ELSEVIER

European Journal of Pharmacology 272 (1995) 269–278

ejp

In vitro effects of capsaicin: antiarrhythmic and antiischemic activity

Albert Joseph D'Alonzo *, Gary J. Grover, Raymond B. Darbenzio, Thomas A. Hess, Paul G. Sleph, Steven Dzwonczyk, Jia L. Zhu, Joseph C. Sewter

Bristol-Myers Squibb Pharmaceutical Research Institute, Department of Pharmacology, P.O. Box 4000, Princeton, NJ00543-4000, USA

Received 23 June 1994; revised 17 October 1994; accepted 21 October 1994

Abstract

The antiarrhythmic effects of vehicle (0.1% dimethyl sulfoxide: DMSO) or capsaicin were evaluated in isolated perfused rat and guinea pig heart preparations. In the rat, capsaicin reduced ischemic ventricular tachycardia from 100% in control to 0%, and ischemic ventricular fibrillation from 60% in control to 0% at 30 μM, and diltiazem reduced the incidence of ischemic ventricular tachycardia and ventricular fibrillation to 55% and 0%, respectively. Reperfusion ventricular fibrillation was reduced from 90% to 20% and 33% for capsaicin and diltiazem, respectively, at these concentrations. In isolated perfused globally ischemic rat hearts, antiischemic efficacy was assessed as a significant extension (36% and 50%) in time to contracture with 30 μM capsaicin and 1 μM diltiazem, respectively. Capsaicin reduced left ventricular developed pressure by 35% in non-ischemic rat hearts, and increased coronary flow by 40%. The increased time to contracture for either compound was not blocked by glyburide (0.1 μM) suggesting a lack of any involvement of ATP-sensitive K^+ channels. In isolated guinea pig hearts subjected to global ischemia, capsaicin and diltiazem reduced reperfusion ventricular fibrillation from 100% to 10% and 0% at 30 and 3 μM, respectively. Electrophysiologic evaluation in guinea pig papillary muscles using standard microelectrode techniques demonstrated significant ($P < 0.05$) action potential durations at 90% repolarization shortening at 1 Hz by 9%, 28% and 39%, and 23%, 37% and 51% at 10, 30, and 100 μM of capsaicin or diltiazem, respectively. Unlike diltiazem, no changes in action potential duration were observed with capsaicin (up to 100 μM) at faster stimulation rates (5 Hz). In conclusion, capsaicin displays both antiarrhythmic and antiischemic efficacy. These data suggest that the effects of capsaicin are mediated primarily through block of Ca^{2+} channels in these preparations.

Keywords: Action potential; K^+ channel blocker; Langendorff; Refractory period; (Rat); (Guinea-pig)

1. Introduction

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) is the active ingredient causing the pungent effects of hot red peppers of the genus *Capsicum*. Capsaicin has long been recognized for its diverse and interesting pharmacologic activity. It predominantly effects sensory neurons where it can produce excitation followed by long-term sensory receptor block or desensitization (Holzer, 1991; O'Neill, 1991). Capsaicin has primary effects on C-fibers, which are slow conducting unmyelinated nerve fibers that carry sensory signals for nociception. The electrophysiologic mechanisms underlying the activity of capsaicin, at least in nerve, have been suggested to result from the opening of a non-selective cation channel (Stretton et al., 1992; Wood et al., 1988). This allows Na^+ and Ca^{2+} to enter the cell and K^+ to efflux via Ca^{2+}-gated channels (O'Neill, 1991; Wood et al., 1988). This increase in ion conductance causes depolarization of the membrane and has been associated with release of neuropeptides such as calcitonin gene-related peptide, substance P, vasoactive intestinal polypeptide and tachykinins (neurokinin A) from peripheral nerve endings, and substance P, somatostatin and calcitonin gene-related peptide from central nerve endings (Holzer, 1991).

Although capsaicin has been extensively studied in the nervous system, few studies with capsaicin have been performed in the myocardium. In the myocardium, capsaicin has been shown to cause release of calcitonin gene-related peptide and substance P from terminal nerve-endings (Franco-Cereceda et al., 1989; Manzini et al., 1989). It has also been reported to block

* Corresponding author. Tel. (609) 252-5115, fax (609) 252-6609.

Elsevier Science B.V.
SSDI 0014-2999(94)00653-9

K^+ ion channel conductances in isolated rat cardiac myocytes. Specifically, capsaicin has been shown to block the transient outward (Ito), delayed rectifier (IK) and inward rectifier (IK1) currents with IC_{50}s of 6.4, 11.5 and 46.9 μM, respectively, in these cells (Castle, 1992). The block of K^+ currents has long been associated with potential antiarrhythmic activity (Hondeghem, 1991; Hondeghem and Snyders, 1990). However, there has been little or no evaluation of capsaicin in models of arrhythmias. In addition, it has been shown that ischemia, as does capsaicin, can cause release of calcitonin gene-related peptide (Franco-Cereceda et al., 1989). Calcitonin gene related peptide has been shown to improve cardiac performance in patients with congestive heart failure (Gennari et al., 1990). Again, there have been no studies performed to evaluate the effects of capsaicin on myocardial function, in particular during ischemia. Thus, the purpose of the present study, was to evaluate the effects of capsaicin on arrhythmias and to determine the preischemic and postischemic functional effects of capsaicin in isolated heart preparations. We found that capsaicin possesses both antiarrhythmic and antiischemic activity in our preparations. Attempts were made to determine the ionic mechanism(s) by which capsaicin was acting. A previous report of this work has been published (Hess et al., 1994).

2. Materials and methods

2.1. Antiarrhythmic testing in isolated rat hearts

Sprague Dawley rats (350–400 g) were anesthetized with Na^+ pentobarbital (75 mg/kg i.p.). Following anesthesia, the skin was removed around the throat, the trachea was intubated and the animal mechanically ventilated with room air. The jugular vein was injected with heparin (100–400 U/kg). A midsternal thoracotomy was made and the ribs retracted to expose the heart. The pericardium was removed and the aorta cleared of any connective tissue. A silk suture (00) was placed around the aorta before its bifurcation. While being ventilated, hearts were perfused in situ with a cannula via retrograde perfusion of the aorta. The cannula was then connected to a reservoir containing oxygenated Krebs-Henseleit bicarbonate buffer with the following composition (mM): 112 NaCl, 3.0 KCl, 11.5 glucose, 25 $NaHCO_3$, 1.2 $MgSO_4$, 1.25 $CaCl_2$, 1.0 KH_2PO_4, at a pH 7.4. The inferior vena cava was clamped just above the diaphragm and a cannula quickly inserted into the aorta and secured with suture. Once the heart was perfused, it was carefully excised from the chest and transferred to the Langendorff apparatus and perfused with Krebs-Henseleit buffer at a constant pressure (80–85 mmHg) and temperature (37 ± 0.2°C).

Once the heart was connected to the Langendorff apparatus a silk suture (000) was looped around the left coronary artery close to its origin and passed through a thin piece of polyethylene tubing (PE60). Each end of the suture was then passed through a hole of a button, and the suture tied loosely. A ventricular electrogram was obtained by inserting small needles just under the epicardial surface and connecting them to an electrocardiogram harness. A pressure transducer connected to the side port of the perfusion apparatus allowed indirect measurement of left ventricular pressure that helped to distinguish ventricular tachycardia from ventricular fibrillation. Pressure and electrocardiogram signals were routed to a chart recorder and oscilloscope. The ambient temperature around the preparation was maintained by a heated vessel (37°C). Flow of the perfusate through the coronary arteries was monitored via an extracorporeal flow probe inserted in line with the flow of the solution.

Each heart was given 10 min equilibration time. After this time, heart rates and coronary flows were recorded. If the control coronary flow was less than 14 ml/min, or the heart rate was less than 260 beats/min, the hearts were excluded from the study. Following equilibration, the hearts were given either vehicle (Krebs-Henseleit or 0.1% dimethyl sulfoxide [DMSO]) or test substance for 7 min prior to occlusion. At the end of 5 min, flow and rate measurements were repeated and the incidence of arrhythmias, if any, were noted. At this time, the artery was ligated by pulling the ends of the strings over the button and knotting it. Adequate occlusion yielded a drop in coronary flow (approximately 45%). If flow decreased less than 40%, attempts were made to tighten the suture to obtain a better ligation. If this failed to yield an acceptable reduction (> 30%) the heart was excluded from the study. Occlusion lasted for 15 min and coronary flow and heart rate were recorded at 1, 3, 5, 7, 10, and 15 min. Arrhythmias were qualified as to their type and time of onset post-occlusion. Arrhythmias were classified as ventricular extrasystoles when isolated events occur, ventricular tachycardia when 5 or more ventricular extrasystoles occur together at a high rate of organized ventricular activity, or ventricular fibrillation when rapid disorganized electrical activity lasting more than 2 s was observed. After 15 min of occlusion, reperfusion was initiated by cutting the tubing and removing the suture. Reperfusion flow served as an index to assure adequate reflow to the heart. Reperfusion arrhythmias were assessed as above for 5 min.

2.2. Antiischemic and cardiac functional testing in isolated perfused rat hearts

Male Sprague-Dawley rats (400–500 g) were anesthetized using 100 mg/kg Na^+ pentobarbital (i.p.).

Following anesthesia, hearts were removed and perfused on a Langendorff apparatus as described above with Krebs-Henseleit containing the following composition (mM): 112 NaCl, 25 $NaHCO_3$, 5 KCl, 1.2 $MgSO_4$, 1 KH_2PO_4, 1.25 $CaCl_2$, 11.5 glucose, and 2 pyruvate at a constant perfusion pressure of 80–85 mmHg. A water-filled latex balloon attached to a metal cannula was then inserted into the left ventricle and connected to a Statham pressure transducer for measurement of left ventricular pressure. The hearts were allowed to equilibrate for 15 min, at which time end-diastolic pressure was adjusted to 5 mmHg and this balloon volume was maintained for the duration of the experiment. Pre-ischemia or pre-drug function, heart rate and coronary flow (extracorporeal electromagnetic flow probe, Carolina Medical Electronics, King, NC) were then measured. Contractile function was calculated by subtracting left ventricular peak systolic pressure from end-diastolic pressure, resulting left ventricular developed pressure. Cardiac temperature was maintained throughout the experiment by submerging the hearts in 37°C buffer which was allowed to accumulate in a stoppered, heated chamber.

The hearts were then divided into vehicle-treated hearts (0.04% DMSO, $n = 4$) and capsaicin-treated hearts (30 μM, $n = 4$). The hearts were pretreated with the respective drug or vehicle 10 min before the initiation of global ischemia. The drugs were administered via the perfusate and pre- and post-drug cardiac function and coronary flow were measured. The hearts were then made globally ischemic by shutting off the perfusate flow. This was continued and the time to contracture was measured as previously described (Grover et al., 1990). The time to contracture was defined as the time during ischemia in which the first 5 mm Hg increase in end-diastolic pressure above baseline was observed.

2.3. Antifibrillatory testing in isolated guinea pig hearts

Hartley guinea pigs (400–800 g) were anesthetized with Na^+ pentobarbital (75 mg/kg i.p.). Following anesthesia, hearts were removed and perfused on a Langendorff apparatus as described above with Krebs-Henseleit containing the following composition (mM): 112 NaCl, 3.0 KCl, 11.5 glucose, 25 $NaHCO_3$, 1.2 $MgSO_4$, 1.25 $CaCl_2$, 1.0 KH_2PO_4, at a pH 7.4. Following a 10 min equilibration period, hearts were administered capsaicin (30 μM) for 10 min, and subjected to global ischemia for 30 min. At the end of this period, hearts were reperfused and the incidence of ventricular fibrillation and the amount of time spent in ventricular fibrillation was measure for 5 min of reperfusion.

2.4. Electrophysiologic testing in isolated guinea pig hearts

Hartley guinea pigs (400–800 g, Hazelton, USA) were anesthetized with Na^+ pentobarbital (75 mg/kg i.p.). Following anesthesia, hearts were removed and perfused on a Langendorff apparatus as described above with Krebs-Henseleit containing the following composition (mM): 112 NaCl, 5.0 KCl, 11.5 Glucose, 25 $NaHCO_3$, 1.2 $MgSO_4$, 1.25 $CaCl_2$, 1.0 KH_2PO_4, at a pH 7.4. Atria were removed and hearts were instrumented with a quadripolar surface patch electrode (Inapres, Norwich NY) and electrocardiogram leads. Two poles of the surface electrode were used for pacing and two for introducing extrastimuli (see below). The heart was horizontally perfused. An electrocardiogram as well as an epicardial monophasic action potential (Franz epicardial Langendorff probe; EP Technologies, Sunnyvale, CA) were continuously recorded throughout the experiment. Monophasic action potential recordings were made from the same location throughout the experiment. Electrocardiogram and monophasic action potential signals were routed to a chart recorder (TA4000; Gould, Cleveland OH) and oscilloscope (DL1200; Yokogawa, Newnan GA). The ambient temperature around the preparation was maintained by a heated vessel (37 $\pm$ 0.2°C; FE 2; Haake, Germany).

2.5. Electrophysiologic determinations

Electrophysiologic measurements were made twice before drug administration, and following sequential administration of test substances. Determinations of the excitation threshold, effective refractory period and action potential duration were made at ventricular pacing rates of 3, 4, 5, and 6 Hz with single pulses of 2-ms duration at twice the threshold current (see below). Stimuli of 2-ms duration were introduced through the an adjacent pair of electrodes on the quadripolar patch to evoke extrasystolic beats and to determine the following electrophysiologic parameters:

Excitation threshold, the minimum current in milliamperes (mA) required to evoke extrasystoles in response to stimuli (S2) placed approximately 90% of the cycle length from the R-wave (S1) of the electrogram. The time between the R-wave and the stimulus is the S1-S2 interval (ms).

Ventricular effective refractory period, the maximum S1-S2 interval at which no extrasystoles were evoked at a current that was twice the excitation threshold.

Action potential duration, was measured at the 90% repolarization level from the plateau region of the monophasic action potential.

Each heart was given 20 min equilibration time. Following equilibration, two control electrophysiologic readings were taken. Hearts were then given vehicle (0.025% to 0.1% DMSO), capsaicin (3, 10, and 30 μM), 4-aminopyridine (300, 1000, and 3000 μM), dofetilide + capsaicin (0.03 + 3, 10 and 30 μM, respectively), or dofetilide + 4-aminopyridine (0.03 + 300, 1000, and 3000 μM) for 10 min. At the end of 10 min

of compound administration, electrophysiologic determinations were repeated. Upon completion of the electrophysiologic determinations, the next concentration of compound was given for 10 min prior to the next reading.

2.6. Microelectrode recording techniques in guinea pig papillary muscle

Male guinea pigs (450–600 g) were killed by cervical dislocation. Hearts were rapidly removed, and rinsed in Krebs-Henseleit bicarbonate buffer solution (room temperature) equilibrated with 95% O_2/5% CO_2. The Krebs-Henseleit was composed as follows (mM): 112 NaCl, 5.0 KCl, 11.5 glucose, 25 $NaHCO_3$, 1.2 $MgSO_4$, 1.25 $CaCl_2$, 5.0 mM Hepes, 1.0 KH_2PO_4, at a pH 7.4. A posterior papillary muscle, 3–5 mm in length and 1 mm or less in diameter, was removed from the right or left ventricle and was pinned to the base of a 5 ml tissue chamber. The papillary muscle was continuously stimulated through a pair of platinum wires with 1-ms square wave pulses set at 200% of threshold voltage. The frequency of stimulation was held constant at 5 Hz during the first hour of equilibration, and paced at 1 Hz during the remaining equilibration period. Muscles were allowed 2–3 h to equilibrate while being superfused with oxygenated Krebs-Henseleit solution (15–25 ml/min at 37 ± 0.2°C).

Standard microelectrode techniques were employed to impale single myocardial cells in a multicellular preparation and to record transmembrane action potentials. Microelectrodes, filled with 3 M KCl and having a tip resistance greater than 10 MΩ, were coupled to an amplifier (Axoclamp-2A; Axon Instruments, Burlingame CA). Electrical potentials were displayed on a digital oscilloscope (Yokogawa model D1200; Newnan, GA). Signals were recorded on a chart recorder (Gould model 2400S) and analyzed with a computer using the digital data obtained from the oscilloscope and a customized BASIC program (Jeffrey R. Itell, Cemtech Energy Control, Conshohocken, PA).

Following equilibration, transmembrane action potentials were recorded and resting membrane potentials, amplitudes, maximum upstroke velocity (V_{max}) and durations at 20%, 50%, and 90% levels of repolarization (APD_{20}, APD_{50}, and APD_{90}, respectively) were measured at stimulating frequencies of 1, 2, 3, 4, and 5 Hz. Either vehicle (DMSO; volume equivalence) or capsaicin (1, 10, 30, and 100 μM) were added to the Krebs-Henseleit solution, and tissues allowed 30 min for equilibration between concentrations. Action potential measurements were repeated.

2.7. Drugs and solutions

Capsaicin was purchased from Sigma Chemical Company, St. Louis MO. Diltiazem, a blocker of L-type Ca^{2+} channels; glyburide, a blocker of the ATP-sensitive K^+ channel; and 4-aminopyridine, a non-selective K^+ channel blocker were also purchased from Sigma. Dofetilide, a selective blocker of the fast component of the delayed rectifier K^+ channel, was synthesized by the Bristol-Myers Squibb chemists. All agents were dissolved in dimethyl sulfoxide (DMSO; Sigma) on their day of use such that the final concentration of DMSO in the Krebs-Henseleit solution did not exceed 0.1%.

2.8. Statistics

Comparisons between vehicle and drug treatment groups were made using an analysis of variance followed by a Dunnett's procedure. A Mann-Whitney U-test was performed on arrhythmia scoring data to test for significant differences between vehicle and capsaicin groups. All data are presented as the means ± S.E.M., and significant differences were determined at the $P < 0.05$ level.

3. Results

3.1. Antiarrhythmic effects of capsaicin in isolated rat hearts

In isolated perfused rat hearts subjected to regional ischemia, capsaicin did not affect either coronary flow or heart rate at 10 μM ($n = 11$; from 19 ± 1 to 20 ± 1 ml/min and from 315 ± 5 to 315 ± 9 bpm) or 30 μM ($n = 10$; from 18 ± 1 to 19 ± 1 ml/min and from 327 ± 9 to 306 ± 10 bpm). Capsaicin reduced the incidence of arrhythmias in this model. The incidence of ischemic arrhythmias decreased from 100% ischemic ventricular tachycardia to 91% and 0% at 10 and 30 μM, respectively. Ischemic ventricular fibrillation decreased from 60% in control hearts to 27% and 0% at 10 and 30 μM, respectively. Reperfusion ventricular tachycardia was not significantly affected by capsaicin. However, there was a reduction in reperfusion ventricular fibrillation. Ventricular fibrillation was reduced from 90% to 20% at 30 μM. Diltiazem at 3 μM ($n = 9$) did not affect coronary flow from a control of 24 ± 1 to 22 ± 1 ml/min. However, diltiazem did significantly reduce heart rate from a control value of 318 ± 7 to 288 ± 10 bpm. Diltiazem did reduce the incidence of arrhythmias in this model. The incidence of ischemic arrhythmias decreased from 100% ischemic ventricular tachycardia to 55%. Ischemic ventricular fibrillation decreased from 60% in control hearts to 0%. Reperfusion ventricular tachycardia was not significantly affected by diltiazem. However, there was a reduction in reperfusion ventricular fibrillation from 90% to 33%.

3.2. Antiischemic and cardiac functional effects of capsaicin in isolated perfused rat hearts

In isolated rat hearts subjected to global ischemia, capsaicin (30 μM) was evaluated for antiischemic activity as well as preischemic changes in contractile function. Capsaicin did not cause any significant change in heart rate. However, it did significantly reduce left ventricular developed pressure (37%), increased coronary flow (27%) and extended time to contracture (36%; Table 1). The extension of the time to contracture was indicative of an antiischemic effect. However, the increased time to contracture was not reversed by glyburide (0.1 μM). Diltiazem (1 μM) did not cause a significant change in heart rate, but it did significantly reduce left ventricular pressure (73%) and increased coronary flow (29%). Time to contracture was also increased with diltiazem by 50%. As with capsaicin, the increase in time to contracture with diltiazem was not reversed by glyburide.

3.3. Antifibrillatory testing in isolated guinea pig hearts

In isolated perfused guinea pig hearts capsaicin elevated coronary flow (from 28.5 ± 2.8 to 34.7 ± 3.3 ml/min) and reduced heart rate (from 234 ± 6 to 194 ± 8 bpm), but only heart rates were statistically significant. When hearts were subjected to global ischemia and reperfusion, capsaicin reduced the incidence of reperfusion ventricular fibrillation (Table 2). One out of ten hearts (10%) fibrillated upon reperfusion whereas eight out of eight (100%) of the vehicle-treated (0.1% DMSO) hearts had ventricular fibrillation upon reperfusion. Diltiazem caused a small elevation in coronary flow (from 21.0 ± 1 to 23.2 ± 1 ml/min) and significantly reduced heart rate (from 201 ± 4 to 134 ± 5 bpm). When hearts were subjected to global ischemia and reperfusion, diltiazem reduced the incidence of reperfusion ventricular fibrillation. None out of six hearts (0%) fibrillated upon reperfusion.

Table 1
The effect of capsaicin (30 μM) or diltiazem (1 μM) with or without glyburide (0.1 μM) on heart rate and cardiac function before ischemia and time to contracture in isolated perfused rat hearts

	n	Predrug	Postdrug	Ischemia
HR (Beats / min)				
Vehicle	11	285 ± 13	279 ± 16	–
Cap	4	289 ± 5	288 ± 21	–
Cap + Gly	6	291 ± 4	289 ± 10	–
Dil	4	303 ± 12	281 ± 10	–
Dil + Gly	4	288 ± 7	245 ± 12	–
LVDP (mm Hg)				
Vehicle	11	135 ± 5	131 ± 6	–
Cap	4	135 ± 3	85 ± 3 [a,b]	–
Cap + Gly	6	129 ± 3	90 ± 3 [a,b]	–
Dil	4	141 ± 5	38 ± 3 [a,b]	–
Dil + Gly	4	131 ± 2	60 ± 5 [a,b]	–
Coronary flow (ml / min per 100 g)				
Vehicle	11	20 ± 1	20 ± 1	–
Cap	4	22 ± 1	28 ± 1 [a,b]	–
Cap + Gly	6	20 ± 1	25 ± 2 [a,b]	–
Dil	4	21 ± 1	27 ± 2 [a,b]	–
Dil + Gly	4	18 ± 1	25 ± 2 [a,b]	–
Minutes to contracture				
Vehicle	11	–	–	17.1 ± 0.6
Cap	4	–	–	23.3 ± 0.2 [b]
Cap + Gly	6	–	–	22.8 ± 0.2 [b]
Dil	4	–	–	25.5 ± 0.2 [b]
Dil + Gly	4	–	–	22.5 ± 1.0 [b]

All values are expressed as the means ± SEM; Cap = capsaicin; Gly = glyburide; Dil = diltiazem. [a] Significantly different from its respective predrug value ($P < 0.05$). [b] Significantly different from its respective vehicle group value ($P < 0.05$).

Table 2
Effects of capsaicin (30 μM) or diltiazem (3 μM) on reperfusion-induced ventricular fibrillation (VF) in isolated guinea pig hearts subjected to global ischemia

Treatment	n	VF	% VF
DMSO (0.1%)	8	8	100
Capsaicin	10	1	10 [a]
Diltiazem	6	0	0 [a]

[a] Significantly different ($P < 0.05$) from corresponding vehicle value. *n* represents number of hearts studied.

3.4. Effects of capsaicin on electrophysiologic parameters in isolated perfused guinea pig hearts

In isolated guinea pig hearts, capsaicin at 10 and 30 μM produced a slight, but non-significant, decrease in action potential duration at low frequencies of stimulation (Table 3). These effects were lost at faster stimulation rates. Effective refractory period values were unchanged at all concentrations relative to their corresponding rates of stimulation. However, at faster stimulation rates, effective refractory period values were slightly but significantly increased above control values. In contrast, 4-aminopyridine prolonged action potential duration in a reverse rate- dependent manner from 21 ± 6% at 3 Hz to 15 ± 2% at 6 Hz (Table 4). Similarly, effective refractory period values were also increased. However, there was no change in the action potential duration/effective refractory period ratio. In the presence of dofetilide, action potential duration and effective refractory period values increased uniformly such that there was no change in the action potential duration/effective refractory period ratio (Table 5). Capsaicin caused no change in either the rate dependency of dofetilide or its effects on action potential duration or effective refractory period. In the presence of dofetilide, 4-aminopyridine did not cause any further prolongation of action potential duration or effective refractory period in this preparation (Table 6).

Table 3
Effects of capsaicin on electrophysiologic characteristics, monophasic action potential durations at 90% (APD) repolarization, effective refractory period (ERP), and ratio of APD/ERP recorded from isolated perfused guinea pig hearts ($n = 5$) at different concentrations (CONC) and frequencies (FREQ, Hz) of stimulation

CONC	FREQ (Hz)	APD (ms)	ERP (ms)	APD/ERP
Control	3	135±4	173±3 (4)	0.77±0.02 (4)
3.0 μM	3	134±4	173±5 (4)	0.75±0.02 (4)
10.0 μM	3	130±4	169±2 (4)	0.75±0.02 (4)
30.0 μM	3	130±5	170±6 (4)	0.75±0.02 (4)
Control	4	129±4	162±2	0.80±0.02
3.0 μM	4	130±4	164±3	0.79±0.01
10.0 μM	4	127±4	166±4	0.77±0.01
30.0 μM	4	126±5	167±5	0.75±0.02
Control	5	118±4	148±2	0.80±0.01
3.0 μM	5	118±4	154±3	0.77±0.02
10.0 μM	5	118±4	155±2 [a]	0.76±0.02
30.0 μM	5	117±4	157±4	0.74±0.02 [a]
Control	6	105±3	137±2	0.77±0.02
3.0 μM	6	107±4	142±2	0.75±0.02
10.0 μM	6	106±4	143±2	0.74±0.02
30.0 μM	6	106±4	144±2 [a]	0.74±0.03

[a] Significantly different ($P < 0.05$) from corresponding control values. Number of hearts are given in parentheses when number was less than 5.

Table 4
Effects of 4-aminopyridine on electrophysiologic characteristics, monophasic action potential durations at 90% (APD) repolarization, effective refractory period (ERP), and ratio of APD/ERP recorded from isolated perfused guinea pig hearts ($n = 6$) at different concentrations (CONC) and frequencies (FREQ, Hz) of stimulation

CONC	FREQ (Hz)	APD (ms)	ERP (ms)	APD/ERP
Control	3	137±4	166±3 (3)	0.83±0.03 (3)
0.3 mM	3	145±4	183±5 (3) [a]	0.83±0.01 (3)
1.0 mM	3	155±4 [a]	187±2 (3) [a]	0.84±0.01 (3)
3.0 mM	3	164±5 [a]	201±6 (3) [a]	0.84±0.04 (3)
Control	4	131±3	154±2	0.85±0.01
0.3 mM	4	141±3 [a]	163±3 [a]	0.87±0.01
1.0 mM	4	150±3 [a]	170±1 [a]	0.88±0.02
3.0 mM	4	156±3 [a]	179±2 [a]	0.87±0.02
Control	5	120±3	142±2	0.84±0.02
0.3 mM	5	127±2	149±3	0.85±0.01
1.0 mM	5	133±2 [a]	153±2 [a]	0.87±0.01
3.0 mM	5	139±2 [a]	162±4 [a]	0.86±0.01
Control	6	108±2	132±1	0.82±0.02
0.3 mM	6	114±2 [a]	136±2	0.84±0.01
1.0 mM	6	118±3 [a]	140±1 [a]	0.84±0.02
3.0 mM	6	123±2 [a]	144±1 [a]	0.86±0.01

[a] Significantly different ($P < 0.05$) from corresponding control values. Number of hearts are given in parentheses when number was less than 6.

3.5. Effects of capsaicin on intracellular action potential characteristics in guinea pig papillary muscles

In isolated guinea pig papillary muscles, capsaicin caused significant reductions in APD_{20}, APD_{50} and

Table 5
Effects of dofetilide (30 nM) and capsaicin on electrophysiologic characteristics, monophasic action potential durations at 90% (APD) repolarization, effective refractory period (ERP), and ratio of APD/ERP recorded from isolated perfused guinea pig hearts ($n = 6$) at different concentrations (CONC) and frequencies (FREQ, Hz) of stimulation

CONC	FREQ (Hz)	APD (ms)	ERP (ms)	APD/ERP
Control	3	142±5	172±3 (4)	0.83±0.01 (4)
Dofetilide +	3	167±8 [a]	201±11 (4) [a]	0.84±0.01 (4)
3.0 μM	3	170±6 [a]	202±8 (4) [a]	0.84±0.02 (4)
10.0 μM	3	168±5 [a]	203±6 (4) [a]	0.83±0.02 (4)
30.0 μM	3	166±8 [a]	208±8 (4) [a]	0.82±0.03 (4)
Control	4	133±5	158±4	0.85±0.01
Dofetilide +	4	157±5 [a]	183±5 [a]	0.86±0.01
3.0 μM	4	157±5 [a]	182±4 [a]	0.86±0.01
10.0 μM	4	158±5 [a]	182±4 [a]	0.85±0.02
30.0 μM	4	157±9 [a]	194±5 [a]	0.80±0.03
Control	5	122±4	145±3	0.84±0.01
Dofetilide +	5	141±4 [a]	166±3 [a]	0.85±0.01
3.0 μM	5	142±4 [a]	167±3 [a]	0.85±0.02
10.0 μM	5	143±4 [a]	170±2 [a]	0.84±0.02
30.0 μM	5	143±5 [a]	170±2 (5) [a]	0.81±0.02
Control	6	108±3	134±2	0.80±0.02
Dofetilide +	6	125±3 [a]	146±2 (5) [a]	0.84±0.02 (5)
3.0 μM	6	127±4 [a]	149±1 [a]	0.86±0.03
10.0 μM	6	128±4 [a]	150±1 (5) [a]	0.84±0.02 (5)
30.0 μM	6	128±4 [a]	149±1 (3) [a]	0.82±0.01 (3)

[a] Significantly different ($P < 0.05$) from corresponding control values. Number of hearts are given in parentheses when number was less than 6.

Table 6
Effects of dofetilide (30 nM) and 4-aminopyridine on electrophysiologic characteristics, monophasic action potential durations at 90% (APD) repolarization, effective refractory period (ERP), and ratio of APD/ERP recorded from isolated perfused guinea pig hearts ($n = 5$) at different concentrations (CONC) and frequencies (FREQ, Hz) of stimulation

CONC	FREQ (Hz)	APD (ms)	ERP (ms)	APD/ERP
Control	3	141±3	175±4 (4)	0.82±0.01 (4)
Dofetilide +	3	170±3 [a]	211±3 (2) [a]	0.83±0.01 (2)
0.3 mM	3	178±6 [a]	208±4 (4) [a]	0.85±0.01 (4)
1.0 mM	3	175±7 [a]	208±4 (4) [a]	0.84±0.02 (4)
3.0 mM	3	167±6 [a]	213±4 (4) [a]	0.79±0.02 (4)
Control	4	131±3	159±3	0.83±0.01
Dofetilide +	4	156±4 [a]	181±5 [a]	0.86±0.02
0.3 mM	4	162±5 [a]	184±5 [a]	0.88±0.01 [a]
1.0 mM	4	161±4 [a]	184±4 [a]	0.87±0.01 [a]
3.0 mM	4	157±4 [a]	187±4 [a]	0.84±0.02
Control	5	122±3	148±3	0.83±0.01
Dofetilide +	5	142±3 [a]	165±4 [a]	0.86±0.01
0.3 mM	5	144±4 [a]	165±4 [a]	0.88±0.01 [a]
1.0 mM	5	143±4 [a]	164±4 [a]	0.87±0.01 [a]
3.0 mM	5	138±5 [a]	166±3 [a]	0.84±0.02
Control	6	112±3	135±4	0.83±0.01
Dofetilide +	6	126±3 [a]	144±3	0.88±0.02 [a]
0.3 mM	6	127±4 [a]	145±2 [a]	0.87±0.02
1.0 mM	6	127±4 [a]	145±2 [a]	0.87±0.02
3.0 mM	6	124±5	148±2 [a]	0.84±0.03

[a] Significantly different ($P < 0.05$) from corresponding control values. Number of hearts are given in parentheses when number was less than 5.

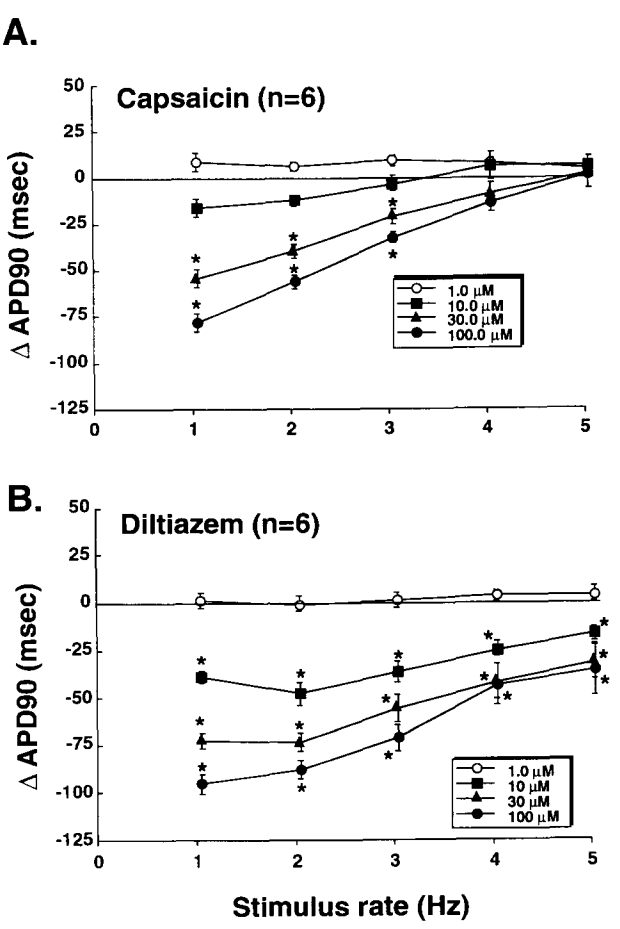

Fig. 1. (A) Effects of capsaicin on intracellular recordings of the action potential duration at 90% repolarization (APD_{90}) represented as a change from control. Capsaicin produced a concentration-dependent shortening of the APD_{90} at 1 Hz. At faster frequencies of stimulation the APD_{90} changes were greatly diminished such that no significant changes were observed above 3 Hz. (B) Effects of diltiazem on APD_{90} represented as a change from control. Like capsaicin, diltiazem also produced a shortening of APD_{90} values at lower frequencies that diminished at faster rates of stimulation. Significant differences ($P < 0.05$) are indicated with an asterisk.

APD_{90} that were noted at 10, 30, and 100 μM at 1, 2, and 3 Hz. At 1 Hz APD_{90} values were reduced by 9%, 28%, and 39% at 10, 30, and 100 μM capsaicin, respectively, from control levels of 205 ± 4, 175 ± 3, and 143 ± 3 ms. The shortening of the action potential duration observed with capsaicin was lessened and subsequently negated at faster rates of stimulation (Fig. 1A). Capsaicin did not affect other action potential parameters at concentrations up to 100 μM. Diltiazem also caused significant shortening of action potential duration values at 10, 30, and 100 μM, but the reduction in action potential duration was observed at all frequencies of stimulation (Fig. 1B). APD_{90} values decreased 23%, 37%, and 51% at 10, 30, and 100 μM at 1 Hz from a control value of 187 ± 3 ms, and at 5 Hz values were decreased 22%, 34%, and 38% from a control of 97 ± 4 ms over respective concentrations. Unlike capsaicin, diltiazem caused a significant reduction in V_{max} (e.g., from 273 ± 31 V/s to 188 ± 38 and 129 ± 28 V/s at 30 and 100 μM and 1 Hz) and action potential amplitude (e.g., from 119 ± 4 ms to 101 ± 5 and 90 ± 5 ms at 30 and 100 μM and 1 Hz) over all frequencies studied. There was a tendency for V_{max} and amplitude to fall greater at faster frequencies of simulation in the presence of diltiazem at these concentrations.

4. Discussion

Since it has been shown that capsaicin blocks a variety of K^+ currents in isolated rat ventricular myocytes (Castle, 1992), we attempted to determine if capsaicin would display antiarrhythmic efficacy in isolated perfused heart preparations. Capsaicin, in our models, did display antiarrhythmic activity at 10 and 30 μM in rat and guinea pig hearts. The rat does not appear to have a prominent delayed rectifier (IK) current (Tande et al., 1990). Thus, the action potential duration of the rat is extremely short due to the predominant contribution of the transient outward current (Ito) at positive potentials (Dukes and Morad, 1989). However, the importance of Ito modulation in this species has not been well studied. Likewise, the guinea pig does not have a prominent Ito current (Sanguinetti, 1992; Varro et al., 1993). However, the importance of Ito in the guinea pig has not been clearly shown in this species. Regardless, capsaicin, as well as diltiazem, were able to demonstrate antiarrhythmic efficacy in both rat and guinea pig isolated heart models, but at concentrations that would involve block of either Ito or IK at least in the rat (Castle, 1992). Concentrations of capsaicin (46.9 μM) needed to block 50% of the inward rectifier (IK1) were much higher than those needed to observe efficacy in our models (30 μM). It is known that Ito as well as IK are composed of two components (Ito1 and Ito2; IKr and IKs, respectively). Ito1 has fast kinetics and is Ca^{2+} insensitive whereas Ito2 has slower kinetics and is sensitive to Ca^{2+} levels (Apkon and Nerbonne, 1991; Tseng and Hoffman, 1989). IKr is a rapidly activating and inactivating current whereas IKs is slowly activating and non-inactivating current (Jurkiewicz and Sanguinetti, 1993; Sanguinetti and Jurkiewicz, 1990). Effects of capsaicin on the components of Ito and IK have not been studied. Since Castle (1992) has demonstrated that capsaicin prolongs action potential duration in the rat, we focused our studies on the guinea pig to determine if K^+ channel modulation with capsaicin could account for the antiarrhythmic activity that we observed in this species.

Electrophysiologically, capsaicin shortened action potential duration values in guinea pig papillary muscles at low frequencies of stimulation. However, there was no difference in action potential duration when stimulation rates were above 4 Hz. The effects on action potential duration in the isolated perfused heart preparation showed little shortening. Differences between these preparations could be related to recording techniques, or the site of recording (epicardium in the whole heart vs. endocardium in the papillary muscle). Regardless, the observations obtained in these preparations are contradictory to the effects of a K^+ channel blocker, i.e., action potential duration prolongation. The shortening of the action potential duration could be explained by a number of mechanisms including block of an inward current (i.e., Na^+ or Ca^{2+}) or enhancement of an outward K^+ current (i.e., IKr, IKs, Ito, IK1, or IK_{ATP}). From our single microelectrode studies, it was observed that there was no significant change in V_{max} or amplitude with capsaicin up to 100 μM indicating no block of fast Na^+ channels. Thus, shortening of action potential duration with capsaicin was not attributed to Na^+ channel blockade. Since resting potentials were not depolarized and action potential amplitudes were not reduced, involvement of IK1 is also an unlikely explanation. The contribution of an activation of IK cannot be ruled out, but it is unlikely, since it has been shown that capsaicin blocks IK in rat (it is not known if the rat has IKr and/or IKs and what the relevance of these channels may be to other species) (Castle, 1992). Although capsaicin could open K^+ATP channels, this is also unlikely to occur (see below). To support a Ca^{2+} channel modulation with capsaicin, diltiazem also shortened action potential duration values. Unlike capsaicin, diltiazem decreased V_{max} and action potential amplitude values at high concentrations (30 and 100 μM) suggesting a block of fast inward Na^+ channels. These data support some form of Ca^{2+} channel blockade with capsaicin, either directly through voltage-gated channels (Castle, 1992) or by some other mechanism(s) that are involved with its activity. Although Castle (1992) did demonstrate some Ca^{2+} channel current inhibition with capsaicin (10 μM), he concluded that the majority of capsaicin's effects are mediated through K^+ channel modulation.

Involvement of the Ca^{2+} channel is further supported by antiischemic effects of capsaicin. In isolated perfused rat hearts subjected to global ischemia, capsaicin and diltiazem were found to extended time to contracture by 36% and 50%, respectively. Since the antiischemic effects of these agents were not reversed by glyburide, it suggests that their mechanisms of action are not likely mediated through K^+ATP channels. In our microelectrode studies, we were also unable to reverse the action potential duration-shortening effects of capsaicin with glyburide (D'Alonzo and Hess, unpublished observation). Ultimately, antiischemic activity is associated with regulation of intracellular Ca^{2+}. It is known that capsaicin can affect Ca^{2+} handling in nerves (Petersen et al., 1989) and heart (Zernig et al., 1984). In our isolated heart preparation, we found that capsaicin as well as diltiazem reduced force of contraction indicating a negative inotropic effect. This reduction in function was not potent for capsaicin, and corresponded to an effect similar to a low dose of Ca^{2+} channel antagonists (Grover and Sleph, 1989). However, in atria, capsaicin has been shown to have positive inotropic effects, which do not appear to be directly mediated through voltage-gated channels and may involve intracellular mechanism(s) (Zernig et al., 1984). Thus, the role of Ca^{2+} channel block versus intracellular Ca^{2+} regulation still needs to be explored in regard to the antiarrhythmic as well as antiischemic activity of capsaicin.

Unlike the rat, guinea pig myocardium has a prominent IK (Sanguinetti and Jurkiewicz, 1990; Tande et al., 1990). However, it is still unclear as to whether Ito contributes significantly to the electrophysiologic activity of the guinea pig heart either alone or in combination with a specific IK blocker. Specifically, a functional Ito may not have been observed experimentally in the guinea pig due to regional differences in the location of this channel within the myocardium (Litovsky and Antzelevitch, 1988; Wang et al., 1991). To better understand the role of Ito, we examined the effects of capsaicin and 4-aminopyridine on the rate dependency of the cardiac action potential duration in isolated perfused guinea pig hearts. Although both capsaicin and 4-aminopyridine are blockers of Ito, they have different kinetics of inactivation. Capsaicin can reduce Ito by enhancing the speed of inactivation and blunting the peak current (Castle, 1992), whereas 4-aminopyridine can reduce peak current amplitude and slow the recovery of inactivation (Hiraoka and Kawano, 1987). In the present study, neither compound demonstrated any positive rate dependency alone nor did they affect the rate dependency of dofetilide, a selective blocker of IKr (Jurkiewicz and Sanguinetti, 1993; Sanguinetti and Jurkiewicz, 1990). Thus, the antiarrhythmic effects of capsaicin in the guinea pig are not likely attributed to Ito blockade, since Ito does not appear to contribute significantly to the electrophysiologic effects in this species as supported by our studies as well as others (Sanguinetti, 1992; Varro et al., 1993).

It is known that capsaicin can release endogenous substances from the myocardium such as calcitonin gene-related peptide, substance P and Neurokinin A (Franco-Cereceda et al., 1989; Hoover, 1987; Hua et al., 1985). We observed no electrophysiological changes in either action potential duration or effective refractory period with the administration of calcitonin gene-

related peptide (10 nM) in the isolated perfused guinea pig heart (unpublished observation). The antiarrhythmic and antiischemic activity of capsaicin are not likely related to the release of endogenous peptides, since the concentrations of capsaicin needed for antiarrhythmic and antiischemic activity (30 μM) were much greater than those necessary to release endogenous peptides (1 μM).

It is clear that the myocardium has a disparate distribution of ion channels that are reflected in the different action potential characteristics recorded within these cells (Antzelevitch et al., 1991; Liu et al., 1993). These differences account for the ability of the heart to be electrically synchronized in such a way that it functions as a single unit or pump. It has been suggested that Ito activation in the mid-myocardium may account for potential proarrhythmic effects and may be implicated in the arrhythmogenic effects of flecainide (Krishnan and Antzelevitch, 1993). Ito in the presence of flecainide can cause profound shortening of the action potential due to an overwhelming effect on the inward currents that promote all or none repolarization. This results in flecainide's proarrhythmic action through enhancement of reentrant arrhythmias. Thus, blocking of Ito may help offset the proarrhythmic response of flecainide (Krishnan and Antzelevitch, 1993) or may synergistically interact with other channel blockers to enhance their antiarrhythmic efficacy. One reason for studying the guinea pig was that no one has conclusively demonstrated that all regions of the guinea pig heart are devoid of Ito conductance. In the guinea pig, we did not observe lengthening of the action potential duration as would be expected of an Ito blocker. In contrast, we observed a shortening of the action potential duration. Thus, other actions of capsaicin in the guinea pig myocardium are occurring. As mentioned above, this likely includes an interaction with Ca^{2+} regulation. Also the prolongation observed with 4-aminopyridine was not changed in the presence of dofetilide suggesting that 4-aminopyridine has IKr-like blocking properties in the guinea pig myocardium. More importantly, since 4-aminopyridine had no additive effects on dofetilide further supports the notion that Ito is not present or is minimally functional in the guinea pig ventricular myocardium.

In conclusion, capsaicin displayed antiarrhythmic efficacy in both isolated perfused rat and guinea pig hearts. Capsaicin shortened action potential duration in the guinea pig whereas 4-aminopyridine prolonged action potential duration. Thus, the antiarrhythmic and antiischemic effects of capsaicin in the guinea pig involves effects on other ionic components, most likely block of voltage-gated Ca^{2+} channels, and not K^+ channels, since it shortened action potential duration values. Regardless of these differences, capsaicin displays both antiarrhythmic as well as antiischemic effects in isolated heart preparations displaying effects that are similar to those of a Ca^{2+} channel antagonist.

References

Antzelevitch, C., S. Sicouri, S.H. Litovsky, A. Lukas, S.C. Krishnan, J.M. DiDiego, G.A. Gintant and D.W. Liu, 1991, Heterogeneity within the ventricular wall: electrophysiology and pharmacology of epicardial, endocardial, and M cells, Circ. Res. 69, 1427.

Apkon, M. and J.M. Nerbonne, 1991, Characterization of two distinct depolarization-activated K^+ currents in isolated adult rat ventricular myocytes, J. Gen. Physiol. 97, 973.

Castle, N., 1992, Differential inhibition of potassium currents in rat ventricular myocytes by capsaicin, Cardiovasc. Res. 26, 1137.

Dukes, I.D. and M. Morad, 1989, Tedisamil inactivates transient outward K^+ current in rat ventricular myocytes, Am. J. Physiol. 257, H1746.

Franco-Cereceda, A., A. Saria and J.M. Lundberg, 1989, Differential release of calcitonin gene-related peptide and neuropeptide Y from the isolated heart by capsaicin, ischaemia, nicotine, bradykinin and ouabain, Acta Physiol. Scand. 135, 173.

Gennari, C., R. Nami, D. Agnusdei and J.A. Fischer, 1990, Improved cardiac performance with human calcitonin gene related peptide in patients with congestive heart failure, Cardiovasc. Res. 24, 239.

Grover, G.J. and P.G. Sleph, 1989, Dissociation of cardiodepression from cardioprotection with calcium antagonists: diltiazem protects ischemic rat myocardium with a lower functional cost as compared with verapamil or nifedipine, J. Cardiovasc. Pharmacol. 14, 331.

Grover, G.J., P.G. Sleph and S. Dzwonczyk, 1990, Pharmacologic profile of cromakalim in the treatment of myocardial ischemia in isolated rat hearts and anesthetized dogs, J. Cardiovasc. Pharmacol. 16, 853.

Hess, T.A., R.B. Darbenzio, J.C. Sewter, G.J. Grover and A.J. D'Alonzo, 1994, In vitro antiarrhythmic and antiischemic effects of capsaicin, a K^+ channel modulator, FASEB J. 8, A609.

Hiraoka, M. and S. Kawano, 1987, Mechanism of increased amplitude and duration of the plateau with sudden shortening of diastolic intervals in rabbit ventricular cells, Circ. Res. 60, 14.

Holzer, P., 1991, Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurons, Pharmacol. Rev. 43, 143.

Hondeghem, L.M., 1991, Ideal antiarrhythmic agents: chemical defibrillators, J. Cardiovasc. Electrophysiol. 2, S169.

Hondeghem, L.M. and D.J. Snyders, 1990, Class III antiarrhythmic agents have a lot of potential but a long way to go, Circ. 81, 686.

Hoover, D.B., 1987, Effects of capsaicin on release of substance P-like immunoreactivity and physiological parameters in isolated perfused guinea-pig heart, Eur. J. Pharmacol. 141, 489.

Hua, X.-Y., E. Theodorsson-Norheim, E. Brodin, J.M. Lundberg and T. Hokfelt, 1985, Multiple tachykinins (neurokinin A, neuropeptide K and substance P) in capsaicin-sensitive sensory neurons in the guinea-pig, Reg. Pept. 13, 231.

Jurkiewicz, N.K. and M.C. Sanguinetti, 1993, Rate-dependent prolongation of cardiac action potentials by a methanesulfonanilide Class III antiarrhythmic agent: specific block of rapidly activating delayed rectifier K^+ current by dofetilide, Circ. Res. 72, 75.

Krishnan, C.C. and C. Antzelevitch, 1993, Flecainide-induced arrhythmia in canine ventricular epicardium. Phase 2 reentry?, Circ. 87, 562.

Litovsky, S.H. and C. Antzelevitch, 1988, Transient outward current prominent in canine ventricular epicardium but not endocardium, Circ. Res. 62, 116.

Liu, D.-W., G.A. Gintant and C. Antzelevitch, 1993, Ionic bases for electrophysiological distinctions among epicardial, midmyocardial, and endocardial myocytes from the free wall of the canine left ventricle, Circ. Res. 72, 671.

Manzini, A., F. Perretti, L. DeBenedetti, P. Pradelles, C.A. Maggi and P. Geppetti, 1989, A comparison of bradykinin- and capsaicin-induced myocardial and coronary effects in isolated perfused heart of guinea-pig: involvement of substance P and calcitonin gene-related peptide release, Br. J. Pharmacol. 97, 303.

O'Neill, T.P., 1991, Mechanism of capsaicin action: recent learnings, Resp. Med. 85, 35.

Petersen, M., G. Wagner and F.-K. Pierau, 1989, Modulation of calcium by capsaicin in a subpopulation of sensory neurones of guinea-pig, Naunyn-Schmied. Arch. Pharmacol. 339, 184.

Sanguinetti, M.C., 1992, Modulation of potassium channels by antiarrhythmic and antihypertensive drugs, Hypertension 19, 228.

Sanguinetti, M.C. and N.K. Jurkiewicz, 1990, Two components of cardiac delayed rectifier K^+ current: differential sensitivity to block by Class III antiarrhythmic agents, J. Gen. Physiol. 96, 195.

Stretton, D., M. Miura, M.G. Belvisi and P.J. Barnes, 1992, Calcium-activated potassium channels mediate prejunctional inhibition of peripheral sensory nerves, Proc. Nat. Acad. Sci. 89, 1325.

Tande, P.M., H. Bjornstad, T. Yang and H. Refsum, 1990, Rate-dependent class III antiarrhythmic action, negative chronotropy, and positive inotropy of a novel IK blocking drug, UK-68,798: potent in guinea pig but no effect in rat myocardium, J. Cardiovasc. Pharmacol. 16, 401.

Tseng, G.-N. and B.F. Hoffman, 1989, Two components of transient outward current in canine ventricular myocytes, Circ. Res. 64, 633.

Varro, A., D.A. Lathrop, S.B. Hester, P.P. Nanasi and J.G.Y. Papp, 1993, Ionic currents and action potentials in rabbit, rat, and guinea pig ventricular myocytes, Basic Res. Cardiol. 88, 93.

Wang, Z., B. Fermini and S. Nattel, 1991, Repolarization differences between guinea pig atrial endocardium and epicardium: evidence for a role of Ito, Amer. J. Physiol. 260, H1501.

Wood, J.N., J. Winter, I.F. James, H.P. Rang, J. Yeats and S. Bevan, 1988, Capsaicin-induced ion fluxes in dorsal root ganglion cells in culture, J. Neurosci. 8, 3208.

Zernig, G., P. Holzer and F. Lembeck, 1984, A study of the mode and site of action of capsaicin in guinea-pig heart and rat uterus, Naunyn-Schmied. Arch. Pharmacol. 326, 58.