ELSEVIER

European Journal of Pharmacology 306 (1996) 99–106

ejp

5-Hydroxydecanoate fails to attenuate ventricular fibrillation in a conscious canine model of sudden cardiac death

Gregory S. Friedrichs, Jose-Nelson Abreu, Shawn C. Black, Liguo Chi, Benedict R. Lucchesi *

The University of Michigan Medical School, Department of Pharmacology, Ann Arbor, MI 48109-0632, USA

Received 13 November 1995; revised 26 January 1996; accepted 8 March 1996

Abstract

The electrophysiologic and antifibrillatory properties of 5-hydroxydecanoate, a K_{ATP} channel antagonist, were studied in a conscious canine model of sudden cardiac death. After a surgically induced myocardial infarction, animals were subjected to programmed electrical stimulation to identify those at risk for sudden cardiac death. 5-Hydroxydecanoate was administered as a bolus (10 mg/kg i.v.) followed by an infusion, 10 mg/kg/h (group 1, $n = 12$) or 30 mg/kg bolus followed by an infusion, 30 mg/kg/h (group 2, $n = 8$) i.v., while vehicle treated animals received a 0.9% sodium chloride solution (group 3, $n = 11$). The administration of 5-hydroxydecanoate did not alter the ventricular effective refractory period or the QTc interval. Anterior wall myocardial infarcts, expressed as a percentage of the left ventricle, did not differ among groups. Infusions of 5-hydroxydecanoate did not confer significant protection from sudden cardiac death (death within 60 min of posterolateral ischemia) due to ventricular fibrillation: group 1, 50%; group 2, 38%; and group 3, 18%. The data demonstrate that a continuous infusion of 5-hydroxydecanoate (10 and 30 mg/kg/h, i.v.) does not provide protection from ischemia-induced ventricular fibrillation in the postinfarcted conscious canine.

Keywords: ATP-dependent K^+ channel blocker; Cardiac arrhythmia; Ventricular fibrillation

1. Introduction

The ATP-dependent K^+ channel is known to remain closed under normal physiologic conditions. In the presence of hypoxia, pH decreases, tissue ATP content is reduced, and the ATP-dependent K^+ channel opens (Noma, 1983). The ATP-dependent K^+ channel becomes functional under pathophysiological conditions accompanied by a decrease in tissue ATP content. If opening the ATP-dependent K^+ channel is responsible for deleterious alterations in cardiac electrophysiology leading to arrhythmogenesis, it may be a potential site for pharmacological modulation for the prevention of ventricular tachyarrhythmias associated with myocardial ischemia. Glyburide, a specific antagonist of the ATP-dependent K^+ channel, has been used in several experimental models to attenuate ventricular arrhythmias and reduce the incidence of ventricular fibrillation (Pogatsa et al., 1988; Billman et al., 1993; Wollenben et al., 1989). The naturally occurring fatty acid, 5-hydroxydecanoate, found in milk (Wyatt et al., 1967), has been used as a pharmacological tool to explore the ATP-dependent K^+ channel. It has been reported that 5-hydroxydecanoate inhibits ATP-sensitive K^+ channel currents in single ventricular myocytes (Notsu et al., 1992a,b). Others (McCullough et al., 1991) have reported the effectiveness of the K_{ATP} channel antagonist, 5-hydroxydecanoate, in abolishing the anti-ischemic effects of K_{ATP} channel agonists. Auchampach et al. (1992) demonstrated that 5-hydroxydecanoate antagonized infarct size reduction of ischemic preconditioning in anesthetized dogs; an event attributed to the inhibition of the ATP-dependent K^+ channel in ventricular myocardium.

The efficacy of 5-hydroxydecanoate as it relates to the prevention of ventricular rhythm disturbances or ventricular fibrillation has not been given thorough attention. However, Niho et al. (1987) reported efficacy of 5-hydroxydecanoate in elevating ventricular fibrillation threshold after coronary ligation in the canine heart as well as suppressing the incidence of ventricular fibrillation in a model of coronary artery ligation of the rat heart. The authors

* Corresponding author. The University of Michigan Medical School, 1301 C Medical Science Research Building III, 1150 West Medical Center Drive, Box 0632, Ann Arbor, MI 48109-0632, USA. Tel.: 313-747-3134; fax: 313-747-4782.

PII S0014-2999(96)00208-7

attributed the antiarrhythmic action of 5-hydroxydecanoate, at least in part, to the suppression of K^+ release from ischemic myocardium by possibly inhibiting the ATP regulated K^+ channel.

The purpose of this investigation was to: (1) determine if 5-hydroxydecanoate, a specific K_{ATP} channel antagonist, could reduce the incidence of ventricular tachyarrhythmias in response to programmed electrical stimulation in the postinfarcted canine heart, and (2) prevent the development of ventricular fibrillation precipitated by a transient, remote, ischemic event superimposed on a previously infarcted myocardium, in the conscious canine.

2. Materials and methods

2.1. Guidelines for research involving the use of animals

The procedures followed in this study were in accordance with the guidelines of the University of Michigan University 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, and the animal care and use program conforms to the standards in 'The Guide for the Care and Use of Laboratory Animals', DHEW Publ. No. (NIH) 86-23, Rev. 1985.

2.2. Surgical preparation / instrumentation

Male mongrel dogs ($n = 37$) weighing 15 ± 0.4 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v., supplements to effect) and ventilated with room air with the use of a cuffed endotracheal tube and a Harvard respirator (Harvard Apparatus Co., S. Natick, MA) adjusted to deliver a tidal volume of 30 ml/kg. Using aseptic technique, the left external jugular vein and left common carotid artery were isolated and cannulated for drug administration and for blood pressure monitoring. A left thoracotomy was performed between the fourth and fifth ribs, the pericardium opened, and the heart suspended in a pericardial cradle. The left anterior descending coronary artery was isolated at the tip of the left atrial appendage and the left circumflex coronary artery was isolated approximately 1 cm from its origin. A Teflon-insulated, copper-coated, silver wire electrode (27 gauge) was inserted into the left circumflex coronary artery and remained in contact with the intimal surface of the vessel.

A critical stenosis was applied to the left anterior descending coronary artery by placing an 18–20 gauge hypodermic needle parallel to the vessel and tying a silk suture around the artery and the needle. The needle is subsequently removed, resulting in a narrowing of the arterial lumen. The diameter of the hypodermic needle was approximately 75% of the diameter of the isolated left anterior descending coronary artery. Anterior wall ischemic injury was produced by total occlusion, for 2 h, of the left anterior descending coronary artery, using a snare formed from a loop of silicone rubber tubing pulled through a polyethylene cylinder. Releasing the snare restored coronary blood flow in the presence of a critical stenosis.

A bipolar pacing electrode was sutured to the surface of the left atrium and used to maintain heart rate constant during refractory period determinations. A bipolar electrode was placed in the region of the right ventricular outflow tract and used to deliver ventricular extrastimuli during programmed electrical stimulation. A bipolar plunge electrode (25 gauge stainless steel posts, 5 mm in length, 3 mm electrode separation) was sutured into the interventricular septum immediately to the right of the anterior descending coronary artery and adjacent to the right ventricular outflow tract. Silver disc electrodes were implanted subcutaneously for ECG monitoring, the thoracotomy incision was closed, and the animals were allowed to recover from surgical anesthesia. Postoperative care was maintained under the supervision of the Veterinary Staff and personnel of the Unit for Laboratory Medicine of the University of Michigan in consultation with the Principle Investigator. Antibiotic therapy (Ampicillin, 200 mg s.c.) was maintained for 5 days after completion of the surgical procedure if deemed necessary on the basis of the animals postoperative course of recovery.

2.3. Electrophysiologic studies and programmed ventricular stimulation in the conscious, canine following myocardial infarction

2.3.1. Electrophysiologic studies

Electrophysiologic monitoring and programmed stimulation were performed between days 3 and 5 after anterior myocardial infarction and after the animal had recovered completely from the effects of surgical anesthesia and was considered to be free of postoperative complications (infection, arrhythmias, etc.). Animals were conscious and unsedated during the studies and were kept resting comfortably in a harness (Alice King Chatham, Hawthorne, CA).

Electrocardiographic (ECG) intervals and electrophysiologic parameters were determined immediately before programmed electrical stimulation. ECG PR, and QRS intervals were determined during sinus rhythm, while a paced QT interval was measured during 2.5 Hz atrial pacing. During atrial pacing, an extrastimulus (S_2) was introduced in late diastole (300 ms) at a minimum current to elicit a ventricular response (V_2). At twice excitation threshold, the basic S_1-S_2 coupling interval was decreased incrementally until S_2 failed to elicit a V_2. The right ventricular outflow tract refractory period was defined as the longest R-S_2 interval at which a $2 \times$ right ventricular outflow tract excitation threshold voltage stimulus of 4 ms duration fails to elicit a V_2 response.

2.3.2. Programmed electrical stimulation

Programmed ventricular stimulation consists of the introduction of single (S_2), double (S_2S_3) and triple ($S_2S_3S_4$) premature ventricular stimuli (4 ms duration, 2 × right ventricular outflow tract excitation threshold voltage) into the interventricular septum near the right ventricular outflow tract using a model S88 stimulator with a modified #4175 delay board and SIU5 stimulus isolation unit (Grass Instruments, Quincy, MA). The S_2 extrastimulus was triggered from the R wave of the ECG which served as the input to a Grass Instruments S88 stimulator. Thereafter, double and triple ventricular extrastimuli are introduced during sinus rhythm at S_2-S_3 and S_2-S_3-S_4 coupling intervals ranging from 182 to 125 ms. Previous work has shown that this method does not lead to the induction of ventricular dysrhythmias in sham-operated animals which do not have previous myocardial ischemic injury (Patterson et al., 1982). Ventricular tachycardia is defined as 'nonsustained' if, by using the protocol described above, five or more repetitive ventricular complexes are initiated reproducibly, but terminate spontaneously. Ventricular tachycardia is defined as 'sustained' if it persists at least 30 s or, in the event of hemodynamic compromise, requires burst pacing for termination. The reproducibility of nonsustained and sustained ventricular tachycardia is confirmed by repeated attempts at programmed electrical stimulation, except in those cases where the induced ventricular tachycardia is associated with hemodynamic compromise. Only dogs which respond to programmed ventricular stimulation with either nonsustained or sustained ventricular tachycardia are entered into the study designed to assess the antifibrillatory activity of 5-hydroxydecanoate.

Resuscitative efforts were not attempted on animals that develop ventricular fibrillation as a result of programmed electrical stimulation in order to avoid the confounding influence of repeated and occasionally prolonged resuscitative efforts on the outcome of subsequent investigations. Furthermore, previous work in this laboratory has demonstrated a relationship between the vulnerability to initiate either nonsustained or sustained ventricular tachycardia and the development of ventricular fibrillation in response to ischemia at a site remote from the previous myocardial infarction (Patterson et al., 1982; Wilber et al., 1985). Finally, during programmed ventricular stimulation, a successful drug response is defined as the post-drug failure to provoke either nonsustained or sustained ventricular tachycardia throughout the entire protocol in a previously responsive animal. Arrhythmogenic responses are defined as the post-drug initiation of relatively more rapid or prolonged VTs or ventricular fibrillation at stimulation parameters comparable to pretreatment parameters.

After determination of the baseline electrophysiologic values and reproduction of sustained or nonsustained ventricular tachycardia in duplicate, the test drug was administered and the electrophysiologic testing was repeated. Failure to induce either sustained or nonsustained ventricular tachycardia prior to drug treatment resulted in the animal being classified as non-inducible and not suitable for inclusion in the second phase of the protocol used to assess the antifibrillatory activity of 5-hydroxydecanoate.

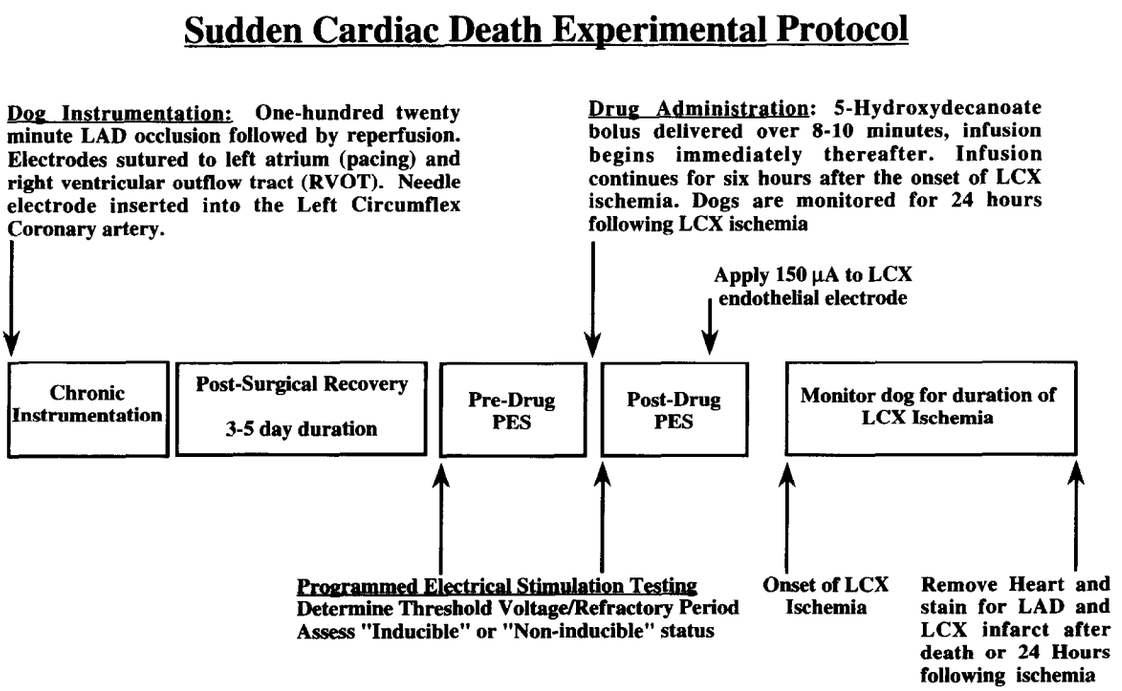

Fig. 1. Experimental protocol used to investigate the antifibrillatory effects of 5-hydroxydecanoate. LAD: left anterior coronary artery; PES: programmed electrical stimulation; LCX: left circumflex coronary artery.

2.4. Experimental protocol and drug dosing regimens

Three groups of dogs were used in this study, each randomly assigned to either a treated group: 10 mg/kg/h (group 1, $n = 12$), 30 mg/kg/h (group 2, $n = 8$), or a vehicle (group 3, $n = 11$) group. All groups underwent identical left anterior descending coronary artery occlusion and programmed electrical stimulation protocols (Fig. 1). The treated group received a single bolus of 10 or 30 mg/kg i.v. followed by a continuous infusion of 10 or 30 mg/kg/h for 6 h after posterolateral ischemia, while the untreated group received vehicle (saline) infusion. 30 min after the infusion of 5 HD or vehicle, programmed electrical stimulation as described above was repeated in all groups of dogs. Upon completion of the second series of programmed electrical stimulation, left circumflex coronary artery wire stimulation and posterolateral ischemia was allowed to develop. Left circumflex coronary artery wire stimulation refers only to the introduction of current to the endothelium of the left circumflex coronary artery. Posterolateral ischemia will result following left circumflex coronary artery wire stimulation AND subsequent thrombus formation in that vessel. One additional untreated group of dogs ($n = 6$) was added to the study which possessed small anterior infarcts (i.e., non-inducible). This group of dogs was also subjected to left circumflex coronary artery wire stimulation and subsequent posterolateral ischemia.

2.5. Ischemia at a site remote from previous myocardial infarction for the induction of ventricular fibrillation

Posterolateral ischemia was initiated in the region of distribution of the left circumflex coronary artery by inducing an anodal direct current of 150 μA to the intimal surface of the vessel using the previously implanted intraluminal electrode. This method has been shown repeatedly by our laboratory (Patterson et al., 1982; Chi et al., 1991; Lynch et al., 1985; Black et al., 1991, 1993a) to induce intimal injury, promoting oscillatory disturbances in coronary artery blood flow and subsequent thrombus formation in the left circumflex coronary artery. In the absence of an appropriate intervention, the procedure results in electrophysiologic disturbances leading to ventricular fibrillation in animals possessing an anterior wall myocardial infarction. Thus, the superimposition of an acute ischemic event in a region remote from a previous myocardial infarction is accompanied by sudden cardiac death manifest as ventricular fibrillation. Sudden cardiac death is defined as ventricular fibrillation occurring within 1 h from the onset of regional ischemia in the distribution of the left circumflex coronary artery as determined from changes in the ST segment (depression and/or elevation) recorded from the Lead II ECG.

Upon the death of the animal, the heart is removed and the thrombus formed in the left circumflex coronary artery is extracted and weighed. The heart is sectioned and the transverse sections are placed in 0.4% triphenyl tetrazolium chloride for 10–15 min maintained at 37°C. Exposure of the cut surface of the ventricular myocardium allows for enzymatic reduction of triphenyl tetrazolium chloride leading to the formation of a brick red formazan precipitate in regions where myocardial tissue remains viable. Infarcted regions of myocardium are unable to reduce triphenyl tetrazolium chloride enzymatically to form the brick red formazan precipitate and appear pale-yellow. The regions demarcated by the triphenyl tetrazolium chloride reaction and the non-reactive regions were traced on acetate sheets. The traced diagrams were digitized using a flatbed scanner, Macintosh computer (Apple, Cupertino, CA) and appropriate software (MacDraft, Innovative Data Design, Concord, CA).

2.6. Verification of K_{ATP} channel antagonism during ischemia

A separate group of anesthetized dogs ($n = 4$) was instrumented with electrodes placed on the atria for pacing and another placed in a region of myocardium supplied by the left anterior descending coronary artery. The latter electrode was used to monitor the monophasic action potential. After a steady state had been established, each dog was subjected to a 2 min period of left anterior descending coronary artery occlusion. Recordings of Lead II ECG and monophasic action potential changes were monitored at 15 s intervals. The ischemic myocardium was reperfused after 2 min of left anterior descending coronary artery occlusion. 30 min was allowed to elapse before the bolus plus infusion of 5-hydroxydecanoate (10 or 30 mg/kg/h) was initiated. 60 min after beginning the 5-hydroxydecanoate infusion, the 2 min interval of ischemia was repeated and the monitored variables were recorded at 15 s intervals. Pilot experiments utilizing repeated episodes of ischemia that were 2 min in duration confirmed that the recovery of the monitored variables is complete if a minimum of 30 min is allowed between ischemic insults. This allowed for each dog to serve as its own control in this phase of the investigation.

2.7. Statistical analysis

The data are expressed as mean $\pm$ S.E.M. The difference between treatment groups for the incidence of sudden cardiac death were analyzed by Fisher's Exact test. Student's t test for paired replicates was performed within groups for electrophysiologic and hemodynamic results before and after drug treatment. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Study groups

A total of 31 postinfarcted dogs met the criteria for inclusion in the sudden cardiac death protocol. The animals were allocated to three separate study groups. Group 1 animals ($n = 12$) received 5-hydroxydecanoate, 10 mg/kg bolus followed by 10 mg/kg/h as a continuous intravenous infusion, while animals assigned to group 2 ($n = 8$) received a 5-hydroxydecanoate as a bolus dose of 30 mg/kg followed by 30 mg/kg/h as a continuous intravenous infusion. Animals in group 3 ($n = 11$) were administered a continuous intravenous infusion of 0.9% sodium chloride solution for injection.

3.2. Pretreatment with 5-hydroxydecanoate

The recorded cardiac electrophysiologic effects at baseline and after the administration of 5-hydroxydecanoate are summarized in Table 1. The recorded parameters included: heart rate (HR), mean arterial blood pressure (BP), atrioventricular conduction as determined by the P-R interval, ventricular conduction as assessed from the duration of the QRS complex, ventricular refractoriness as calculated from changes in the paced QTc interval and the effective refractory period determined by the extrastimulus method. Induced ventricular tachycardia cycle length was also determined before and after drug treatment. There were no changes in any of the measured variables.

Dogs that were at risk for sudden cardiac death, (i.e. inducible) determined via programmed electrical stimulation testing before 5-hydroxydecanoate treatment, were not affected by administration of the drug at either dose. Twelve animals were inducible before administration of 5-hydroxydecanoate. After administration of 5-hydroxydecanoate, 11 of 12 animals in group 1 remained inducible. Of the eight inducible animals belonging to group 2, five remained inducible after 5-hydroxydecanoate administration (Table 2).

Table 1
Hemodynamic and electrophysiological parameters (post-infarcted conscious canine) in the presence and absence of 5-hydroxydecanoate

Treatment	Vehicle ($n = 11$)	10 mg/kg/h ($n = 12$)	30 mg/kg/h ($n = 8$)
HR (baseline)	131 ± 5	124 ± 6	135 ± 9
HR (post-drug)	132 ± 5	123 ± 5	133 ± 11
BP (baseline)	104 ± 5	93 ± 5	100 ± 4
BP (post-drug)	105 ± 5	90 ± 4	96 ± 9
P-R (baseline)	93 ± 3	101 ± 4	104 ± 4
P-R (post-drug)	95 ± 4	101 ± 6	104 ± 6
QRS (baseline)	41 ± 2	42 ± 2	41 ± 1
QRS (post-drug)	41 ± 1	39 ± 1	41 ± 1
QTc (baseline)	298 ± 9	294 ± 7	272 ± 10
QTc (post-drug)	302 ± 10	294 ± 7	271 ± 7
PQT (baseline)	199 ± 8	198 ± 6	181 ± 6
PQT (post-drug)	203 ± 9	200 ± 7	183 ± 6
ERP (baseline)	123 ± 6	126 ± 4	124 ± 8
ERP (post-drug)	120 ± 5	121 ± 3	122 ± 8
VTCL (baseline)	176 ± 27	176 ± 25	206 ± 61
VTCL (post-drug)	192 ± 23	184 ± 25	159 ± 11

Data are mean ± S.E.M. * $P < 0.05$ relative to predrug value. HR: heart rate (b/min); BP: mean arterial blood pressure (mmHg); P-R: P-R interval (ms); QRS: QRS interval (ms); QTc: corrected QT interval; PQT: paced QT interval (ms); ERP: effective refractory period (ms): VTCL: ventricular tachycardia cycle length (ms).

Table 2
Incidence of programmed electrical stimulation-induced ventricular tachycardia in the presence and absence of 5-hydroxydecanoate

Treatment	Pre-treatment (% of inducible animals)	Post-treatment (% of inducible animals)
Vehicle ($n = 11$)	100	91
5-Hydroxydecanoate 10 mg/kg/h ($n = 12$)	100	92
5-Hydroxydecanoate 30 mg/kg/h ($n = 8$)	100	63

Data are mean ± S.E.M. * $P < 0.05$ relative to predrug value.

Anterior infarct size was determined in each animal. There were no differences in infarct size between groups, 10 mg/kg/h, 23 ± 3%; 30 mg/kg/h, 27 ± 3%; and vehicle, 28 ± 3% of the left ventricle.

The results obtained for the sudden death portion of the protocol are shown in Fig. 2 (24 h). The percent of animals surviving sudden death in group 3 was 18%, those belonging to groups 1 and 2 had a 50% and 38% incidence of survival, respectively. The percent survival of those identified as at risk for sudden cardiac death in the presence of 5-hydroxydecanoate, was not found to be increased significantly from vehicle treated animals ($P = 0.18$, Fishers Exact test). Since 50% mortality was observed in group 1 dogs, it was determined that surviving animals of group 1 possessed significantly smaller infarcts compared to non-survivors. Survivors possessed infarcts representing 15 ± 1% of the left ventricle compared to infarcts of non-survivors, 32 ± 3% ($P < 0.05$ vs. survivors).

3.3. Non-inducible dogs and posterolateral ischemia

Dogs possessing small anterior infarcts have a reduced incidence of sudden cardiac death in response ischemia in a region remote from the infarct related vessel. Six additional dogs were classified as non-inducible after complete programmed electrical stimulation testing and were subjected to subsequent posterolateral ischemia in the absence of any drug treatment. Of the six dogs entered into the protocol, five survived (83% survival) the first hour of posterolateral ischemia and four of the latter animals were observed for the next 24 h. Anterior and posterolateral myocardial infarct size was 13 ± 2% and 26 ± 5 of the left ventricle, respectively. The size of the anterior wall infarct in this group of animals was not different from the anterior

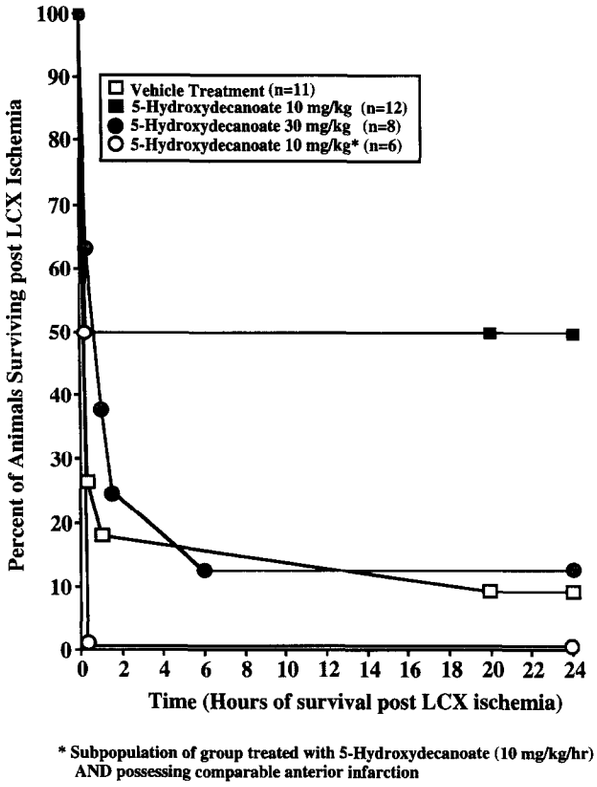

Fig. 2. Survival curve for 5-hydroxydecanoate (10 and 30 mg/kg) and vehicle-treated animals after the onset of ischemia in the posterolaternal myocardium remote from the infarct related artery. Data shown are 24 h survival after the onset of posterolateral ischemia. A subpopulation is included depicting only those animals treated with 10 mg/kg *and* possessing anterior wall infarcts comparable to all other groups (* $P <$ 0.05 vs. vehicle treatment).

wall infarct in a subpopulation of survivors in the 5-hydroxydecanoate (10 mg/kg/h) treated group.

3.4. Verification of K_{ATP} channel antagonism during ischemia in the anesthetized dog

To ensure that 5-hydroxydecanaote was capable of action potential modulation during ischemia, four dogs were subjected to brief periods of myocardial ischemia before and after intravenous infusions of 5-hydroxydecanoate (10 or 30 mg/kg/h). Action potential duration was reduced significantly after 90 s of ischemia, a reduction that continued throughout the ischemic period (Table 3). Intravenous administration of 5-hydroxydecanoate (10 mg/kg bolus + 10 mg/kg/h) 60 min before induction of regional myocardial ischemia resulted in the attenuation of action potential duration shortening. A similar trend was observed at a dose of 30 mg/kg bolus + 30 mg/kg/h infusion. Ischemia in the absence of 5-hydroxydecanoate resulted in a maximal monophasic action potential shortening of 28 ± 5% from baseline values, compared to 5-hydroxydecanoate treatment (30 mg/kg/h) which resulted in a 14 ± 3% reduction in monophasic action potential duration compared to pre-ischemic values.

3.5. Adverse drug reactions

Administration of 5-hydroxydecanoate was tolerated by all animals in the study. The intravenous infusion of 5-hydroxydecanoate was not associated with obvious signs related to the gastrointestinal, neuromuscular, central nervous, or cardiovascular systems. Conscious animals receiving 5-hydroxydecanoate rested quietly throughout the procedure. During the infusion of 5-hydroxydecanoate there was no evidence of proarrhythmic activity in the postinfarcted hearts.

4. Discussion

Inhibition of the delayed rectifier K^+ current (I_K) has been the desired target of new antiarrhythmic agents in an attempt to prolong the duration of the cardiac action potential, without altering ventricular conduction velocity (Escande and Henry, 1993). Prolonging the action potential duration via putative Class III antiarrhythmics is effective in attenuating reentrant ventricular arrhythmias associated with sudden cardiac death in experimental animal models (Chi et al., 1991; Black et al., 1991, 1993a; Friedrichs et al., 1995). Class I antiarrhythmic agents have been viewed with guarded enthusiasm since they possess

Table 3
Action potential duration during myocardial ischemia in the absence and presence of 5-hydroxydecanoate

Treatment	Baseline	Ischemia			
		30 s	60 s	90 s	120 s
Vehicle ($n = 4$)	173 ± 6	178 ± 7	159 ± 8	149 ± 7 **	130 ± 9 **
5-Hydroxydecanoate 10 mg/kg/h ($n = 4$)	168 ± 4	175 ± 2	170 ± 5	159 ± 7	154 ± 6 *
Vehicle ($n = 4$)	169 ± 10	160 ± 9	153 ± 9 **	131 ± 8 **	121 ± 8 **
5-Hydroxydecanoate 30 mg/kg/h ($n = 4$)	173 ± 5	171 ± 11	169 ± 12	155 ± 11 *	148 ± 9

Data are mean ± S.E.M. * $P < 0.05$ relative to vehicle treated value; ** $P < 0.05$ relative to respective baseline value. Action potential duration was determined at 90% repolarization. All values are expressed in milliseconds.

characteristics which include negative inotropy and potential proarrhythmic effects (Woosley, 1991).

The therapeutic potential of modulating ATP-dependent K^+ channels to manage ventricular arrhythmias has received limited attention. Billman et al. (1993) have described significant reductions in the occurrence of ventricular fibrillation in conscious dogs subjected to exercise plus ischemia after treatment with glyburide. It has been suggested that other commonly used drugs (quinidine, verapamil, and amiodarone) may inhibit the ATP-sensitive potassium channel and produce their effects via K_{ATP} channel antagonism (Haworth et al., 1989). Notsu and co-workers (Notsu et al., 1992a,b) showed that 5-hydroxydecanoate inhibits ATP-sensitive K^+ channel currents in single ventricular myocytes. McCullough et al. (1991) have reported the effectiveness of the K_{ATP} channel antagonist, 5-hydroxydecanoate, in abolishing the anti-ischemic effects of K_{ATP} channel agonists. 5-hydroxydecanoate suppression of regional ischemia-induced shortening of monophasic action potential was also demonstrated in an anesthetized dog model (Moritani et al., 1994). The efficacy of 5-hydroxydecanoate as it relates to the prevention of ventricular rhythm disturbances or ventricular fibrillation has not been given thorough attention. In a limited study, an antiarrhythmic effect of 5-hydroxydecanoate in rabbit isolated hearts subjected to 30 min of global ischemia was not observed (Wilde et al., 1994). However, 5-hydroxydecanoate has been reported to increase the ventricular fibrillation threshold after coronary ligation in the canine heart as well as reducing the incidence of ventricular fibrillation in a model of coronary artery ligation of the rat heart (Niho et al., 1987). The reported antiarrhythmic effects were considered to be related to the inhibition of the ATP-dependent K^+ channel by 5-hydroxydecanoate. There have been no other reports describing efficacy of 5-hydroxydecanoate on ventricular arrhythmias, though it has been suggested by several investigators that 5-hydroxydecanoate may have the potential to suppress ventricular arrhythmias (Notsu et al., 1992a; Moritani et al., 1994). It was our goal, therefore, to characterize the ATP-dependent K^+ channel antagonist, 5-hydroxydecanoate (a component of hydroxy fatty acids found in milk (Wyatt et al., 1967)), during programmed electrical stimulation as well as its potential to prevent ventricular fibrillation in a conscious canine model of sudden cardiac death.

The susceptibility of programmed electrical stimulation-induced ventricular tachycardia was not reduced by 5-hydroxydecanoate administered as a continuous infusion. Furthermore, 5-HD did not reduce the incidence of sudden cardiac death when administered in doses of 10 or 30 mg/kg/h. The 50% incidence of survival obtained in the presence of 5-hydroxydecanoate at a dose of 10 mg/kg/h was not significantly different from that of vehicle treated animals. Careful examination of the data revealed a significant difference in anterior wall infarct size between survivors and non-survivors of the treated group. Infarct size is an important determinant in the characterization of animals determined to be at risk for lethal ventricular arrhythmias, i.e., ventricular fibrillation (Wilber et al., 1985; Legato, 1993; Black et al., 1993b). We reasoned therefore, that the ability of post-infarcted dogs treated with 10 mg/kg/h 5-hydroxydecanoate to survive posterolateral myocardial ischemia may have been related to the small anterior wall infarcts in a subset of the total population and not due to drug treatment since administration of 30 mg/kg/h did not afford any protection against sudden cardiac death. Our conclusion is supported by results obtained in a separate group of dogs ($n = 6$) with small anterior wall infarcts that were subjected to myocardial ischemia in a region remote from the infarct related artery. One dog succumbed to ventricular fibrillation within 1 h of posterolateral ischemia. A second animal survived 8 h, and the remaining four dogs survived 24 h or more despite the presence of posterolateral ischemia which progressed to myocardial infarction. The mean anterior wall infarct size in the six animals was not different from the infarct size in the group of dogs treated with 5-hydroxydecanoate (10 mg/kg/h). Therefore, the observed increase in the number of surviving animals in the group treated with 10 mg/kg/h 5-hydroxydecanoate can be explained by the presence of a small anterior wall infarct size and not due to the presence of the drug. Dogs treated with 30 mg/kg/h, possessed an anterior wall infarct which was comparable to that of the vehicle treated animals. In the latter instance, there was no significant difference in the observed incidence of ventricular fibrillation between the controls and those animals treated with 5-hydroxydecanoate.

The doses chosen for this study were arrived upon following an extensive literature search. Niho et al. (1987) reports the incidence of VF induced by coronary ligation in rats was reduced following a single dose (200 mg/kg), and elevating the ischemically decreased VF threshold in coronary ligated dogs (3 or 10 mg/kg). Other investigators (Moritani et al., 1994), used 30 mg/kg in the dog to attenuate ischemia-induced reductions in the monophasic action potential. The cited study employed a single i.v. bolus of 5-hydroxydecanoate. The electrophysiologic effect of 5-hydroxydecanoate dissipated as a result of its short biological half life. Our own investigation utilized a protocol similar to Moritani et al. (1994), demonstrating the strongest evidence that a continuous infusion of 5-hydroxydecanoate (10 or 30 mg/kg/h) was capable of attenuating the shortening of action potential duration in the ischemic myocardium. Thus, the doses employed in the sudden death portion of the investigation were sufficient to antagonize myocardial K_{ATP} channels.

As mentioned earlier, the K_{ATP} channel remains closed under resting conditions and therefore an infusion of 5-hydroxydecanoate would not be expected to produce observable changes in monitored parameters. The onset of posterolateral ischemia in the post-infarcted heart is rapid, and most often results in sudden death. If the K_{ATP} channel

opens during this period of ischemia and is important in the development or maintenance of ventricular fibrillation, we can conclude from our findings: (1) 5-hydroxydecanoate is not an effective antagonist of the K_{ATP} channel in our model, (2) the K_{ATP} channel is not important in the development of ventricular fibrillation, or (3) the doses of the drug used in our investigation could antagonize myocardial K_{ATP} channels but were not sufficient to attenuate the development of ventricular fibrillation. Our results demonstrate that 5-hydroxydecanoate was not effective in this model of sudden cardiac death and contend that 5-hydroxydecanoate, while a specific K_{ATP} channel antagonist, does not possess the capacity to attenuate ventricular arrhythmias in a setting of ischemia superimposed on a vulnerable substrate.

While the rationale of modulating the ATP-dependent K^+ channel in the treatment of lethal cardiac arrhythmias is reasonable, we have not found that specific treatment with 5-hydroxydecanoate (10 and 30 mg/kg/h) is effective in preventing ventricular fibrillation in the post-infarcted conscious canine. It remains to be determined whether or not increased doses of 5-hydroxydecanoate may be useful in preventing lethal ventricular dysrhythmias, or if exclusive modulation of the ATP-dependent K^+ channel is ineffective in this model.

Acknowledgements

This study was supported by a grant from the National Institutes of Health, Heart, Lung and Blood Institute, #HL05806-35; and an educational grant from Warner-Lambert/Parke-Davis Pharmaceutical Research Division, Ann Arbor, Michigan. During the course of the study, GSF was supported by a Merck Postdoctoral Fellowship in Pharmacology; SCB (now with Merck Frosst Canada, Quebec) was a Fellow of the Heart and Stroke Foundation of Canada.

References

Auchampach, J.A., G.J. Grover and G.J. Gross, 1992, Blockade of ischemic preconditioning in dogs by the novel ATP-dependent potassium channel antagonist sodium 5-hydroxydecanoate, Cardiovasc. Res. 26, 1054.

Black, S.C., L. Chi, D.-X. Mu and B.R. Lucchesi, 1991, The antifibrillatory actions of UK-68,798, a class III antiarrhythmic agent, J. Pharmacol. Exp. Ther. 258, 416.

Black, S.C., J.L. Butterfield and B.R. Lucchesi, 1993, Protection against programmed electrical stimulation-induced ventricular tachycardia and sudden cardiac death by NE-10064, a class III antiarrhythmic drug, J. Cardiovasc. Pharmacol. 22, 810.

Black, S.C., G.S. Friedrichs and B.R. Lucchesi, 1993, The autonomic nervous system, myocardial infarct size and ventricular fibrillation. J. Mol. Cell. Cardiol. 25, 1291.

Billman, G.E., C.E. Avendana, J.R. Halliwill and J.M. Burroughs, 1993, The effect of the ATP-dependent potassium channel antagonist, glyburide, on coronary blood flow and susceptibility to ventricular fibrillation in unanesthetized dogs, J. Cardiovasc. Pharmacol. 21, 197.

Chi, L., D.-X. Mu and B.R. Lucchesi, 1991, Electrophysiology and antiarrhythmic actions of E-4031 in the experimental animal model of sudden coronary death, J. Cardiovasc. Pharmacol. 17, 285.

Escande D. and P. Henry, 1993, Potassium channels as pharmacological targets in cardiovascular medicine, Eur. Heart J. 14 (suppl.), B2.

Friedrichs, G.S., L. Chi, M.R. Gralinski, S.C. Black, G.C. Basler, D.-X. Mu, S.R. Pewitt, C.R. Johnson and B.R. Lucchesi, 1995, MS-551 protects against ventricular fibrillation in a chronic canine model of sudden cardiac death, J. Cardiovasc. Pharm. 25, 314.

Haworth, R.A., A.T. Goknur and H.A. Berkoff, 1989, Inhibition of ATP-sensitive potassium channels of adult rat heart cells by antiarrhythmic drugs, Circ. Res. 65, 1157.

Legato, M.J., 1993, The anatomic matrix as a factor in susceptibility to lethal arrhythmias in a canine model of sudden cardiac death, J. Mol. Cell. Cardiol. 25, 501.

Lynch, J.J., L.A. Coskey, D.G. Montgomery and B.R. Lucchesi, 1985, Prevention of ventricular fibrillation by dextrorotatory sotalol in a conscious canine model of sudden coronary death, Am. Heart J. 109, 949.

McCullough, J.R., D.E. Normandin, M.L. Conder, P.G. Sleph, S. Dzwonczyk and G.J. Grover, 1991, Specific block of the anti-ischemic actions of cromakalim by sodium 5-hydroxydecanoate, Circ. Res. 69, 949.

Moritani, K., T. Miyazaki, S. Miyoshi, M. Asanagi, L. Zhao, H. Mitamura and S. Ogawa, 1994, Blockade of ATP-sensitive potassium channels by 5-hydroxydecanoate suppresses monophasic action potential shortening during regional myocardial ischemia, Cardiovasc. Drugs Ther. 8, 749.

Niho, T., T. Notsu, H. Ishikawa, H. Funato, M. Yamazaki, H. Takahashi, I. Tanaka, M. Kayamoto, T. Dabasaki and F. Yamasaki, 1987, Study of mechanism and effect of sodium 5-hydroxydecanoate on experimental ischemic ventricular arrhythmia, Nippon Yakurigaku Zasshi. 89, 155.

Noma, A., 1983, ATP-regulated K^+ channels in cardiac muscle, Nature, 305(5930), 147.

Notsu, T., K. Ohhashi, I. Tanaka, H. Ishikawa, T. Niho, K. Fukutake and M. Mizota, 1992, 5-Hydroxydecanoate inhibits ATP-sensitive K^+ channel currents in guinea-pig single ventricular myocytes, Eur. J. Pharmacol. 220, 35.

Notsu, T., I. Tanaka, M. Takano and A. Noma, 1992b, Blockade of the ATP-sensitive K^+ channel by 5-hydroxydecanoate in guinea pig ventricular myocytes, J. Pharmacol. Exp. Ther. 260, 702.

Patterson, E., K. Holland, B.T. Eller and B.R. Lucchesi, 1982, Ventricular fibrillation resulting from ischemia at a site remote from previous myocardial infarction. A conscious canine model of sudden coronary death, Am. J. Cardiol. 50, 1414.

Pogatsa, G., M.Z. Koltai, I. Balkanyi, I. Devai, V. Kiss and A. Koszneghy, 1988, The effect of various hypoglycemic sulfonylureas on the cardiotoxicity of glycosides and arrhythmogenic activity due to myocardial ischemia, Acta Physiol. Hung. 71, 243.

Wilber, D.J., J.J. Lynch and B.R. Lucchesi, 1985, Postinfarction sudden death: Significance of inducible ventricular tachycardia and infarct size in a conscious canine model, Am. Heart J. 109, 8.

Wilde, A.A.M., T. Opthof, C.A. Schumacher and M.J. Janse, 1994, Blocking K-ATP channels with 5 hydroxydecanoate: Effect on extracellular K^+ and pH during ischemia: Comparison with glibenclamide, Circulation 90, I-466 [abstr].

Wollenben C.D., M.C. Sanguinetti and P.K. Siegl, 1989, Influence of ATP-sensitive potassium channel modulators on ischemia-induced fibrillation in isolated rat hearts, J. Mol. Cell. Cardiol. 21, 783.

Woosley, R.L., 1991, Antiarrhythmic drugs, Ann. Rev. Pharmacol. Toxicol. 31, 427.

Wyatt, C.J., R.L. Pereira and E.A. Day, 1967, The lactone precursor in fresh milk fat: Identification of the hydroxy fatty acids, Lipids 2, 208.