9[a]
1.0 μM ($n = 6$)	-	177 ± 15	184 ± 15	189 ± 9[a]
3.0 μM ($n = 6$)	-	190 ± 15	203 ± 13[a]	213 ± 16[a]

Data are mean ± s.e.mean. [a]$P<0.05$ relative to predrug value.

Action potential durations (APD) at 90% repolarization were determined at baseline, 10 min after addition of clofilium, and 5 min after pinacidil plus clofilium in groups II–IV. Table 3 summarizes the results. The changes in APD were not as profound in our preparation as were the changes in measured refractory period after clofilium exposure. An important result was obtained in this portion of the study. While we measured only slight increases in APD after clofilium alone, there was a significant change in APD after pinacidil had been administered. After the lowest 2 concentrations of clofilium had been administed (0.1 and 1.0 μM), pinacidil reduced APD ($P<0.05$ vs corresponding baseline). In the presence of 10.0 μM clofilium, there was no reduction in APD compared to baseline. These findings indicate that the prevention of APD shortening by 10.0 μM clofilium may contribute to its antifibrillatory action.

Chemical defibrillatory effects of clofilium

Hearts in Group VI were used to explore the ability of clofilium to achieve chemical defibrillation in the isolated perfused heart. Ventricular fibrillation was induced by direct current pulses applied to the RVOT of the isolated heart while coronary flow was maintained constant. Ventricular fibrillation was permitted to persist for a period of 30 s to ensure the presence of a persistent state of fibrillation after which a volume of vehicle buffer was administered just proximal to the aorta. Repeated administration of the drug diluent did not influence the fibrillation status of the isolated heart. Isolated perfused hearts, while in ventricular fibrillation, were given a single dose of clofilium (0.35 or 0.60 ml of a 10 mM solution) rapidly, immediately above the aorta. Conversion of the electrically-induced VF to normal sinus rhythm occurred in 10 of 11 hearts (Figure 4). The mean time to conversion was 14 ± 2 s in 5/5 hearts ($P<0.05$ vs vehicle), using 0.35 ml of 10.0 mM clofilium. Time to conversion after 0.60 ml of 10.0 mM clofilium was 42 ± 13 s in 5/6 hearts ($P<0.05$ vs vehicle). The longer time to conversion in the latter group was related to sinus arrest associated with exposure to 0.6 ml of

Table 3 Action potential duration (ms) on paced rabbit isolated hearts during baseline, and in the presence of clofilium and pinacidil

Concentration	*Baseline*	*Clofilium* (10 min)	*Clofilium + pinacidil*
0.1 μM ($n = 6$)	139 ± 6	143 ± 6 (3 ± 2)	123 ± 8[a] (−9 ± 5)
1.0 μM ($n = 7$)	141 ± 9	150 ± 9 (7 ± 3)	134 ± 8[a] (−4 ± 5)
10.0 μM ($n = 5$)	143 ± 13	150 ± 12 (5 ± 2)	142 ± 11 (+4 ± 2[b])

Data are mean ± s.e.mean at 90% repolarization (ms). Values in parentheses represent % change from baseline. [a]$P<0.05$ vs corresponding clofilium (10 min) value. [b]$P<0.05$ relative to 0.1 μM clofilium + pinacidil (1.25 μM) % change.

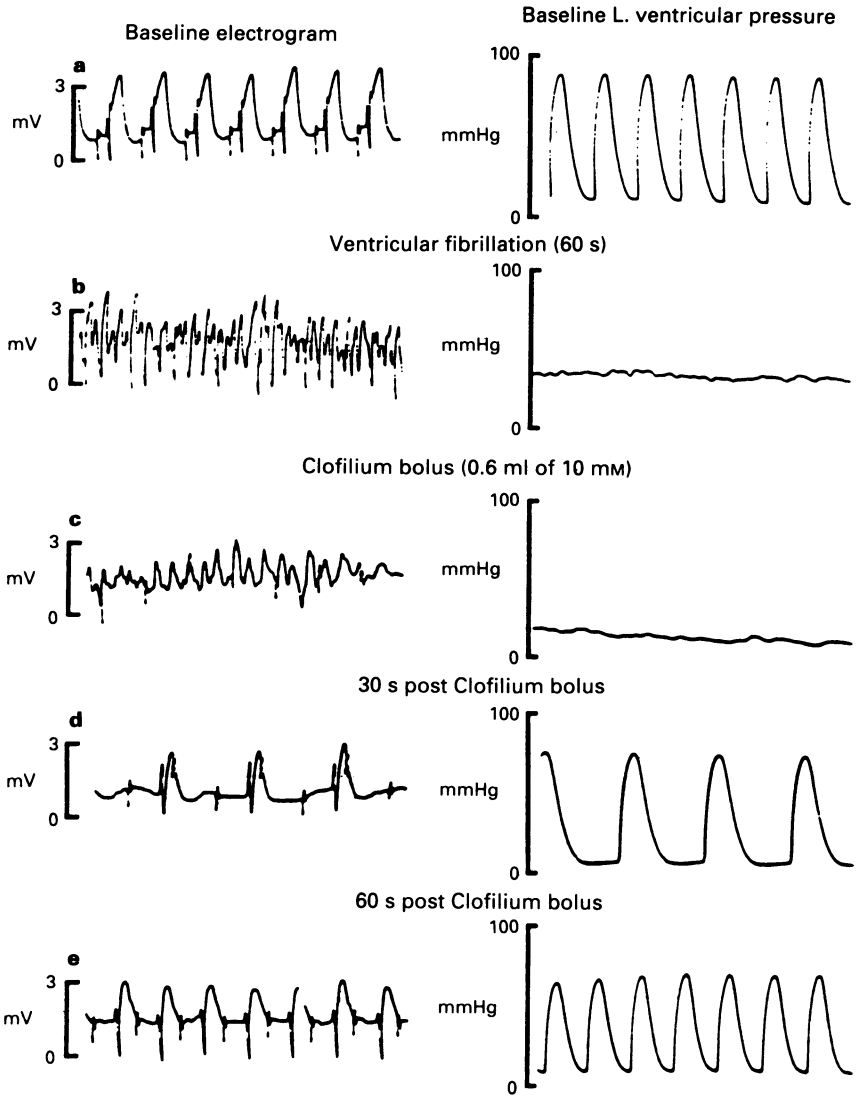

Figure 4 (a) Electrogram (left), and corresponding left ventricular pressure (right); under baseline conditions. (b) Electrogram after 60 s of sustained ventricular fibrillation (L) and left ventricular function (R). (c) Electrogram after induction of clofilium bolus (0.6 ml of 10 mM) to the heart (L) and left ventricular function (R). (d) Electrogram 30 s post administration of clofilium (L) and left ventricular function (R). (e) Electrogram 60 s post administration of clofilium (L) and left ventricular function (R).

10 mM clofilium. The administration of a non-drug containing volume of perfusion buffer was used as a vehicle control. Introduction of the placebo buffer never resulted in the conversion of ventricular fibrillation to sinus rhythm.

Discussion and conclusions

The inefficacy of many Class I agents for the prevention of sudden cardiac death has prompted a renewed effort in the development of antiarrhythmic drugs. Class III antiarrhythmic therapy targeting lethal arrhythmias is receiving much attention. Several Class III agents in various stages of development have been reported to be efficacious in animal models characterized by the development of ventricular fibrillation (Lynch *et al.*, 1984; Chi *et al.*, 1990a; Black *et al.*, 1991). It is novel however to find an agent that possesses not only an antiarrhythmic/antifibrillatory capacity, but a defibrillatory capacity. Reports in the past have ascribed this effect to bretylium (Bacaner, 1968; Sanna & Arcidiacono, 1973), but the effects of bretylium on the sympathetic nervous system, i.e., release of catecholamines from sympathetic nerve terminals (Boura & Green, 1959) and side effects of hypotension contributed to its lack of widespread use. Clofilium, another quaternary antiarrhythmic agent has been shown to prolong the effective refractory period or action potential duration in animal models (Steinberg & Molloy, 1975; Steinberg *et al.*, 1981; Kowey *et al.*, 1985; Wu *et al.*, 1989; Li *et al.*, 1990) as well as increase the refractory period in man without affecting conduction time or haemodynamics (Greene *et al.*, 1983). The role of clofilium as an antifibrillatory agent has been shown to be beneficial by lowering defibrillation requirements (Kopia *et al.*, 1985; Dorian *et al.*, 1991). To date, there has not been a report that systematically describes the chemical defibrillatory ability of clofilium. We have evidence that clofilium, which up to now has been considered to be a I_{to}/I_K (Arena & Kass, 1988; Castle, 1991) channel blocker is not only efficacious as an antifibrillatory agent, but as a defibrillatory agent as well.

While it is generally understood that ATP-dependent potassium channels (glibenclamide-sensitive channels) are closed under normal conditions (Noma, 1983), blockade of channels that are closed would be expected to produce few measurable effects. Under normal conditions, the rabbit isolated heart treated with graded concentrations of clofilium responds by a progressive increase in the ventricular effective refractory period (Table 2). We observed slight increases in the duration of the monophasic action potential after

clofilium administration (Table 3). If the mechanism of action of clofilium were K_{ATP} channel blockade, we would expect to find very little change in either measure. This was not the case, which implies that clofilium does not block the ATP dependent K^+ channel under normal conditions during which the intracellular ATP concentration is not perturbed. Blockade of delayed rectifier current by clofilium in ischaemic Purkinje fibres has been reported by several investigators (Gough *et al.*, 1988; Reeve, 1992). In the present study, clofilium produced a dose-dependent increase in the effective refractory period, whereas changes in the monophasic action potential duration (90% repolarization) failed to reach statistical significance after 10 min of exposure. This is not surprising since it has been reported that clofilium causes substantial prolongation of action potential duration in the Purkinje fibre, but the duration was unchanged in the ventricular muscle cell (Carlsson *et al.*, 1992).

Addition of pinacidil before induction of hypoxia unmasked effects of clofilium not associated with blockade of channels associated with the transient outward (I_{to}) or delayed rectifier currents. In the presence of lower concentrations of clofilium (0.1 and 1.0 μM), pinacidil exposure produced a reduction in the monophasic action potential duration. The highest dose of clofilium (10.0 μM), however, prevented the pinacidil-induced reduction in the monophasic action potential duration. Therefore the functional data implicate clofilium in the blockade of ATP-dependent potassium channels in the presence of the K_{ATP} channel opener, pinacidil (Arena & Kass, 1989; Martin & Chinn, 1990). In support of our functional data is a recent report in which clofilium was capable of inhibiting glibenclamide-sensitive K^+ channels in voltage-clamped *Xenopus* oocytes (Sakuta *et al.*, 1993). However, we cannot exclude the possibility of a functional antagonism by clofilium. The action potential duration shortening by pinacidil may be counteracted by blockade by clofilium of transient outward and/or delayed rectifier currents, the net result being little change in the duration of the monophasic action potential.

Membrane channel activity becomes more complex as the biochemical cascade of events associated with hypoxia ensues. In our experimental model, control hearts subjected to hypoxia in the absence of pinacidil had a 20% incidence of ventricular fibrillation as compared to the significant increase when pinacidil was present in the perfusion medium. The results indicate that opening of the K_{ATP} channel by pinacidil, together with a previous hypoxic insult and decrease in intracellular ATP content, is pivotal in the promotion of ventricular fibrillation (Chi *et al.*, 1993). Clofilium (10.0 μM) consistently prevented the development of ventricular fibrillation under the same experimental conditions that otherwise proved to be arrhythmogenic. Moreover, administration of two additional agents possessing known pharmacological activity on myocardial potassium channels add support to our findings. UK-68,798 which has been shown to block selectively the delayed rectifier current (Gwilt *et al.*, 1989; Rasmussen *et al.*, 1992; Carmeliet, 1992) was not effective in reducing the incidence of ventricular fibrillation in response to pinacidil plus hypoxia. In contrast, the administration of 5-hydroxydecanoate, a well characterized inhibitor of the ATP-dependent K^+ channel (Niho *et al.*, 1987; Notsu *et al.*, 1992; Notsuto *et al.*, 1992) was equally as effective as clofilium in preventing the development of ventricular fibrillation.

It is reasonable to believe that clofilium is able to block not only the transient outward and/or the delayed rectifier potassium currents, but the metabolically active ATP-dependent potassium channel under conditions of decreased intracellular ATP that would promote its opening. In the present experimental model, pinacidil administration in the presence of a reduced intracellular ATP content, would be expected to facilitate opening of the ATP-dependent potassium channel. The increase in the outward potassium current *via* the ATP-dependent potassium channel most likely accounts for the initiation of ventricular fibrillation; an event that can be prevented by glibenclamide (Chi *et al.*, 1993), and 5-hydroxydecanoate. It follows that clofilium may owe *part* or *all* of its antifibrillatory action to the prevention of action potential shortening *via* blockade of ATP-dependent K^+ channels. The prevention of ventricular fibrillation in the present experimental model may be dependent upon a very specific electrophysiological derangement initiated by opening of the ATP-dependent potassium of glibenclamide-sensitive channel. This specific action of clofilium may not occur under conditions in which the initiation of ventricular fibrillation is not associated with opening of the ATP-dependent potassium channel. Hypoxia-induced decrease in myocardial ATP content increases the responsiveness of the ATP-dependent K^+ channel to the opening effects of pinacidil. The experimental model, therefore, utilizes the vulnerability of the heart under a specific set of conditions to explore the role of the ATP-dependent K^+ channel in the genesis of ventricular fibrillation. Using this approach, it becomes possible to identify those pharmacological interventions capable of preventing ventricular fibrillation arising from opening of the ATP-dependent potassium channel. Clofilium, in addition to its other potential effects on myocardial membrane potassium channels, appears to have a salutary effect similar to that of glibenclamide and 5-hydroxydecanoate, and radically different from that of dofetilide (UK68,798) in terms of preventing ventricular fibrillation. These observations support the concept that clofilium has the potential to modulate the ATP-dependent potassium channel and to prevent those electrophysiological changes leading to ventricular fibrillation associated with decreases in myocardial tissue ATP content.

It is noteworthy that clofilium is capable of reverting electrically induced sustained ventricular fibrillation to sinus rhythm. This group of hearts was an addendum to our primary study after we had noted that 4/9 hearts in the 10.0 μM clofilium group reverted to sinus rhythm after transient episodes of spontaneous ventricular flutter during the 40 min reperfusion period, a phenomenon that has not been observed in vehicle-treated rabbit hearts. A bolus of clofilium (0.35 ml of 10 mM) slowed the rate of fibrillation, until the heart resumed normal sino-atrioventricular conduction within 14 ± 2 s. This event was observed in 5 isolated hearts. A larger quantity of administered clofilium ($n = 5$, 0.6 ml of 10 mM) to the fibrillating heart, not only slowed the frequency of fibrillatory waves, but elicited sinoatrial arrest. Since coronary perfusion was maintained constant in our preparation, and oxygen delivery uncompromised, the heart was capable of resuming normal sinus rhythm in 42 ± 16 s.

The ability of clofilium to antagonize the coronary vasodilator action of pinacidil should not be overlooked. Before induction of hypoxia, pinacidil alone produced vasodilatation and a negative inotropic effect on the rabbit isolated perfused heart. The first derivative of left ventricular pressure, $+dP/dt$ is reduced approximately 10% within 5 min after pinacidil was added to the perfusion medium. The presence of clofilium in the perfusate attenuated this effect (see Table 1). It is well known that pinacidil is capable of specific K_{ATP} channel activation (Arena & Kass, 1989), while others report a profibrillatory effect of pinacidil (Chi *et al.*, 1990a, 1993). This evidence suggests that pinacidil will promote ventricular fibrillation given the proper substrate or altered physiological conditions. Clofilium prevents these responses, thereby leading us to suggest that clofilium (10.0 μM) may, in part, block the metabolically activated ATP-dependent potassium channel. This conclusion is supported by the observation that UK-68,798 (specific delayed rectifier current antagonist) was not effective, while 5-hydroxydecanoate (specific ATP-dependent K^+ current antagonist) was effective in attenuating the development of ventricular fibrillation. It may be that clofilium has been overlooked as a potentially efficacious antifibrillatory agent.

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