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Effects of Class III antiarrhythmic agents in an in vitro rabbit model of spontaneous torsades de pointe

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Abstract

Acquired long QT syndrome develops as a result of pharmacological interventions that prolong action potential duration. Excessive action potential prolongation may lead to torsade de pointes, a potentially fatal arrhythmia. To study this arrhythmia, in vivo models have been developed, but are difficult to interpret due to the complex nature of the intact metabolic, nervous and humoral systems. To more clearly examine the propensity of various Class III agents to elicit torsades de pointe, an in vitro model of spontaneous torsades de pointe was used in isolated perfused rabbit hearts. Male New Zealand white rabbits were anesthetized with sodium pentobarbital, and hearts isolated and perfused in a Langendorff apparatus. Electrocardiogram and epicardial monophasic action potentials were continuously recorded, and methoxamine (30 nM) and acetylcholine (0.3 μM) were given throughout the experiment. After 10 min of methoxamine and acetylcholine perfusion, Class III agents, dofetilide (0.1 to 0.7 μM), E-4031 (0.1 to 0.5 μM), D-sotalol (10 to 30 μM), or clofilium (0.1 to 0.3 μM), were given. All agents, except D-sotalol, induced torsades de pointe in 100% of hearts tested. D-Sotalol (30 μM) elicited a low incidence of torsades de pointe (25%). This could be explained by the limited prolongation of action potential duration with D-sotalol as compared to other Class III agents under these conditions. Dofetilide, a selective Class III agent, alone did not induce torsades de pointe. Nadolol (3 μM), a β-adrenoceptor antagonist, increased the propensity of dofetilide to elicit torsades de pointe. In conclusion, increases in action potential duration (i.e., using Class III agents) in combination with a low heart rate (muscarinic receptor stimulation) and increases in intracellular Ca^{2+} (α-adrenoceptor stimulation) are needed to develop torsades de pointe in this model. Modulating these systems may provide us with new insights into preventing the initiation or maintenance of this arrhythmia.

Keywords: Dofetilide; Clofilium; E-4031; D-Sotalol; Langendorff; Arrhythmia; Action potential, monophasic; Heart rate

1. Introduction

There is a therapeutic need for more effective and safe antiarrhythmic agents. Recent development of antiarrhythmic agents has been focused in the area of Class III agents. These compounds exert their antiarrhythmic effects by delaying action potential repolarization (i.e., increasing action potential duration) and increasing refractoriness. One such class of compounds act via blockade of an outward repolarizing K^+ current known as the delayed rectifier (Vaughan-Williams, 1970; Colatsky and Follmer, 1989; Vaughan-Williams, 1989, 1992). The delayed rectifier is composed of two components: a rapidly activating and inactivating component, and a slowly activating and deactivating component (Sanguinetti and Jurkiewicz, 1990). Most, if not all, of the Class III agents that block the rapid component of the delayed rectifier, have been associated with proarrhythmic activity known as torsades de pointe (Buchanan et al., 1993; Vos et al., 1995). Furthermore, the survival with oral D-sotalol (SWORD) trial demonstrated an increased risk of mortality with D-sotalol, a prototypical Class III agent known to block the rapid component of the delayed rectifier (Waldo et al., 1995). SWORD raised caution to the use of all Class III agents, because of their potential for proarrhythmic effects. Thus, the development of any Class III agent must be weighted against its liability to induce proarrhythmic responses. To further uncover the liabilities of these agents, a better understanding of the mechanism(s) of the arrhythmias that they induce is warranted.

Torsades de pointe is usually initiated with a slowing of the heart rate in combination with an increase in action potential duration or QT intervals (Roden et al., 1986;

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Cranefield and Aronson, 1988; Cranefield and Aronson, 1991; Roden, 1993). Torsades-like arrhythmias are characterized by electrocardiograms exhibiting a series of rapid repetitive polymorphic QRS complexes with characteristic undulating peaks. Concerns regarding torsades de pointe are founded in the propensity of this arrhythmia to lead to ventricular fibrillation and sudden cardiac death.

Due to the possible proarrhythmic liabilities of Class III antiarrhythmic agents, newly developed agents should be tested in animal models that address their proarrhythmic potential. Recently, investigators have described an in vivo rabbit model in which polymorphic ventricular tachycardia, similar to torsades de pointe, could be consistently initiated by a combination of an α-adrenoceptor agonist and a Class III antiarrhythmic agent (Carlsson et al., 1990). More recently, an in vitro model of torsades de pointe in the isolated perfused rabbit heart has been described (Zabel et al., 1997). The purpose of this study was to study the potential of Class III agents: clofilium, E-4031, dofetilide and D-sotalol to generate torsades de pointe in our isolated rabbit heart model, and to determine some of the contributing factors to the development of this arrhythmia in our model. A preliminary report using this model has been published previously (Zhu et al., 1997).

2. Materials and methods

2.1. Surgical procedure

All experiments in this study conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1985). Male New Zealand white rabbits (2.0–2.5 kg) were anesthetized with sodium pentobarbital (50 mg/kg i.v.) and anticoagulated with sodium heparin (500 U i.v.) injected into a marginal ear vein. Following anesthesia, the neck skin was opened, a tracheotomy was performed, and the animal was ventilated with room air. 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. A cannula was connected to a three-way stopcock that was connected to a reservoir containing a modified Krebs–Henseleit bicarbonate buffer solution (room temperature) equilibrated with 95% O_2/5% CO_2. The Krebs–Henseleit solution was composed of the following (mM): 112 NaCl, 2.0 KCl, 11.5 glucose, 25 $NaHCO_3$, 0.2 $MgSO_4$, 2.4 $CaCl_2$, 1.0 KH_2PO_4, at pH 7.4. The inferior vena cava was clamped just above the diaphragm and an incision made in the aorta. The cannula was quickly inserted into the opening made in the aorta, and secured with suture. Once the heart was perfused, it was carefully excised from the chest, transferred to the Langendorff apparatus, and perfused with Krebs–Henseleit solution via a pump (Ismatec) at a constant flow (30 ml/min) and temperature (37 ± 0.2°C).

The heart was mounted in a horizontal position. The electrocardiogram and an epicardial monophasic action potential (Franz epicardial Langendorff probe; EP Technologies, Sunnyvale, CA) were continuously recorded 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).

2.2. Experimental protocol

The hearts were allowed to equilibrate for 20 min before control measurements were taken. A perfusion of acetylcholine (0.3 μM) and methoxamine (30 nM) were continuously given throughout the experiment following control recordings. After 10 min of methoxamine and acetylcholine perfusion, Class III agents were given at increasing concentrations at 30-min intervals until torsades de pointe occurred or the highest concentration was reached (Fig. 1). Heart rate, QT-intervals, and monophasic action potential duration at 90% repolarization were measured at specific time points throughout the experiment. Torsades

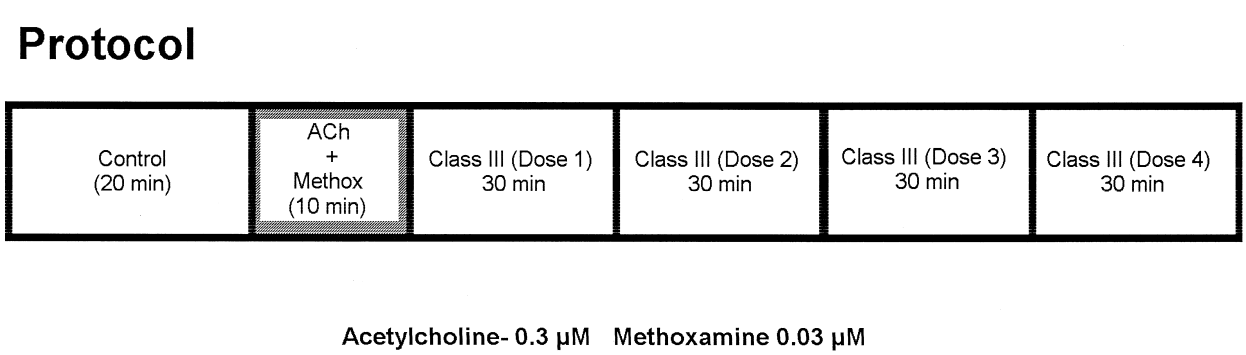

Fig. 1. Protocol design for the induction of torsades in the isolated perfused rabbit heart. Following a 20-min control period in which baseline readings were taken. Acetylcholine (0.3 μM; ACh) and methoxamine (0.03 μM; Methox) were given for 10 min and throughout the experiment. Readings were repeated at the end of the initial 10-min period. Increasing concentrations of a Class III antiarrhythmic agents were administered for 30 min or until the initiation of torsades was noted.

de pointe was defined as a polymorphic ventricular tachycardia of greater than five beats as shown in Fig. 2.

2.3. Drug preparation and administration

Stock solutions of acetylcholine and methoxamine (Sigma, St. Louis, MO) were prepared in dimethylsulfoxide (Fisher Scientific) at concentrations of 10^{-1} M and 10^{-2} M, respectively. A working solution was prepared by dissolving 0.09 ml of each stock solution to 100 ml of Krebs–Henseleit solution, and administered at a rate of 0.1 ml/min. This yielded a final concentration of 0.3 and 0.03 μM for acetylcholine and methoxamine, respectively, that was delivered to the heart. Stock solutions of Class III agents were prepared in dimethylsulfoxide at 10^{-2} M.

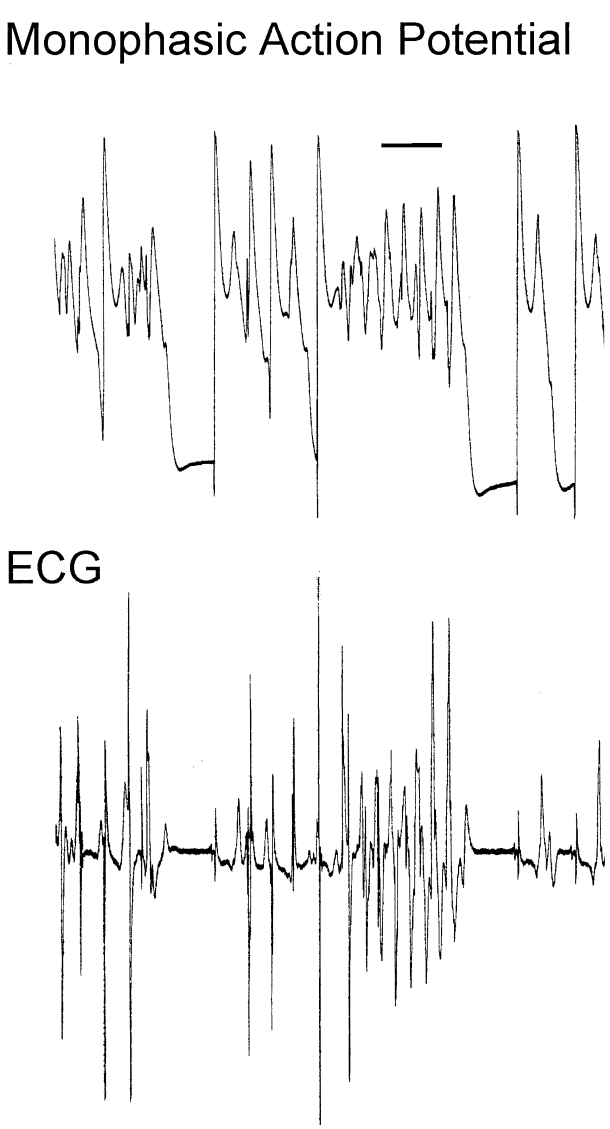

Fig. 2. An example of dofetilide-induced torsades de pointe in the isolated perfused rabbit heart. Monophasic action potential recordings and electrocardiogram were simultaneously recorded. Torsades de pointe was characterized as a polymorphic ventricular tachycardia of at least five beats. Time bar represents 2 s.

D-Sotalol was also prepared as a 10^{-1} M stock solution. Working solutions were prepared by dissolving the stock solution into 100 ml Krebs–Henseleit solution to achieve appropriate final concentrations. All agents were administered through a sideport on the Langendorff apparatus using an infusion pump. The concentration of dimethylsulfoxide delivered to the heart did not exceed 0.05% and flow rates to deliver agents did not exceed 1 ml/min. Dofetilide, D-sotalol, and clofilium were synthesized by the Bristol-Myers Squibb chemists. E-4031 was obtained from Eisai Pharmaceuticals.

2.4. Statistics

Data are represented as mean ± S.E.M. Percent changes in the QT interval and action potential duration were calculated relative to the methoxamine data. Comparisons were made using a Tukey's test. Significant differences were determined at $P < 0.05$ level.

3. Results

3.1. Torsades in the isolated perfused rabbit heart

Treatment with acetylcholine and methoxamine caused a slight reduction in heart rate (7% to 11%) and prolongation of action potential duration between 8% and 18% (Table 1). In none of the treatment groups did pretreatment with acetylcholine, methoxamine or their combination elicit arrhythmogenic activity. Dofetilide, E-4031 and clofilium all caused a concentration-dependent increase in the incidence of torsades de pointe. Extra systolic beats (couplets) and early afterdepolarizations generally preceded torsades de pointe. Each agent caused a 100% incidence of torsades de pointe at concentrations below 1 μM. Rates of torsades de pointe ranged from 170 to 220 bpm. Only D-sotalol was unable to elicit a high incidence of torsades de pointe (25% incidence at 30 μM). Administration of 100–300 μM D-sotalol produced no further increase in the incidence of torsades de pointe. All agents caused a further reduction in heart rate relative to acetylcholine and methoxamine pretreatment. Heart rates were decreased by approximately 30% with each agent except clofilium (17% reduction in heart rate). Clofilium treated hearts immediately developed torsades de pointe, which prevented an accurate measurement of heart rate. Action potential duration values were increased with all Class III agents. However, D-sotalol was least effective ($36 \pm 6\%$) in increasing action potential duration values compared to dofetilide ($76 \pm 9\%$), E-4031 ($85 \pm 15\%$) and clofilium ($55 \pm 4\%$). Consequently, D-sotalol was the least proarrhythmic agent in this model. It is important to note that changes in QT paralleled those of the action potential duration. Thus, changes in QT are not presented.

Table 1
Effects of methoxamine (Methox), acetylcholine (ACh) and Class III agents on heart rate (HR) and action potential duration measured at 90% repolarization (APD) in isolated rabbit hearts

Treatment	HR (bpm)	ΔHR (%)	APD (ms)	ΔAPD (%)	EB (%)	EAD (%)	TdP (%)
Dofetilide (n = 10)							
Control	185 ± 7	0 ± 0	130 ± 2	0 ± 0	0	0	0
Methox + ACh	172 ± 7	−7 ± 2	139 ± 5	9 ± 3	0	0	0
0.1 μM	158 ± 5[a]	−13 ± 2[a]	167 ± 5[a]	29 ± 5[a]	10	0	0
0.3 μM	133 ± 5[a]	−27 ± 2[a]	210 ± 10[a]	61 ± 5[a]	40	50	40
0.5 μM	122 ± 4[a]	−31 ± 3[a]	231 ± 15[a]	76 ± 9[a]	90	80	90
0.7 μM	N/A	N/A	N/A	N/A	90	80	100
E-4031 (n = 6)							
Control	180 ± 8	0 ± 0	117 ± 5	0 ± 0	0	0	0
Methox + ACh	160 ± 6	−11 ± 2	137 ± 6[a]	18 ± 6[a]	0	0	0
0.1 μM	138 ± 8[a]	−24 ± 4[a]	195 ± 12[a]	68 ± 11[a]	17	33	33
0.3 μM	128 ± 3[a]	−32 ± 4[a]	210 ± 12[a]	85 ± 15[a]	33	50	83
0.5 μM	N/A	N/A	N/A	N/A	50	100	100
Clofilium (n = 6)							
Control	182 ± 5	0 ± 0	117 ± 8	0 ± 0	0	0	0
Methox + ACh	168 ± 5	−8 ± 2	126 ± 6	8 ± 5	0	0	0
0.1 μM	151 ± 7[a]	−17 ± 3[a]	180 ± 10[a]	55 ± 4[a]	67	83	83
0.3 μM	N/A	N/A	N/A	N/A	83	83	100
D-Sotalol (n = 8)							
Control	185 ± 7	0 ± 0	122 ± 1	0 ± 0	0	0	0
Methox + ACh	168 ± 7	−9 ± 2	135 ± 3	11 ± 2	0	0	0
10 μM	143 ± 4[a]	−23 ± 2[a]	154 ± 6[a]	26 ± 4[a]	13	0	0
30 μM	129 ± 8[a]	−31 ± 4[a]	167 ± 8[a]	36 ± 6[a]	38	13	25
300 μM	N/A	N/A	N/A	N/A	50	13	25

The percentage of hearts exhibiting extra beats (EB), early afterdepolarizations (EAD) and torsades de pointes (TdP) are given.
N/A: not available due to the presence of arrhythmias.
[a]Significantly different ($P < 0.05$) from corresponding control values.

3.2. Contribution of acetylcholine and methoxamine to induce torsades de pointe

To further understand the role of acetylcholine and methoxamine in our model, we compared the effects of various combinations of these agents with dofetilide. Acetylcholine alone caused a slight, but non-significant, reduction in heart rate relative to control, whereas methoxamine alone had no apparent effect on heart rate. Neither acetylcholine nor methoxamine alone caused any significant change in action potential duration. Dofetilide alone or in combination with acetylcholine and/or methoxamine caused a similar decrease in heart rate of approximately 30% (Table 2). These data demonstrate that the major effects on heart rate were due to administration of dofetilide alone. Interestingly, dofetilide alone did not cause as much prolongation of action potential duration as when combined with acetylcholine and/or methoxamine. Furthermore, at 0.5 and 0.7 μM dofetilide alone, action potential duration values decreased relative to preceding concentrations (Table 1). Dofetilide (0.7 μM) alone caused extra beats and formation of early afterdepolarizations, but failed to elicit torsades de pointe. In the presence of acetylcholine, dofetilide demonstrated a 25% incidence of torsades de pointe, although action potential duration values remained prolonged. In the presence of methoxamine, dofetilide elicited torsades de pointe in 67% of the hearts, but at 0.7 μM action potential duration values were decreased relative to preceding concentrations. The combination of all three agents (acetylcholine, methoxamine and dofetilide) produced additive effects in which torsades de pointe was developed in 100% of hearts. Action potential duration values remained prolonged and were longest in this group of hearts.

3.3. Effects of β-adrenoceptor blockade on dofetilide-induced torsades de pointe

Finally, the effects of β-adrenoceptor stimulation were investigated. Specifically, we looked at the effects of nadolol, a β-adrenoceptor antagonist, plus acetylcholine and methoxamine on the ability of dofetilide to elicit torsades de pointe. Heart rates were reduced by 7% following acetylcholine and methoxamine treatment. The addition of nadolol (3 μM) caused a further reduction (6%) in heart rate to 13%. However, heart rates were decreased 27 ± 2% at 0.3 μM dofetilide in the absence of nadolol and 26 ± 1% following treatment with 0.1 μM dofetilide

Table 2
Effects of acetylcholine (ACh; 300 nM), methoxamine (Methox; 30 nM) and dofetilide (0.1 to 0.7 μM) alone or in combination on heart rate (HR), action potential duration measured at 90% repolarization (APD) and incidences of extra beats (EB), early afterdepolarization (EAD) and torsades de pointes (TdP) in isolated rabbit hearts

Treatment	HR (bpm)	ΔHR (%)	APD (ms)	ΔAPD (%)	EB (%)	EAD (%)	TdP (%)
Dofetilide (n = 4)							
Control	184 ± 7	0 ± 0	132 ± 4	0 ± 0	0	0	0
0.1 μM	139 ± 4[a]	−24 ± 1[a]	177 ± 5[a]	34 ± 4[a]	0	0	0
0.3 μM	128 ± 5[a]	−31 ± 2[a]	193 ± 9	46 ± 4[a]	0	0	0
0.5 μM	123 ± 5[a]	−33 ± 2[a]	173 ± 16[a]	34 ± 9[a]	0	0	0
0.7 μM	123 ± 6[a]	−33 ± 3[a]	161 ± 18	25 ± 10	75	25	0
Acetylcholine + Dofetilide (n = 4)							
Control	190 ± 4	0 ± 0	123 ± 5	0 ± 0	0	0	0
ACh	175 ± 5[a]	−8 ± 1[a]	142 ± 6	16 ± 4	0	0	0
0.1 μM	143 ± 4[a]	−25 ± 2[a]	176 ± 9[a]	44 ± 8[a]	25	25	25
0.3 μM	135 ± 3[a]	−30 ± 1[a]	193 ± 4[a]	55 ± 5[a]	25	25	25
0.5 μM	128 ± 2[a]	−34 ± 1[a]	211 ± 11[a]	69 ± 6[a]	25	50	25
0.7 μM	130 ± 0[a]	−33 ± 1[a]	213 ± 17[a]	69 ± 9[a]	25	75	25
Methoxamine + Dofetilide (n = 6)							
Control	176 ± 4	0 ± 0	120 ± 4	0 ± 0	0	0	0
Methox	167 ± 4	−5 ± 1	133 ± 5	11 ± 4	0	0	0
0.1 μM	153 ± 4[a]	−13 ± 3[a]	175 ± 19[a]	48 ± 18[a]	17	33	17
0.3 μM	138 ± 6[a]	−20 ± 3[a]	184 ± 26[a]	52 ± 19[a]	33	33	67
0.5 μM	128 ± 8[a]	−28 ± 3[a]	202 ± 3[a]	63 ± 10[a]	33	67	67
0.7 μM	125 ± 4[a]	−30 ± 2[a]	N/A	N/A	33	67	67
Acetylcholine + Methoxamine + Dofetilide (n = 10)							
Control	185 ± 7	0 ± 0	130 ± 2	0 ± 0	0	0	0
ACh + Methox	172 ± 7	−7 ± 2	139 ± 5	9 ± 3	0	0	0
0.1 μM	158 ± 5[a]	−13 ± 2[a]	167 ± 5[a]	29 ± 5[a]	0	0	0
0.3 μM	133 ± 5[a]	−27 ± 2[a]	210 ± 10[a]	61 ± 5[a]	40	50	40
0.5 μM	122 ± 4[a]	−31 ± 3[a]	231 ± 15[a]	76 ± 9[a]	90	80	90
0.7 μM	N/A	N/A	N/A	N/A	90	80	100

N/A: not available due to the presence of arrhythmias.
[a]Significantly different ($P < 0.05$) from corresponding control values.

in the presence of nadolol (Table 3). Action potential duration values were increased slightly more in the presence of nadolol at similar concentrations of dofetilide (29 ± 5% and 36 ± 5% increases in action potential duration with 0.1 μM dofetilide in the presence and absence of nadolol, respectively). Thus, nadolol shifted the incidence graph of torsades de pointe with dofetilide to the left.

Table 3
Effects of methoxamine (Methox) plus acetylcholine (ACh) and dofetilide in the presence and absence of nadolol (Nad; 3 μM) on heart rate (HR), action potential duration measured at 90% repolarization (APD) and incidence of torsades de pointes (TdP) in isolated perfused rabbit hearts

Treatment	HR (bpm)	ΔHR (%)	APD (ms)	ΔAPD (%)	TdP (%)
Without nadolol (n = 10)					
Control	185 ± 7	0 ± 0	130 ± 2	0 ± 0	0
Methox + ACh	172 ± 7	−7 ± 2	139 ± 5	9 ± 3	0
Dofetilide 0.1 μM	158 ± 5[a]	−13 ± 2[a]	167 ± 5[a]	29 ± 5[a]	0
Dofetilide 0.3 μM	133 ± 5[a]	−27 ± 2[a]	210 ± 10[a]	61 ± 5[a]	40
With nadolol (n = 6)					
Control	186 ± 6	0 ± 0	117 ± 5	0 ± 0	0
Nad + Methox + ACh	157 ± 9[a]	−13 ± 3[a]	140 ± 4	11 ± 2	0
Dofetilide 0.1 μM	138 ± 3[a]	−26 ± 1[a]	172 ± 9[a]	36 ± 5[a]	50
Dofetilide 0.3 μM	N/A	N/A	N/A	N/A	100

N/A: not available due to the presence of arrhythmias.
[a]Significantly different ($P < 0.05$) from corresponding control values.

4. Discussion

Class III antiarrhythmic agents exert their effects by delaying myocardial repolarization and increasing refractoriness (Vaughan-Williams, 1970; Colatsky and Follmer, 1989; Vaughan-Williams, 1989, 1992). However, by selectively delaying repolarization, there exists the liability of inducing a potentially fatal arrhythmia known as torsades de pointe. To better understand the mechanism(s) underlying torsades de pointe, we used an in vitro model of spontaneous torsades de pointe in the isolated perfused rabbit heart. In the present study, we describe the potential of various Class III agents to elicit torsades de pointe. Second, we evaluated the contributions of acetylcholine (acetylcholine receptor stimulation) and methoxamine (α-adrenoceptor stimulation) on their potential to induce tor-

sades de pointe either alone or in combination with dofetilide. Finally, we evaluated the effects of β-adrenoceptor blockade as it relates to dofetilide-induced torsades de pointe in this model.

4.1. Proarrhythmic effects of Class III agents

All Class III antiarrhythmic agents, except D-sotalol, provoked torsades de pointe in 100% of hearts tested. The order of potency to induce arrhythmias was clofilium > E-4031 > dofetilide > D-sotalol. The reason why D-sotalol was less effective in eliciting torsades de pointe can be explained by the reduced ability of this compound to increase action potential duration in these hearts. D-Sotalol at 100 μM did not increase action potential duration values relative to 30 μM (data not shown). We have observed that it was also more difficult to elicit torsades de pointe in vivo in the anesthetized rabbit with D-sotalol as compared to other Class III agents such as dofetilide and E-4031 (unpublished observations). The reduced incidence of torsades de pointe with D-sotalol may have been due to its antimuscarinic activity (Mori et al., 1995; Uemura et al., 1995). However, this alone is unlikely the reason for D-sotalol's lack of effect, since cholinergic stimulation accounts for only 25% of the torsades de pointe activity observed with dofetilide-induced torsades de pointe in our model (see below). Furthermore, the concentrations needed to block muscarinic M_2 receptors are above those needed to develop torsades de pointe in vivo. Another possibility, is that D-sotalol requires an intact and active autonomic tone not present in either isolated hearts or anesthetized animals (Vos et al., 1995). At this time, it not known why D-sotalol causes a minimal degree of torsades de pointe in this in vitro model. It has recently been reported that D-sotalol at high concentrations elicits consistently torsades de pointe in an isolated rabbit heart (Zabel et al., 1997), and the difference between this model and ours may be attributed to the use of AV nodal ablation to achieve slower heart rates.

4.2. Contribution of cholinergic and α-adrenoceptor stimulation to the development of dofetilide-induced torsades de pointe

In our initial attempts to develop this in vitro model, we observed that the combination of methoxamine, an α_1-adreceptor agonist, with dofetilide did not consistently elicit torsades de pointe. However, the addition of acetylcholine, a muscarinic receptor agonist, yielded a 100% incidence of torsades de pointe, i.e., the combination of acetylcholine, methoxamine and dofetilide. These results suggest that the development of torsades de pointe in this model is dependent upon at least three general processes: (1) a prolongation of the action potential duration, (2) an increase in intracellular Ca^{2+}, and (3) a slowing of heart rate. Class III agents contribute to each of these processes. Once a consistent model of torsades de pointe was developed, studies were designed to determine the relative contribution of α_1-adrenoceptor and muscarinic M_2 receptor stimulation to the development of torsades de pointe.

4.2.1. Contribution of α-adrenoceptor stimulation

There are two types of α-adrenoceptors, α_1 and α_2. However, α_2-adrenoceptors, have not been conclusively found in the heart (Fedida et al., 1993). There are at least two subtypes of α_1 receptors, α_{1A} and α_{1B}, of which the latter is predominant in the heart (Benfey, 1990). For the purposes of this discussion α_1-adrenoceptors subtypes will not be distinguished. Both muscarinic M2 receptors and α_1 adrenoceptors mediate their effects through distinct pathways (Hathaway and March, 1989; Berridge, 1993; Yost, 1993).

Stimulation of myocardial α-adrenoceptors increases levels of intracellular inositol triphosphate and diacylglycerol through a G-protein (Gq) sensitive mechanism. Inositol triphosphate releases Ca^{2+} from inositol triphosphate sensitive stores in the sarcoplasmic reticulum. Diacylglycerol increases protein kinase C activity, which enables the activation of a wide variety of Ca^{2+}-dependent kinases. Both the activities of inositol triphosphate and diacylglycerol serve to increase intracellular calcium concentrations either directly (through inositol triphosphate) or indirectly (through diacylglycerol) by an increase in protein kinase C. In addition to elevation of intracellular Ca^{2+}, increases in inositol triphosphate and diacylglycerol can regulate the activity of several ion channels including the inward rectifier and transient outward currents (Braun et al., 1990, 1992). How these processes contribute to the development of torsades de pointe can now be addressed in future studies using the isolated heart. Specifically, agents used to alter the actions of inositol triphosphate, protein kinase C, or diacylglycerol may be rapidly metabolized, require large amounts of compound or have deleterious effects when given to the intact animal, but can be tested easily in an in vitro heart model (Zhu et al., 1997).

4.2.2. Contribution of cholinergic stimulation

Cholinergic stimulation of the heart is predominantly mediated through muscarinic M2 receptors (Peralta et al., 1987). As with α_1-adrenoceptor stimulation, muscarinic receptor stimulation also activates G-proteins. However, acetylcholine activation is through the inhibitory GTP-binding protein, Gi, which is independent of the α_1-adrenoceptor pathway (Ashkenazi et al., 1987). Muscarinic M_2 receptor stimulation leads to a decrease in adenyl cyclase activity. In addition, it has been implicated in the slowing of heart rate by increasing conductance through acetylcholine-sensitive potassium channels (Krapivinsky et al., 1995), and stimulation of muscarinic M_2 receptors in the rat heart has been associated with increases in inositol triphosphate (Brown et al., 1985). However, the amount of inositol triphosphate generated through muscarinic stimula-

tion is less than that through α_1-adrenoceptor stimulation. The reason for this discrepancy is currently unknown. However, it does not appear to be due differences in receptor density, since α_1-adrenoceptors are less abundant than muscarinic M_2 receptors as determined by radiolabeled binding (Buxton and Brunton, 1985; Buxton et al., 1985). Nonetheless, the action of muscarinic M_2 receptor stimulation is likely through two different receptors (muscarinic M_1 and M_2 receptors) coupled to different G-proteins, Gq and Gi, respectively. However, it would appear that α-adrenoceptor stimulation contributes more to the development of torsades de pointe, since there is a reduced incidence of torsades de pointe observed in our model with the combination of acetylcholine and dofetilide (25%) relative to the combination of methoxamine and dofetilide (67%). Further work is needed to more fully understand the contribution of muscarinic M_2 receptors and α-adrenoceptor stimulation in the development of torsades de pointe under these conditions.

4.3. Contribution of β-adrenoceptor blockade to the development of dofetilide-induce torsades de pointe

There are two major forms of torsades de pointe associated with long QT syndrome: acquired and hereditary long QT syndrome (Tan et al., 1995). Acquired long QT syndrome, as the name implies, is typically the result of pharmacological or toxicological interventions. For example, Class III antiarrhythmics (as stated above) contribute to this form of torsades de pointe. These arrhythmias are pause-dependent, i.e., bradycardia-induced, and are exacerbated by conditions such as hypokalemia, bradycardia and hypomagnesia. In contrast, increasing K^+, heart rate or Mg^{2+} levels can ameliorate torsades de pointe. Blocking Na^+ channels with lidocaine (which shortens action potential duration and reduces Ca^{2+} influx) or blocking of Ca^{2+} channels can also suppress torsades de pointe. Due to the bradycardic nature of β-adrenoceptor antagonists, they are contradicted in acquired long QT syndrome. However, β-adrenoceptor agonists or electrical pacing may be used to suppress torsades de pointe. It is postulated that our in vitro model closely mimics the acquired long QT syndrome.

In support of a model of acquired long QT syndrome in our isolated heart, it was shown that nadolol, a non-selective β-adrenoceptor antagonist, was found to increase the sensitivity of the myocardium to dofetilide-induced torsades de pointe. Specifically, nadolol shifted the dofetilide-induced torsades de pointe response curve to the left. These data demonstrate that in the isolated heart there is a background level of catecholamines, since heart rates were further decreased in the presence of nadolol. The ability of nadolol to enhance the proarrhythmic action of dofetilide may be explained: (1) a further reduction in heart rate; (2) a reduction in norepinephrine's effect on β-adrenoceptor. This would prevent Ca^{2+} from being sequestered as efficiently as in the presence of adrenergic stimulation. Stimulation of β-adrenoceptors would increase Ca^{2+}-ATPase pump activity through a cAMP mediated effect. Thus, β-adrenoceptor blockade would tend to maintain elevated calcium levels and allow torsades de pointe to be more easily be generated; and (3) the effects of β-adrenoceptor blockade may reduce the current of the slow component of the delayed rectifier activity (Sanguinetti et al., 1991), which has been shown to contribute to repolarization in the rabbit heart (Salata et al., 1996). Further studies are needed to more fully address these issues.

4.4. Limitations of the study

There are several limitations of this study. First, recordings of the monophasic action potential were made from the epicardial surface. Arrhythmias initiating from other areas of the myocardium would go undetected. Furthermore, regional changes in action potential duration with Class III agents might have been missed due to limited recording of the monophasic action potential electrode. Second, no measurements of electrical dispersion were made in these studies. Thus, we were unable to determine if differences in inducibility were related to changes in the degree dispersion created with these agents. Third, since no mapping of the heart was made, we could not discern the underlying mechanism of torsades de pointe in our model, i.e., triggered activity or reentry.

4.5. Conclusions

In conclusion, we employed an in vitro model of torsades de pointe in the isolated perfused rabbit heart. There are three major factors that are needed for torsades de pointe to occur: (1) an increase in action potential duration, (2) an increase in intracellular calcium, and (3) a decrease in heart rate. This model represents a unique opportunity for the study of various systems and their involvement in the generation of torsades de pointe as characterized in the isolated rabbit heart. Future studies will attempt to determine the importance of these systems in the generation of arrhythmias, and may provide a molecular target(s) for prevention or suppression of torsades de pointe when developing new antiarrhythmic agents. This may be achieved by probing the underlying biophysical and biochemical pathways involved in torsades de pointe through molecular or pharmacologic manipulation.

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