Acquired Torsade de Pointes arrhythmias : role of early afterdepolarizations and dispersion of repolarization

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Acquired Torsade de Pointes arrhythmias role of early afterdepolarizations and dispersion of repolarization

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Acquired Torsade de Pointes arrhythmias role of early afterdepolarizations and dispersion of repolarization

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aan de Rijksuniversiteit Limburg te Maastricht,
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volgens het besluit van het College van Dekanen,
in het openbaar te verdedigen
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List of Abbreviations

Δ APD	: Dispersion of action potential duration
APD	: Action potential duration
AV	: Atrioventricular
AT	: Activation time
CL-IVR	: Cycle length of the idioventricular rhythm
EAD(s)	: Early afterdepolarization(s)
EB(s)	: Ectopic beat(s)
LV	: Left ventricle
MAP	: Monophasic action potential
PES	: Programmed electrical stimulation
PF	: Purkinje fibers
RV	: Right ventricle
SEM	: Standard error of mean
SR	: Sinus rhythm
SD	: Standard deviation
SLS	: Short long short sequence
TdP	: Torsade de Pointes arrhythmia(s)
VT	: Ventricular tachycardia

Preface

Every human being experiences cardiac arrhythmias during his or her life. The severity may vary from innocent occasional ectopic beats to life threatening arrhythmias. Treatment of cardiac arrhythmias should preferably be based on knowledge about the different responsible mechanisms. Several mechanisms are known, of which reentry is studied best, but there is also abnormal automaticity and triggered activity. The relevance of these different mechanisms for every clinically occurring arrhythmia is not clear. The studies described in this thesis are focussing on the phenomenon of the appearance of a new and different arrhythmia occurring after treatment with drugs aimed to prevent and/or suppress heart rhythm disturbances. This is called a proarrhythmic effect of antiarrhythmic drugs.

The name of the particular arrhythmia, which is the focus of this dissertation, is Torsade de Pointes (TdP). In the past several other names have been used to describe this arrhythmia because of its typical configuration. It is known for a long time that TdP arrhythmias occur in the presence of prolonged ventricular repolarization which can be recognized on the electrocardiogram as a prolonged QT interval. They are more common during bradycardia and are characterized by a specific twisting of the QRS complexes like a corkscrew around the baseline of ECG. The arrhythmia often terminates spontaneously but may deteriorate into ventricular fibrillation and thereby lead to the death of the patient.

Although investigations have been performed to study the circumstances leading to this arrhythmia and how to prevent it, no clinically relevant animal model existed in the early nineties in which the mechanism of TdP could be reproducibly studied.

The aim of the research reported in this thesis was to evaluate the value of an animal model of acquired TdP arrhythmias in which the mechanism of initiation, continuation and termination of the arrhythmia could be studied. Moreover such a model could allow the comparison of potential of different (new) antiarrhythmic drugs to induce TdP under well controlled and reproducible circumstances.

This thesis consists of 8 chapters. In chapter 1 a review is given of the literature on different models and the mechanism(s) of acquired Torsade de Pointes arrhythmias that appeared until 1992 (the start of this PhD thesis). The dog model developed in Maastricht to study acquired TdP is described in chapter 2. The role of early after-depolarizations (EADs) and interventricular dispersion of repolarization (Δ APD) in the generation of TdP is discussed in chapters 3 and 4. Using a group comparison (inducible versus non inducible dogs) the role of these parameters was determined. In chapter 3 the suppressive effect of magnesium is also reported. In chapter 4, two drugs (*d*-sotalol and almokalant) were administered in two separate experiments in the same dog to

evaluate the relevance of EADs and interventricular Δ APD for the induction of TdP. The contribution of EADs to interventricular Δ APD is the subject of chapter 5. This was done by studying the dynamic changes in between reproducible induced TdP episodes. Chapter 6 relates to the treatment of TdP by two drugs that are known to influence intracellular calcium, flunarizine and ryanodine. The influence of the electrophysiologic changes induced by chronic complete AV block on Torsade de Pointes incidence is discussed in chapter 7, by studying the dog at two different time points (0 and 5 weeks) after AV block. Chapter 8 contains a general discussion using the data presented in this thesis and the most recent literature. This is followed by a summary in English and Dutch.

Chapter 1

Review of the literature

1.1 Introduction

Torsade de Pointes arrhythmias (TdP) are defined as polymorphic tachycardias showing a specific rotating pattern of the QRS complexes around the baseline in the presence of a prolonged repolarization (figure 1). TdP was first used by Dessertenne to describe a polymorphic tachycardia in a patient with acute third degree AV block (1). Since that time the name TdP has been used, although initially other names were also given like cardiac ballet, aspecific polymorphous tachycardia, atypical tachycardia, and transient or self terminating ventricular fibrillation (2,3).

TdP can be found in two different patient populations: 1) patients with a congenital and therefore genetically determined cause of prolonged repolarization and 2) patients with an acquired form of prolonged repolarization (4).

The congenital long QT syndrome can be either familial or idiopathic (4). The familial type consist of two subgroups: 1) the Jervell-Lange Nielsen syndrome which is associated with deafness and 2) the Romano Ward syndrome with normal hearing (5). These syndromes are found in a small part of the population. Recently there has been a marked progress concerning their genetic basis (6-8).

Acquired or drug induced TdP is a less rare phenomenon. Its occurrence is higher when synergistic circumstances as bradycardia, hypokalemia and/or hypomagnesemia are present (9). This in contrast to congenital TdP, in which bradycardia does not have to be an essential component (4).

This review will deal with the acquired or drug induced form of TdP. First the clinical circumstances and drugs leading to TdP will be discussed (§1.2). This is followed by a description of several in vitro, in vivo and computer models of TdP (§1.3), which were published before 1992 and formed the background for the development of our dog model of acquired TdP. Thereafter, the importance of ventricular heterogeneity of repolarization for the generation of TdP will be discussed (§1.4). Finally the different possible treatments will be summarized (§1.5).

1.2 Torsade de Pointes arrhythmias in patients

The use of antiarrhythmic drugs can be associated with different proarrhythmic side effects (13). As pointed out by Wellens et al. (13) five different proarrhythmic responses may occur after administration of a drug 1) Worsening of an arrhythmia which is already present; 2) The induction of sinoatrial or atrioventricular block or the suppression of escape mechanisms; 3) Uncovering of a hidden arrhythmia mechanism; 4) Induction of a new arrhythmia mechanism; 5) Combination of 1 to 4. The TdP arrhythmias fall in the fourth category.

Depending upon the antiarrhythmic drug used, TdP arrhythmias may occur in about 1 to 10% of patients (11). In those patients, episodes of TdP are often repetitive till preventive and/or suppressive measures are taken (4). These episodes often end spontaneously but can also deteriorate into ventricular fibrillation (4). In sudden arrhythmic death documented by a Holter recording, a TdP arrhythmia was found in 12% of cases as the initiation of the arrhythmia which led to the death of the patient (12).

Apart from the specific undulating pattern of the QRS complexes, TdP often shows a typical initiation sequence, the so-called short long short sequence (see figure 1) that is present in more than 95% of patients (13-15). It has been suggested to put more emphasis on this mode of initiation, when defining TdP arrhythmias (16).

Another feature which may be observed in the TdP patient is the change in the TU wave morphology. Not only a prolongation of the QT time is seen, but this is often associated with the appearance of a prominent U wave (4, figure 1). On the electrocardiogram, the U wave is the wave that directly follows the T wave, and has under normal circumstances the same direction as the T wave. Abnormalities in repolarization may lead to U waves which are directed opposite to the T wave or a fusion between the T and U wave. The exact origin of the U wave is still not known.

Understanding the mechanism(s) involved in TdP can help to determine the risk of an individual patient and to develop drugs which are devoid of this proarrhythmic effect. The most frequent cause of TdP arrhythmias is the administration of class Ia and class III antiarrhythmic drugs, but also other drugs which prolong repolarization may lead to TdP (see table 1). The antiarrhythmic effect of class Ia and III drugs (table 2) is at least partly based on the prolongation of repolarization. Because no alternative antiarrhythmic drugs are presently available to replace these drugs, prevention of TdP induction by removing these drugs from the market is not a realistic possibility.

In contrast to earlier suggestions (17) more specific K^+ channel antagonists do not seem to have a lower incidence of TdP (11, table 2). Channel specificity can be defined as one channel e.g. Na^+ , Ca^{2+} or K^+ but there is also a subdivision of K^+ channels. However, a drug like amiodarone which blocks many channels, like K^+ , Ca^{2+} and Na^+ channels, has a very low incidence of TdP. Despite a similar lengthening in QT, there are even reports in which amiodarone was safely and effectively administered to patients who developed TdP after other antiarrhythmic drugs (18,19).

Figure 1. Episode of acquired TdP.

The ECG shows in seven simultaneously recorded leads a typical example of a Torsade Pointes arrhythmia after quinidine. Note the QT prolongation, the pronounced U waves (arrows) and the short long short sequence which precedes the TdP.

So although TdP is per definition linked to a prolonged QT duration, (QT values above 600 ms (20) or a QT_c time above 440 ms (15) have been described as a definite risk for development of TdP), no definite QT time can be established that has sufficient specificity and sensitivity to predict TdP.

The development and the use of monophasic action potentials (MAP) catheters, which are able to reliably record the configuration and duration of the action potential (APD) of the heart in specific regions (21-23), offered new opportunities to investigate the electrophysiological mechanism(s) involved in TdP generation. In patients with congenital long QT, the MAP recordings (24,25) showed a "distinct hump" in the repolarization phase. Careful examination of the MAP recordings also showed regional differences in right ventricular APD (24,25), leading to intraventricular dispersion. Similar findings were observed in case reports of patients with acquired TdP (26,27,28). These distinct humps were described as early afterdepolarizations (EADs), and have been

Table 1. Drugs associated with the development of Torsade de Pointes (4,5)

drug group	drug name
Antiarrhythmic drugs	See table 2
Antihistamines	Astemizole Terfenadine
Neuroleptic therapy/antidepressant drugs	Haloperidol Tricyclic antidepressants Tetracyclic antidepressants
Antimicrobial agents	Erythromycin Trimethoprim-sulphamethoxazole
Vasodilatory agents	Papaverine Prenylamine
Apnea therapy	Suxamethonium
Antimalaria treatment	Halofantane
Organophosphate Insecticides	Dimethoate Methylparathion
Cholesterol lowering drugs	Probucol
Prostaglandine	
Diuretics	Furosemide
Cocaine	
Antifungal agents	Ketoconazole Itraconazole
Serotonine antagonist	Tropisetron Ketanserin

defined as a depolarizing potential that begins prior to the completion of repolarization and causes interruption or retardation of repolarization (29). Some studies showed a link between the U wave on the ECG and the EADs on the MAP (26). In one patient with procainamide induced TdP there was a clear difference between the right and left ventricular APD, so called interventricular dispersion (27). In most cases EADs were only seen on the longest APD (responsible for the ventricular dispersion) (27).

1.3 Models of TdP

The mechanism of TdP has been studied in several models. Those studies can be divided in three subgroups: 1) QRS morphology studies using computer models and in vivo data aimed at mimicking the specific TdP configuration, 2) EAD based models by applying toxins and high dosages of drugs in vivo and in vitro, and 3) animal models which try to reproduce the clinical circumstances such as bradycardia and the administration of TdP producing drugs in therapeutic dosages (pharmacological models).

Table 2. Specificity of antiarrhythmic drugs in blocking potassium channels relative to development of Torsade de Pointes arrhythmias (11,17,85)

Class	Drug	I _K	I _{K1}	I _{to}	TdP
Ia	Quinidine	yes	yes	yes	2-8%
	Disopyramide	yes	yes	yes	2-3%
Ib	Lidocaine	no	no	no	0
Ic	Flecainide	yes	no	no	*
	Encainide	yes	no	no	*
II	β blocker	no	no	no	0
III	Sotalol	yes	yes	yes	1-5%
	d-sotalol	yes	yes	yes	1-2%
	Amiodarone	yes	yes	no	<1%
	Clofilium	yes	yes	no	10%
	Almokalant	yes	no	no	6%
	E 4031	yes	no	no	*
	Dofetilide	yes	no	no	*
	Ibutilide	?	?	?	8%
IV	Bepidil				*

*: exact incidence not yet known.

1.3.1 QRS Morphology studies

In the early description of Dessertenne it was suggested that two competing foci were responsible for the twisting morphology of TdP (1). This hypothesis has been tested in a porcine Langendorff heart (30) and in a dog heart (31) in situ. In both cases pacing from a left and right ventricular site at similar cycle length but slightly out of phase resulted in a TdP like configuration (30,31). In a dog with "normal" quinidine concentrations and prolonged APD, application of acotinine on two sites of the heart, leading to two triggering foci, also led to TdP (32). Other studies have stressed the importance of alterations in ventricular activation site rather than two competing foci (33). Thirdly, the TdP morphology can be explained by a reentrant mechanism caused by dispersion caused by differences in duration of repolarization at different sites (34-35). As far as the QRS morphology is concerned, all these theories (the two competing foci, changing activation patterns of one focus or a reentrant circuit) can be possible.

1.3.2 EAD based models © 2015

The registration of EADs in patients with TdP by MAP catheters (24,25) led to investigations as to the presence and causal mechanisms of these humps. In vitro models using canine Purkinje fibers superfused with cesium (36,37), barium (38), quinidine (13,37-40) or procainamide (41) were the first tested models. These studies also allowed evaluation of the frequency dependence of EADs, that demonstrating a clear bradycardia dependence. Many studies are only capable of showing EADs at a cycle length exceeding 2000 ms (36,42). The APD of Purkinje fibers is longer than that of myocardial cells (figure 2). Therefore Purkinje fibers seem to be more sensitive for the generation of EADs and have been most extensively used in vitro.

In vitro studies provide the ideal circumstances to test the ionic background of the EAD. It was demonstrated (table 3) that EADs can be generated under several circumstances, ranging from drugs that block potassium channels, drugs that delay Na^+ -channel inactivation (43) to drugs that open Ca^{2+} channels (44). Each induced EAD seems to have a rather specific way to be suppressed, although in all cases shortening of the APD by acceleration of the rate is effective (table 3). A drug like isoprenaline can be used to induce (45), facilitate (46) or inhibit EADs (46), depending whether its dominating effect was an increase in repolarization or an increase in rate.

EADs have been suggested to occur at different membrane potentials: low membrane potentials: phase 2, in the plateau phase with a take off potential about -30 mV and at higher membrane potentials: phase 3 EADs (-60 mV). After cesium both kinds of EADs (figure 3) have been observed in vitro (36) and in vivo (47). The phase 2 EADs can also be evoked by the Ca^{2+} agonist Bay K 8644 (44), and are logically suppressed by Ca^{2+} antagonists like nitrendipine (48). With ryanodine and the Ca^{2+} chelator BAPTA the phase 2 EAD seem not suppressible (48). These findings suggest that the Ca^{2+} window current is a likely mechanism for the phase 2 EADs (44).

Phase 3 EADs are not only induced by cesium but also occur after treatment with isoprenaline (45). In contrast to phase 2 EADs the phase 3 EADs are responsive to treatment with ryanodine (45). This observation indicates that different channel(s) and/or mechanisms are involved as suggested by Szabo and co workers. They have put emphasis on the $\text{Na}^+/\text{Ca}^{2+}$ exchange (49).

Suppression of EADs is also accomplished with magnesium (37), and substances which are not directly related to ion channels such as prostaglandins (50) and/or α adrenergic blockade (51). The effects of α adrenergic blockade could point to the involvement of an altered intracellular Ca^{2+} handling in the cell.

Most drugs which induce EADs in vitro were also used in the intact dog to investigate whether their application would lead to TdP in vivo. Cesium has been the most often

Figure 2. Frequency dependent behavior of action potential from the different cell types. The action potential duration (APD) of the four different cell types of the canine heart is depicted at cycle lengths ranging from 300 to 5000 ms. With an increase in cycle length the transmural difference increases, because the APD of the Purkinje and to a lesser extent of the M cell prolong relatively more in response to a prolongation of the cycle length. Data modified from references 76 and 84.

used drug (47,50,52-58), but other drugs like anthopleurine A (59) or toxic dosages of quinidine were also administered (33,60).

Because rate is an important factor in clinical TdP, several interventions were made to slow heart rate, like vagal stimulation (58), crushing the sinus node, or the creation of complete AV block (32,47,52). Some drugs like cesium have the advantage of not only prolonging the cycle length but also to create long pauses because of induction of AV block (55) which may act as a trigger for TdP. The limitations of cesium induced polymorphic VT are described in the article of Nayeypour et al. (61).

1.3.3. Models using drugs that are clinically used

Originally, administration of clinically used drugs only lead to the appearance of TdP like morphologies when toxic dosages were used or when they were combined with ischemia (31), or after the application of aconitine (32, see table 4). In the sixties, Roberts

Table 3. In vitro studies reporting on the evoking of early afterdepolarizations

drug/condition	channel affected	phase 2 EADs	phase 3 EADs	cells	antagonist	not affected by	enhanced by
Cesium ^{36,51,52,53}	K ⁺	+	+	PF	Ca ²⁺ blockade ⁴⁸ , fast pacing ⁵² , Na ⁺ blockade ⁵² , Mg ²⁺ , α blockade ⁵¹	ryanodine ⁴⁸	Long CL, Bay K 8644 ⁴⁵
Quinidine ^{37,38,39}	Na ⁺ , K ⁺	+	+	PF	Mg ²⁺ , fast pacing		
Isoprenaline ⁴⁵	?	+	+	myocytes	ryanodine		
ATXII ⁴³	Na ⁺			PF	Na ⁺ blockade	Ca ²⁺ blockade	ryanodine
ryanodine Bay K 8644 ⁴⁴	Ca L			PF	Ca ²⁺ blocker		
Acidosis ⁸²			+	PF	high K, TTX, lidocaine	verapamil manganese	low K ⁺ , low Ca ²⁺
Sotalol/Brétylium/Clonidine ⁴²	K ⁺	+		PF			ischemia
Bepridil/Lidoflazine ⁴⁶	Ca L, Na ⁺		+	PF	high epinephrine		low epinephrine, long CL
Ischemia ⁸³				PF	pacing, lidocaine	ryanodine	quinidine, long CL

Abbreviations: PF: Purkinje fibers, CL: cycle length

Table 4. Review of in vivo studies TdP using dog models

drug	anesthesia	diuretics	heart rate	stimulation	MAP	suppression
cesium ^{57,61}	+	no	SR	decrease of rate by vagal stim	-	MVT, PVT, TdP rare
quinidine ³³	+	no	SR	PES	-	PVT
cesium ⁵²	+	no	SR/AV block		-	PVT
quinidine ³¹	+	no	SR	PES; ischemia	-	PVT
cesium ⁴⁷	+	no	AV block	stellate ganglion stim	EADs	VT/PVT
anthopleurine ⁵⁹	+	no	SR (480-2400ms)		EADs endo/epi	
cesium ⁵³	+	no	SR		EADs	VT
Acotinine/quinidine ³²	+	no	SR/AV block		-	QT=long→TdP QT=short→PVT
cesium ⁵⁶	+	no			EADs	PVT
cesium ⁵⁸	+	no	SR	vagal stim	EADs/DADs	VT
cesium ⁵⁴	+	no	800 ms atrial pacing	left and right anseal subclavia stim	EADs LV > EADs RV	VT
sotalol/quinidine ^{63,64}	awake	yes/no	AV block			TdP

Abbreviations: SR: sinus rate, AV: complete atrioventricular, PES: programmed electrical stimulation, MAP: monophasic action potential, EADs: early afterdepolarizations, MVT: monomorphic ventricular tachycardia, PVT: polymorphic ventricular tachycardia, VT: ventricular tachycardia, stim: stimulation.

Figure 3. Occurrence of early afterdepolarizations after cesium administration in a dog. On the left side a smooth monophasic action potential signal is shown during the control state. After cesium a high membrane potential early afterdepolarization is shown in panel A and B and a low membrane potential early afterdepolarization in panel C (47). Figure reproduced with permission from the American Heart Association and the authors.

et al. reported that in awake dogs with complete AV block quinidine administration led to ectopic activity, short runs of ventricular tachycardia, and to periods of syncope (62). It took another 30 years before two models were published in which clinically used drugs led to TdP. These models were developed independently from our own model, one is an awake dog model while the other uses rabbits (63-65).

In hypokalemic awake dogs with chronic AV block, administration of quinidine only leads to TdP after prolonged infusion or after a further deceleration in ventricular rate using β blockade (63). Similarly it was also shown that administration of *dl*-sotalol leads to spontaneous TdP (64).

In the other model, rabbits are pretreated with the α agonist methoxamine, followed by the infusion of several class III agents (65). Spontaneous episodes of TdP evolve following ectopic beats despite the fast heart rate of the rabbits. Omission of the α agonist requires a ten to hundred fold increase of the class III dose leading to TdP (65).

From all these model studies, consensus was reached about the necessity of EADs for the occurrence of ectopic beats preceding TdP. The mechanism of continuation and termination remained to be elucidated.

1.4 Ventricular heterogeneity in repolarization

The clinical observation of a difference in duration of repolarization between different ventricular regions (27) in patients with TdP has been neglected in the experimental studies during the period between 1980 and 1990. Dispersion in repolarization is a common phenomena which has been shown to be present during baseline situation in patients (24,66,67-69), see table 5. It is thought to represent a measure of non homogeneous recovery of excitability which can include differences in APD, differences in refractory period, and/or differences in activation times. Finally it can be present as transmural, interventricular as well as intraventricular dispersion.

It was hypothesized that differences in repolarization duration could initiate reexcitation, because cells with a prolonged APD caused by local EADs were capable to reexcite the neighboring cells (70). Other groups disapproved of this mechanism and suggested that triggering EADs were also responsible for the continuation (29).

Surawicz (71) pointed to the pro's and con's of both EADs and dispersion of repolarization in the mechanism of the TdP. In the *in situ* experiments, most attention was directed to the development and behavior of EADs. Although in some experimental studies more than one MAP catheter was placed, no attention was given to differences in duration, despite the observation of the preferential increase of LV EAD compared with the RV EAD after stellate ganglion stimulation (54).

A study using MAPs at four different endocardial sites in dogs, did not find an increased difference in repolarization after administration of a total of 18 mg quinidine (72). Although none of the dogs developed EADs and/or TdP, these authors suggested that other factors than the intrinsic APD prolongation after quinidine must be responsible for TdP induction (72).

Dispersion as a mechanism for arrhythmias has been more extensively studied in other fields, e.g. an increase in dispersion of APD by regional cooling and warming of the ventricle facilitates tachycardia induction (73). No specific attention was ever placed to interventricular dispersion. *In vitro* studies showed that the different antiarrhythmic drugs increase or decrease the difference in APD between the Purkinje and ventricular muscle cells in a cycle length dependent fashion (74). Besides the difference between

Table 5. Data on intraventricular dispersion in patients without QT prolongation

number of subjects/source	registration site	dispersion	technique
n=28 ⁶⁷			
Heart catheterization	intra RV endocardial	0-38 ms	suction MAP rate dependent
n=40 ²⁴			
Heart catheterization	intra RV endocardial	0-40 ms	suction MAP
n=7 ⁶⁸			
Heart catheterization	intra LV endocardial 5-11 sites	43 ± 18 (21-64) ms	contact MAP RV APD < LV APD
n=3 ⁶⁸			
CABG	intra LV epicardial 5-10 sites	59 ± 11 (45-73) ms	epi APD < endo APD inverse relation AT and APD
n=10 ⁶⁹			
CABG	intra LV epicardial 10 sites	40 ± 19 (21-89) ms	contact MAP inverse relation AT and APD
n=8 ⁶⁶			
Electrophysiologic study	intra LV endocardial 2-3 sites	0-40 ms	contact MAP

Abbreviations: APD : action potential duration, AT: activation time, CABG: coronary artery bypass grafting, LV: left ventricle, RV: right ventricle, MAP: monophasic action potential.

ventricular myocytes and Purkinje fibers, recent investigations also showed difference between the different subpopulations of the myocardial cells: epicardial, endocardial and M cells (75,76). In figure 2, the frequency dependent changes are summarized, at slower rates the APD prolongation is most pronounced in the Purkinje fibers, followed by the M cell region, which creates an enlarged difference in intramural APD. The M cells might also provide a possible explanation for the U wave formation (75). In addition, administration of K⁺ channel blocking drugs induces EADs more easily in M cells than in endocardial and epicardial myocytes (75).

1.5 Treatment of TdP

Treatment of acquired TdP in patients consists of withdrawal of the offending drug. For direct suppression, every measure that shortens the cycle length is capable of suppressing episodes of TdP (4). This can be achieved by isoprenaline infusion and ventricular pacing. The most often used medicament is magnesium (20) which is a very

effective suppressor of TdP. In addition, the calcium antagonist verapamil and lidocaine have administered to prevent TdP (77). In vitro (see also table 3), all interactions that shorten action potential duration are effective in prevention of the EADs. Most used is the Na⁺ channel blocker tetrodotoxin (TTX), but also several Ca²⁺ antagonists have proven their efficacy (44,48,52,53).

In 1990 we started to develop our TdP model in dogs with chronic AV block. This AV block dog was used because in Maastricht much experience had been obtained using this model to study different mechanisms of ventricular arrhythmias under awake and anesthetized circumstances (78-81).

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Chapter 2

Reproducible induction of early afterdepolarizations and Torsade de Pointes arrhythmias by *d*-sotalol and pacing in the dog with chronic atrioventricular block

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ABSTRACT

Background: It has been well established that (antiarrhythmic) drugs can also have proarrhythmic effects such as Torsade de Pointes arrhythmias (TdP). It was the purpose of this study to create an animal model with a high incidence of reproducible TdP that occur under clinically relevant circumstances.

Methods: Experiments were performed in anesthetized dogs that had been in chronic atrioventricular (AV) block for 9 ± 6 weeks. Inducibility of TdP was attempted using different pacing modes before and after the administration of 2 mg/kg *d*-sotalolol (dS). In some experiments, endocardial monophasic action potentials (MAP) were recorded.

Results: dS increased the cycle length of the idioventricular rhythm (CL-IVR: 1475 ± 460 to 1730 ± 570 ms, $P < 0.01$) and the QT time (390 ± 65 to 480 ± 85 ms, $P < 0.01$). In 10% of the experiments "spontaneous" TdP occurred after dS. The incidence of "pacing dependent" TdP was 52% ($P < 0.01$). In the inducible group CL-IVR and QT time were significantly longer despite equal percentual increases in these parameters after dS in both groups. The pacing modes consisting of more than one frequency change had a higher induction rate of TdP ($P < 0.05$). Reproducibility of induction of TdP (≥ 3 times using the same pacing train) remained present for about 60 minutes after dS and was $>90\%$ within the same animal over weeks. Induction of TdP was related to the presence of early afterdepolarizations (EADs) on the MAP recordings: 5/6 in the inducible group vs. 2/6 in the non responders. Inducibility could be further increased to 89% when a second bolus of dS was administered to non inducible dogs. On the other hand, decreasing QT time by faster basic pacing, isoprenaline or $MgSO_4$ prevented induction of TdP. This effect of $MgSO_4$ coincided with disappearance of EADs.

Conclusions: Our animal model shows a high incidence of reproducible acquired TdP arrhythmias allowing study of mechanism and treatment of TdP. Induction of TdP was related to the combination of 1) a slow ventricular rate, 2) prolongation of QT time, 3) a sudden induced rate change which often requires ≥ 2 cycle length changes, and 4) the presence of EADs.

INTRODUCTION

Torsade de Pointes arrhythmias (TdP) can be congenital or acquired (1). The latter often occur in the presence of drugs which prolong ventricular repolarization, such as class Ia and III drugs. By definition, one of the characteristics of the drug induced TdP is the prolongation of the QT(U) duration. Prolongation of the refractory period of cardiac muscle is at the same time the desired antiarrhythmic goal to prevent reentrant tachycardias.

Several canine models exist (2-10), which enable the study of the morphological or mechanistic characteristics of TdP arrhythmias. Initiation of TdP is often associated

with early afterdepolarizations (EADs) (2-5). Still, we had the impression that the addition of a model that mimicks the clinical circumstances of TdP is of importance for a) the comparison of the proarrhythmic potential of drugs to initiate TdP, and b) to study mechanisms and treatment of TdP. The clinical conditions known to favor acquired TdP are 1) bradycardia, 2) therapeutic dosages of drugs that prolong QT duration, 3) sequences of short long short intervals and 4) hypokalemia and/or hypomagnesemia (1).

In the dog we developed a highly reproducible TdP model by mimicking the first three circumstances by 1) chronic AV block, 2) administration of 2 mg/kg *d*-sotalol, and 3) specific pacing modes. To investigate the model further, we increased basic heart rate by pacing, we administered $MgSO_4$ and isoprenaline and we recorded monophasic action potentials (MAP) to demonstrate involvement of EADs. Finally, different pacing modes were used to clarify the relevance of the number of cycle length changes (e.g. the short long short sequence) needed to initiate TdP.

METHODS

The study protocol was approved by the Committee for Experiments on Animals of the University of Limburg, Maastricht, The Netherlands and conducted in accordance with the guidelines of the American Physiological Society.

The experiments were performed on 18 adult mongrel dogs of either sex having a body weight between 20-35 kg (26 ± 5). In a preliminary surgery, a right thoracotomy was performed to induce a permanent complete AV block by injecting 37% formaldehyde into the AV junction (11). During the same session, pacing electrodes (Bakken Research Center, Medtronic, Maastricht, The Netherlands) were inserted epicardially into the basal portion of the right ventricle and the apex of the left ventricle. The wires were exteriorized through the back of the neck.

Six surface electrocardiographic leads and one local electrocardiogram were continuously registered and stored on optical disk. All drugs were administered through a canula in a cephalic vein.

Anaesthesia was induced by 1) premedication i.m. (1 ml/5 kg: 10 mg oxycodon, 1 mg acepromazine, and 0.5 mg atropine) and 2) sodium pentobarbital (20 mg/kg i.v.). The dogs were artificially ventilated through a cuffed endotracheal tube using a mixture of oxygen, nitrous oxide and halothane (vapor concentration 0.5%) by a respirator. Ventilation was controlled by continuous reading of the carbon dioxide concentration in the expired air. A thermal mattress was used to maintain adequate body temperature. Proper care was taken postoperatively, including antibiotics (1000 mg ampicilin) and analgetics (0.3 mg i.m. buprenorfine).

Pacing modes

Stimulation was done preferably from the right ventricular lead with a programmable stimulator having a synchronizing circuit (12). Unipolar stimuli were given using a pulse width of 2 ms and a stimulus strength of twice the diastolic threshold. As indifferent electrode, we used a needle placed through the skin.

Pacing consisted of *four different pacing modes*, which represent respectively one, two and three cycle length changes. The pacing modes for the one cycle length change consisted of 1) 3 stimuli (NVS=3), and 2) continuous pacing for 30 seconds (CP). Both were performed with equal interstimulus intervals and shortened from 1200 to 400 ms in steps of 100 ms for NVS=3, while CP was started just below the cycle length of the idioventricular rhythm (CL-IVR) and reduced to 1200, 900, 600 and 400 ms. The pacing modes with more than one frequency change were: 3) a basic train of 8 stimuli followed by an extrastimulus (8+1), and 4) a short long short sequence (SLS). The extrastimulus was shortened from 350 ms down in steps of 10 ms until the effective refractory period (VERP) was reached. VERP was defined as the longest stimulus interval which was not followed by a ventricular complex. VERP was determined during IVR and at paced rates similar to the CP protocol. SLS consisted of a) 4-8 paced beats with an interval of 600 ms followed by a beat with an interval of 1200 ms and finally an extrastimulus (4-8x600/1200/extra), b) 400/800/extra, and/or c) 2x300/900/extra. The pacing protocol needed 30-40 minutes for completion, while the different pacing modes were applied in a random manner.

Incidence of TdP was related to a specific pacing train and mode. Moreover, because TdP frequently started before the pacing train was completed, induction was also classified to the specific change in cycle length that seemed to be responsible for the initiation of TdP. When TdP was induced during the first 8 paced beats of either CP or 8+1, it was classified as being induced after one frequency change. A dog was considered inducible when a TdP arrhythmia could be reproducibly induced by the same pacing train for at least 3 times.

Definition TdP

A Torsade de Pointes arrhythmia was defined as a polymorphic ventricular tachycardia (VT) consisting of ≥ 5 beats twisting around the baseline having a rate of more than 200 beats/min which occur in the setting of a prolonged QT(U) duration (1). These VTs either stop spontaneously or degenerate into ventricular fibrillation. The VT was terminated using cardioversion (60-70 Joule) when it lasted longer than 10 sec. Defibrillation was performed maximally 6 times per experiment. TdP was differentiated from VF using the following characteristics: 1) twisting QRS behaviour using all 6 ECG-leads, 2) the (change in) amplitude of the QRS complex in a single ECG lead, and 3) the frequency of the arrhythmia.

Experiments

At least two weeks after the creation of complete AV block, the dogs were anaesthetized (see above). A blood sample was taken 1) to measure plasma levels of the electrolytes: calcium, sodium, potassium and magnesium, and 2) to establish their kidney function by measuring ureum and kreatinine. Two defibrillation patches, which were connected to a defibrillator, were attached at both sides of the chest. Most dogs were tested at different weeks to investigate reproducibility of their response.

After the baseline pacing protocol, a bolus *d*-sotalol (2 mg/kg/5 min) was administered. Thereafter, 5 minutes elapsed before pacing was resumed. The experiments were divided in two groups: inducible (I) and non inducible (NI). In group I, we distinguished between TdP which occurred spontaneously after *d*-sotalol ("spontaneous" TdP) and TdP which was pacing dependent ("pacing dependent" TdP). Because the number of cardioversions per experiment was limited, it was often not possible to repeat the complete pacing protocol in group I.

In the majority of the experiments belonging to the group NI, we added another bolus *d*-sotalol (2 mg/kg/5 min) and repeated the pacing protocol.

When TdP was inducible, we performed three procedures. First, the inducibility and reproducibility of TdP arrhythmias was tested further using different pacing modes. Also the length of the period (min) was determined during which a single bolus of *d*-sotalol in combination with pacing was able to induce TdP. Second, the basic ventricular rate was accelerated by: 1) pacing, or 2) administration of isoprenaline i.v. (10 µg/5 min). Thereafter, the specific pacing train responsible for TdP induction was repeated. Third, MgSO₄ (100 mg/kg/2 min) was administered to assess its suppressive effect against "spontaneous" TdP and to study its preventive effect against "pacing dependent" TdP.

Monophasic Action Potentials

A single endocardial MAP recording was made before and after *d*-sotalol to detect EADs in 12 experiments. Either through the jugularis vein (n=11) or the carotid artery (n=1), the MAP catheters (Franz combination catheter, EPT # 1650) were placed randomly under fluoroscopy (13). The MAP was amplified by a DC-coupled differential amplifier, which is provided with a 20 mV calibration pulse. The MAP signals were sampled at a rate of 1 KHz. The recording sites were chosen based on stability, quality of the signal and an amplitude which had to exceed 15 mV (13). The presence of an EAD on the MAP recording was defined as a retardation in repolarization (2-5).

Data analysis

The following parameters were measured in each experiment by two independent observers: CL-IVR, QRS duration, and QT(U) duration. QT(U)_c duration was calculated according to Bazett's formula (14).

Statistics

For determination of statistical difference ($P < 0.05$), we used analysis of variance (ANOVA) for comparisons between more than two groups, followed by Bonferroni's *t*-test when the F-value permitted. Paired Student's *t*-test was applied to compare data between two groups and χ^2 testing was used when the data were presented as percentages. All data are presented as mean $\pm$ standard deviation (SD), or when indicated as mean $\pm$ standard error of the mean (SEM).

RESULTS

In 18 dogs, we performed 42 experiments (2.5 ± 1.2). The mean time interval between the first experiment and AV block was 9 ± 6 weeks, whereas the time in between experiments was 14 ± 9 days. All dogs had normal electrolytes except for a slightly decreased potassium level of 3.4 mmol/l in one dog receiving diuretics because of heart failure.

Pacing under control conditions

We were not able to induce TdP by pacing under baseline conditions. However in 3 dogs, we noticed transient episodes of repolarization abnormalities (occurrence of U-waves) in the first spontaneous beats directly following pacing.

Electrophysiologic effects of d-sotalol

Administration of *d*-sotalol resulted in a marked increase in the CL-IVR from 1475 ± 460 to 1730 ± 570 ms ($P < 0.01$) and QT duration from 390 ± 65 to 480 ± 85 ms ($P < 0.01$), often occurring in the presence of U waves.

In a subset of dogs ($n=8$), we investigated the duration of these electrophysiologic changes (figure 1). The increases in CL-IVR and QT duration diminished in time, but remained present during the investigational period of 50 min. Also, QT_c showed a similar behaviour. No changes were observed in QRS duration.

Figure 1. Time dependent electrophysiologic effects of one bolus of *d*-sotalol (2 mg/kg).

The time dependent electrophysiological effects of *d*-sotalol are described for CL-IVR, QT, QT_c and QRS duration (panels 1-4 respectively). The time after *d*-sotalol is presented on the x-axis for 0-50 minutes. CL-IVR, QT duration and QT_c time were all maximally increased 10 minutes after *d*-sotalol (panels 1-3). From that period on, the effect diminished, but remained statistically significant for 40 minutes, i.e. 50 minutes after the start of *d*-sotalol. Reproducible induction of TdP arrhythmias was possible for a similar time period. Finally, QRS duration did not change after *d*-sotalol (panel 4).

VERP during spontaneous IVR increased from 265 ± 60 to 325 ± 65 ms ($P < 0.05$). The effect of *d*-sotalol on VERP was reverse-use dependent: the relative increase of VERP was greater at slower heart rates (+23%) than at faster ones (+2%, $P < 0.01$).

Figure 2. "Spontaneous" TdP arrhythmia after *d*-sotalol.

Six surface electrocardiographic leads and a local electrogram from the left ventricular (LV) apex are recorded simultaneously in an anaesthetized dog with chronic AV block. Paper speed is 25 mm/sec. Panel 1: spontaneous idioventricular rhythm. Panel 2: 10 minutes after *d*-sotalol CL-IVR and QT time are increased and ectopic beats with different QRS configurations occur at the end of the TU wave. Panel 3: spontaneous induction of a TdP arrhythmia. Note the "typical" short long short sequence which precedes the polymorphic VT.

Spontaneous TdP arrhythmias after d-sotalol

Following *d*-sotalol administration, we noticed four "spontaneous" appearances of a TdP arrhythmia: $4/42=10\%$ (figure 2, panel 3). These episodes were repetitive and often needed interventions, such as cardioversion and back-up pacing at faster rates to suppress the arrhythmia. More frequently (50%), ectopic beats with different configurations often arising from the end of the TU wave were observed (figure 2, panel 2).

Inducibility of TdP arrhythmias after d-sotalol

After the first bolus of *d*-sotalol, pacing resulted in reproducible TdP in 22/42 experiments (52%, $P < 0.01$ vs "spontaneous"), which appeared in 12 of the 18 dogs. An example is presented in figure 3. In all dogs with "spontaneous" TdP, pacing also resulted in the induction of TdP. Inducibility remained present using similar pacing modes for at least 60 minutes in the 3 dogs, in which this was tested.

Inducibility was related to CL-IVR and QT(U) duration (table 1). In the NI group ($n=20$), CL-IVR and QT time were significantly shorter, although the relative increase induced by *d*-sotalol was comparable for both parameters in both groups. Correction for QRS duration (JT interval) revealed no differences in the quantification of the parameter of repolarization.

Only in two dogs, the (in)ability to induce TdP was not reproducible in subsequent experiments: one inducible and one non inducible dog that reversed their response. Therefore, the reproducibility is quite high ($38/42=90\%$).

Registration of EADs by MAP recordings

EADs were registered in 7/12 experiments (figures 4-6). In half of the MAP experiments, TdP could be induced. The occurrence of ectopic beats was related to the presence of EADs in 4/5 experiments (figure 4). EADs were seen in 5/6 inducible and in 2/6 non inducible experiments. In figure 5, an example is presented of a TdP induction by "pacing" which is initiated by EAD-dependent triggered beats. Often, pronunciation of EAD amplitude was seen during the pacing train.

Effect of the pacing mode on inducibility of TdP arrhythmias

In a subgroup of subsequent, inducible experiments ($n=11$), we analyzed the induction of 55 episodes of TdP in relation to the pacing mode. Defibrillation was performed in 21 cases, while spontaneous termination occurred in 34 TdP. Induction either occurred during the pacing train or directly thereafter (figures 3 and 5). In case of CP, induction was always seen in the beginning of the pacing train. The incidence of TdP in relation to pacing mode is presented in the left part of table 2. It is shown that the modes 8+1 and SLS are equally effective and possess a greater ability to induce TdP than the other two modes ($P < 0.05$), while NVS=3 is more successful than CP ($P < 0.05$).

Induction was related to the number of cycle length changes: Half of the inductions occurred after one change in cycle length, i.e. starting during or directly after protocols 1 or 2, or during the first frequency change of protocols 3 and 4 (right part of table 2, presented as first change). However, inducibility was only possible in the other half of the experiments by at least 2 frequency changes, as illustrated in figures 3 and 5.

Table 1. Electrophysiologic changes of *d*-sotalol (2mg/kg)

Inducible experiments (N=22)	Control	D-SOT (10')	%
CL-IVR (ms)	1745 ± 465	2055 ± 580*	+18
QT(U)	420 ± 60	535 ± 80*	+27
QT _c	325 ± 45	380 ± 60*	+17

Non inducible experiments (N=20)	Control	D-SOT (10')	%
CL-IVR (ms)	1175 ± 190 [#]	1370 ± 255* [#]	+17
QT(U)	350 ± 45 [#]	425 ± 50* [#]	+21
QT _c	325 ± 45	365 ± 40*	+12

* P<0.001 vs control; [#] P<0.001 vs inducible, mean ± SD

Figure 3. Induction of a TdP arrhythmia directly after pacing in the presence of *d*-sotalol.

Six simultaneously recorded ECG-leads are shown at a paper speed of 10 mm/sec. After *d*-sotalol, the idioventricular rhythm is interrupted by a short long short sequence (400/800/extra). Directly following this pacing protocol, a self terminating TdP occurs. We have classified this sequence as a TdP induced by three cycle length changes.

Figure 4. Registration of EADs on MAP recordings during spontaneous ectopic activity after *d*-sotalol. Three surface ECG-leads and an endocardial MAP registration from the apex of the left ventricle are simultaneously seen at a paper speed of 10 mm/sec. After *d*-sotalol (middle and right panel), spontaneous ectopic beats from different origin occur during the idioventricular rhythm. In the enlarged MAP recording of the middle panel, phase 2 subthreshold EADs (↓) can be clearly seen. Also phase 3 EADs (↑) can be seen which trigger and induce (an) ectopic beat(s).

Inducibility in the non inducible group after a second bolus of d-sotalol

After administration of a second bolus of *d*-sotalol in 14 of the 20 non inducible experiments, inducibility increased to a total of 32/36 (89%, $P < 0.01$ vs first bolus *d*-sotalol). This was accompanied by increases in CL-IVR from 1340 ± 190 to 1690 ± 500 ms, $p \leq 0.05$ and QT(U) duration from 450 ± 60 to 490 ± 55 ms, $p \leq 0.05$.

Figure 5. Registration of EADs on MAP recordings during “pacing dependent” TdP in the presence of *d*-sotalol.

In the same experiment as in figure 4, a pacing protocol which consisted of 8x600/1200/extra was performed. Paced beats are indicated by S. Ectopic beats interfered with the paced complexes. Resulting in a self terminating TdP. When we look at the MAP signal, again we see subthreshold phase 2 EADs (second “paced” beat) and triggering phase 3 EADs (last part of the pacing train) that are responsible for the initiation of ectopic beats and eventually TdP. This induction of TdP was classified to have started after two cycle length changes.

Table 2. The relevance of the pacing mode for the inducibility of TdP arrhythmias in a subgroup of inducible animals

	incidence	1st change	2nd change	3rd change	total
SLS	90%*	9	7	9	25
8+1	100%*	6	11	–	17
NVS=3	63%#	10	–	–	10
CP	20%	3	–	–	3
total (%)		50%	30%	20%	55

CP: continuous pacing for 30 seconds, 8+1: 8 stimuli + 1 extra, NVS=3: three stimuli, SLS: short long short. #p<0.05 vs CP; *p<0.05 vs NVS=3 and CP

Figure 6. Administration of MgSO₄.

This is the same experiment as in figures 4-5. At the time of MgSO₄ administration, ectopic beats are still present (panel 1). Also, subthreshold and triggered EADs can be seen on the MAP registration. Following MgSO₄, the QT duration shortens to 470 ms, while the cycle length increases. The ectopic beats completely disappear and the repolarization phase of the action potential becomes smooth (panels 2-3). Pacing is no longer able to induce ectopic beats, EADs, or a TdP arrhythmia (panel 4). Note the repolarization abnormality (negative T wave) in the first spontaneous beat postpacing.

Table 3. Electrophysiologic results of magnesium (100mg/kg, n=6)

	Control	D-SOT (10')	D-SOT (40')	Mg (3')	Mg (5')
CL-IVR	1710 ± 390	2025 ± 490*	1910 ± 540	2730 ± 1475	2030 ± 655
QT(U)	405 ± 75	490 ± 110*	485 ± 95	420 ± 80#	435 ± 110#
QTc	310 ± 35	345 ± 60*	355 ± 65	270 ± 65#	300 ± 60#*

* P < 0.05 vs Control; # P<0.05 vs D-SOT 40'; #P< 0.05 vs Mg 3', mean ± SD

Prevention of TdP

increasing heart rate: The basic heart rate was increased by continuous pacing to ± 65% of spontaneous CL-IVR (n=3). Inducibility was completely prevented but returned when continuous pacing was stopped. Administration of isoprenaline (n=2) decreased CL-IVR to ± 70% and resulted in complete prevention of inducibility. Thirty minutes after isoprenaline, CL-IVR and QT duration returned to their original value and TdP arrhythmias became inducible again.

administration of MgSO₄: MgSO₄ was administered to 6 “pacing dependent” TdP experiments. Two of these dogs also showed “spontaneous” TdP. In 2 experiments, a MAP signal was registered. At the time of MgSO₄ administration, spontaneous ectopic beats were seen in two experiments (figure 6, panel 1). MgSO₄ suppressed the ectopic beats (panels 2-3) and prevented the induction of TdP in all dogs tested (panel 4). This was accompanied by disappearance of the (subthreshold) EADs on the MAP recordings. Also, the pacing protocol was completed without the occurrence of ectopic beats. Electrophysiologically, MgSO₄ shortened the QT time (table 3). The QT_c duration decreased even more due to the fact that MgSO₄ increased the CL-IVR. In two experiments, reinduction of TdP was seen after the effect of MgSO₄ had disappeared.

DISCUSSION

When we summarize the results in this animal model, it becomes obvious that to reproducibly induce TdP arrhythmias, the CL-IVR and the QT time have to be sufficiently long at the start of the experiment. Shortening of the QT duration by either an increase in the heart rate by pacing, administration of isoprenaline or MgSO₄ will prevent induction, whereas a second bolus of *d*-sotalol, which further lengthens QT time and CL-IVR, will tend to make the non susceptible animals inducible. Both the “spontaneous” TdP as the “pacing dependent” TdP rely for their induction on the

presence of EADs. Finally, a sudden rate change often consisting of ≥ 2 cycle length changes is needed to induce TdP under the above conditions.

In recent years, the interest for (animal models of) Torsade de Pointes-arrhythmias has increased considerably (1-10). Attention was focussed on 1) the (ionic) mechanism(s) underlying the induction and perpetuation of TdP arrhythmias, 2) screening for parameters and/or circumstances which could predict possible proarrhythmic effects of antiarrhythmic drugs, especially new class III drugs, and 3) prevention of TdP by specific interventions, including changes of the activity of the autonomic nervous system, specific antiarrhythmic drugs, including new drugs like I_k -activators (2-10,15-19).

The intervention most widely used to induce TdP arrhythmias in animals is cesium administration (3,5,7,9,18). In anaesthetized dogs with chronic AV block, we developed a TdP model that shows a high incidence of TdP inducibility together with a high reproducibility. Depending on the number of doses of *d*-sotalol the incidence of TdP is 52% or 89% respectively. Reproducibility of TdP induction was present during an experiment for about 60 minutes and in repeated experiments over weeks in 90% of the attempts. In the majority of experiments, inducibility was dependent on (abrupt) changes in the cycle length, applied by pacing.

During the course of our experiments, an article was published which reported a similar approach (10), although important differences exist between the two methodologies. Weissenburger et al. (10) studied spontaneous induction of TdP arrhythmias in conscious dogs with chronic AV block. To achieve that, they needed higher dosages of the antiarrhythmic drugs, in the presence of either hypokalemia, β blockade or both.

The electrophysiologic effects of *d*-sotalol are in agreement with a therapeutic dose of this drug and they do represent clinically achieved values (20). The observed reverse use-dependency of VERP has also been described as a property of (*d*)-sotalol (21,22). Next to the increases in QT time and VERP, *d*-sotalol slowed the heart rate (increase in CL-IVR). Similar findings for *d*-sotalol but also other class III agents have recently been reported for dogs in sinus rhythm (23-24). This negative chronotropic effect of class III-agents has been attributed almost entirely to prolonged repolarization.

Relevance of the number of cycle length changes

Spontaneous, drug induced TdP arrhythmias often show a specific initiating sequence that is related to the occurrence of spontaneous ectopic beats: short long short intervals which represent 3 abrupt cycle length changes in a short period of time (1,6,10,15,19). Using a similar pacing mode, we tried to imitate this sequence. Because there is controversy in the literature about the specificity of this sequence (1,25-27), we also used other pacing modes to study their (possible) relevance. Two pacing modes were clearly superior in initiating TdP arrhythmias in our model: SLS and 8+1. Especially,

the equal incidence of the 8+1 pacing mode is of interest, because this is worldwide the most often used pacing mode during electrophysiologic studies.

Using the MAP catheter, we saw the occurrence of (triggered) EADs directly after the start of pacing (figure 5) when the rate was relatively fast and the APD relatively short in comparison to the idioventricular rhythm. This observation indicates that (inadequate) adaptation of the duration of the action potential to abrupt changes in cycle length are of importance for the induction of TdP. This has also been suggested by others (28). Support for this line of thinking comes from the results of the two pacing modes with fixed interstimulus intervals but differences in duration (NVS=3 vs CP). The higher inducibility of NVS=3 underscores the importance of (an) abrupt frequency change(s) rather than the duration of pacing. Additional confirmation comes from 1) the observation that, in case of CP, TdP always started within the first 10 beats, and 2) that one cycle length change already induced TdP in half of the experiments. When however a second or a third interval change is added, inducibility is doubled.

The fact that prolonged pacing is often used to prevent induction of spontaneous drug induced TdP is another argument to stress the importance of the abruptness of the cycle length change.

Interpretation of these data should however be done with some caution. Because we allowed ourselves only a small number of cardioversions per experiment, we could not always study all pacing modes in the same experiment. We tried to correct this by using the other pacing modes in a subsequent experiment of the same animal. Still, we cannot answer questions concerning cycle length dependence (interstimulus interval) and/or possible interference of different pacing modes on inducibility of TdP.

The most effective treatment for clinical acquired TdP are rapid ventricular pacing, isoproterenol or magnesium administration (1). They were all tested and considered effective in preventing TdP arrhythmias.

EAD dependent TdP

Several studies have addressed the involvement of triggered activity resulting from EADs in the initiation of TdP (2-5,15,18,19). And although the contribution of EADs is still not completely resolved, evidence is becoming more convincing that EADs play an important role in the induction of acquired TdP. As seen in this study, the presence of EADs seems to be related to as well "spontaneous" as "pacing dependent" TdP. It is however difficult to demonstrate the causal relation between the presence of EADs, triggered beats elicited by EADs, and the actual TdP. The continuous shift in site of origin of EADs and the elicited beats together with the speed of the arrhythmias complicate the MAP registrations. This technique itself has also some technical difficulties, like stability and the possibility of registering artefacts. We relied on some good fortune to be at the site of origin of the EAD (figures 4-6). Still, we are convinced that

these recordings in a few experiments are representative for the model. We also believe that the experiments with MgSO_4 demonstrate that the EADs are no artefacts. Therefore, we think that initiation of TdP is EAD dependent. Whether the same mechanism is also responsible for the perpetuation of the arrhythmia has to be established.

The relevance of this animal model

A relatively low incidence of "spontaneous" TdP arrhythmias occurring after *d*-sotalol administration was observed in these experiments. We defined TdP as ≥ 5 beats. More frequently ($\pm 50\%$), ectopic beats occurred arising from the TU-wave that seem to be dependent on EADs. These beats occurred as singles, doubles or triplets. Reproducible initiation of TdP arrhythmias required cycle length changes which were induced by pacing. Still, we believe that this "pacing dependent" TdP can be used to study mechanism and treatment of "spontaneous" acquired TdP because 1) in dogs showing "spontaneous" TdP, pacing was also able to induce TdP reproducibly, 2) both forms of TdP seem to be dependent for their initiation on the presence of EADs (figures 4 and 5), 3) "pacing dependent" TdP showed the same specific ECG characteristics as "spontaneous" TdP and often terminated spontaneously, and 4) both forms of TdP could be suppressed by MgSO_4 (figure 6), levromakalim (17), flunarizine (29) and by increasing the heart rate.

In this model, we have tested the dogs at a relatively long period after application of AV block (9 ± 6 weeks). The bradycardia related volume overload and subsequent biventricular hypertrophy (personal observations) could be of importance for the inducibility of TdP. Currently, we are documenting these changes over time, both echocardiographically and electrophysiologically and we are testing the relevance of these changes for the inducibility of TdP. The observation by Strauss et al. (21) that application of acute AV block together with a similar dose of *d*-sotalol did not result in TdP arrhythmias, indicates that additional changes have to be present.

At this moment, we are also testing other drugs that prolong action potential duration. Our results indicate that this animal model can be used to compare the proarrhythmic potential of different drugs. Whether this model is suitable to predict the occurrence of TdP in patients treated with these drugs has to be determined.

In conclusion, this animal model shows a high incidence of reproducible, acquired Torsade de Pointes arrhythmias, that allows study of mechanism and treatment of these triggered tachycardias.

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Chapter 3

Role of interventricular dispersion of repolarization for the induction of acquired early afterdepolarization dependent Torsade de Pointes arrhythmias: reversal by magnesium

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ABSTRACT

Background: The mechanism of acquired Torsade de Pointes arrhythmias (TdP) is not clear. To study the importance of 1) triggered activity by early afterdepolarizations (EADs) and 2) interventricular dispersion in duration of repolarization (Δ APD) for the initiation of TdP, both parameters were recorded in our recently described animal model of acquired TdP. In half of the anesthetized dogs with chronic complete AV block, the combination of *d*-sotalol and pacing leads to reproducible induction of TdP (≥ 3 times TdP using the same pacing mode).

Methods: Eleven inducible (I) and nine non inducible dogs (NI) after *d*-sotalol were compared. From at least two sites, endocardial monophasic action potentials (MAPs) were registered to record EADs and to measure regional differences in action potential duration (APD). The rate dependent behavior of the interventricular Δ APD (APD of left minus right ventricle) was studied in seven dogs in which no EADs were visible. MgSO_4 (100 mg/kg) was administered to 7 *d*-sotalol treated dogs (4 I and 3 NI dogs).

Results: In the absence of EADs, we demonstrated that interventricular Δ APD is bradycardia dependent (greater at longer paced cycle lengths (PCL)). *d*-Sotalol prolonged the spontaneous cycle length of the idioventricular rhythm (CL-IVR), QT time and the APD of both ventricles, but did not increase interventricular Δ APD when the PCL was kept constant. After *d*-sotalol the I dogs demonstrated a longer CL-IVR: 2115 ± 320 vs 1615 ± 265 ms, ($P < 0.05$), increased QT time (540 ± 80 vs 450 ± 70 ms, $P < 0.05$), a higher incidence of EADs (14/22 MAPs vs 5/18 MAPs, $P < 0.05$), an equal number of triggered ectopic beats induced by EADs (8/11 vs 4/9) and an increased interventricular Δ APD (135 ± 55 vs 60 ± 40 ms, $p < 0.05$). Interventricular Δ APD was always larger than the simultaneously recorded APD differences within one ventricle. Although CL-IVR increased after MgSO_4 (1885 ± 320 to 2400 ± 755 ms, $P < 0.05$), this drug prevented induction of TdP (4/4) by suppressing EADs, decreasing APD and the interventricular Δ APD from 110 ± 45 to 55 ± 60 ms ($P < 0.05$).

Conclusions: 1) In the absence of EADs, Δ APD demonstrates frequency dependence 2) Increased interventricular Δ APD in the presence of local EADs is an important factor in the initiation of TdP and 3) MgSO_4 diminishes both interventricular Δ APD and presence of EADs.

INTRODUCTION

Prolongation of QT duration with (antiarrhythmic) drugs has been associated with cardiac arrhythmias, like Torsade de Pointes (1,2). Because repolarization parameters will lengthen when heart rate slows, the incidence of acquired TdP will increase under bradycardic circumstances. In recent years, evidence has accumulated that early afterdepolarizations (EADs) play a decisive role in the genesis of ectopic beats and the U

wave changes that often precede the spontaneous occurring TdP (3-8). It has been demonstrated in vitro and in vivo that EADs are bradycardia dependent and originate preferably in cells with prolonged action potential duration (APD) (8-12). No evidence was provided that the EAD dependent triggered activity also led to TdP (13).

In patients with congenital TdP, intraventricular dispersion of APD has been observed (14,15). Also, these studies provided evidence that EADs occurred site specifically, thereby explaining (in part) the observed intraventricular dispersion (14-16). More recently, interventricular dispersion was seen in combination with EADs in a patient with acquired TdP (17).

In our animal model of pacing induced TdP (18), our main objective was to investigate the contribution of two parameters i.e. EADs and interventricular dispersion of APD for the initiation of TdP. For this purpose, we compared inducible vs non inducible experiments after *d*-sotalol and we administered $MgSO_4$ because of its antiarrhythmic effect on TdP (18-21). Our additional goals were to determine the frequency dependence of the interventricular dispersion in the absence of EADs and, secondly to differentiate between inter- and intraventricular dispersion in APD. For the latter goal, multiple (simultaneous) endocardial MAP recordings were made in some dogs.

METHODS

All experiments were performed according to the principles of the American Physiological Society and approved by the Committee for Experiments on Animals of the University of Limburg, Maastricht, the Netherlands.

General

The experiments were performed on 20 anesthetized adult dogs with chronic AV block (5 ± 3 weeks) of either sex with a body weight between 18 and 34 kg. Each dog had one epicardial electrode (Bakken Research Center, Medtronic, Maastricht, The Netherlands) implanted at the apex of the left ventricle, when a right sided thoracotomy was performed to produce a complete AV block by injection of formaldehyde (0.37%) in the AV groove (18). Between AV block and the experiment a minimum of two weeks elapsed.

Pacing was done with a programmable stimulator capable of pacing synchronously to the QRS complexes. Unipolar stimuli were given using a pulse width of 2 ms and a stimulus strength of twice the diastolic threshold. As indifferent electrode a needle was placed through the skin. Six surface electrocardiographic leads and ≥ 2 endocardial MAP signals were simultaneously registered and stored on hard disc. All drugs were administered through a canula in the cephalic vein.

Anaesthesia was induced by 1) premedication i.m. (1 ml/5 kg: 10 mg oxycodon, 1 mg acepromazine and 0.5 mg atropine) and 2) sodium pentobarbital (20 mg/kg i.v.). The dogs were artificially ventilated through a cuffed endotracheal tube using a mixture of oxygen, nitrous oxide and halothane (vapor concentration 0.5%-1%) by a respirator. Ventilation was controlled by continuous reading of the carbon dioxide concentration in the expired air. A thermal mattress was used to maintain adequate body temperature. Proper care was taken after the experiments, including administration of antibiotics (1000 mg ampicillin) and analgesics (0.3 mg i.m. buprenorfine). A temporary pacemaker (with a maximum of 24 hours) was given after the AV block operation and also after the experiments, because the animals are tested repeatedly.

Induction of Torsade de Pointes arrhythmias

A detailed description of the Torsade de Pointes protocol is described elsewhere (18). In short, anesthetized animals received two defibrillation patches that were attached to both sides of the chest and connected with a defibrillator. Half an hour after the onset of anaesthesia, pacing was done from the epicardial electrode and consisted of two different pacing modes: 1) short long short sequence and 2) 8 basic stimuli followed by an extra stimulus. The interstimulus interval was 400, 600 or 1200 ms, the extrastimulus was shortened from 500 ms with steps of 50 ms till 300 ms. After completing the pacing protocol, a bolus of *d*-sotalol (2 mg/kg/5 min) was administered. Pacing was resumed 10 minutes after the start of the bolus. A Torsade de Pointes arrhythmia was defined as a polymorphic ventricular tachycardia consisting of ≥ 5 beats, which twisted around its baseline in the presence of a prolonged QT(U) duration. When TdP did not stop spontaneously in 10 seconds or when it deteriorated into ventricular fibrillation, cardioversion (60-70 J) was performed.

This animal model of "pacing induced" TdP results in reproducible TdP arrhythmias in half of the animals (18). In 20 consecutive experiments in which stable MAP recordings could be made, we induced TdP in eleven animals (inducible group). Inducibility was defined as the initiation of TdP for at least 3 times using the same pacing mode.

MgSO₄ administration

MgSO₄ (100 mg/kg/2 min) was administered within 45 minutes after *d*-sotalol to 7/20 animals, 4 inducible and 3 non inducible dogs. We have demonstrated that one bolus of *d*-sotalol is capable of reproducible induction of TdP for at least 60 minutes (18). At 5 minutes after the start of the injection of MgSO₄, the pacing modes which led to TdP induction were repeated.

Monophasic Action Potentials

Endocardial monophasic action potentials were recorded simultaneously to observe the occurrence of EADs and to measure the (differences in) the duration of the action potential (APD). A quadripolar contact electrode (Franz combination catheter, EPT # 1650), that provides both pacing and MAP recording (22) capabilities was placed endocardially in the left and in the right ventricle. Placement was based on quality of the signal. Therefore a carotid and/or femoral artery and an external jugular vein were dissected free and the 7 French MAP catheter was introduced under fluoroscopy. After the experiment the carotid artery and the external jugular vein were sewed-up. The MAP signals were amplified with an isolated DC coupled differential amplifier with calibrated gains of 10, 20, 50, 100, 200 and 500 (custom made, University of Limburg). The offset of the amplifier is variable and can be adjusted to the recorded signal. All amplifiers are provided with a 20 mV calibration pulse. Data acquisition of the MAP signals is achieved by an 8 bits Analog to Digital Converter resulting in a 0.39% resolution of full scale. The MAP signals are sampled at a rate of 1 kHz per signal. MAP phases were defined according to the definitions used for transmembrane potentials (9). Amplitude was defined between phase 4 and 2 of the signal. Besides a minimal amplitude of 15 mV, the MAP had to have a constant configuration and a smooth shape during control circumstances. After placement of the MAP catheter, 5 paced beats were given using the MAP electrode to register the ECG (QRS morphology). This morphology was used to determine the site at which the MAP catheter was placed and also used for correct repositioning, when amplitude decreased and adjustment was necessary. EADs were defined as an interruption of the smooth contour of phase 2 or 3 of the action potential (4,9). The presence of EADs was examined in all MAPs.

Inter- and intraventricular difference in APD

In all experiments, two MAP catheters were placed randomly to measure the APD in the left and in the right ventricle. After the first 10 minutes of *d*-sotalol, we replaced these catheters to several sites (23) to measure intraventricular differences in APD in seven experiments. To more carefully assess LV intraventricular differences especially in regard to EADs. We placed an additional LV MAP catheter, while the first MAP catheter remained at a stable site in 4 experiments.

*Frequency related changes of APD and interventricular Δ APD after *d*-sotalol*

In 7 experiments a paced ventricular rhythm (on the epicardial electrode) with a paced cycle length (PCL) of 1000 and 500 ms was used to investigate the rate related changes of the APD and the interventricular Δ APD. At steady state (> 2 minutes pacing) the

APD was determined as the average of 5 beats. After administration of *d*-sotalol the APD measurements were repeated, and we could include a PCL of 1500 ms.

Data analysis

All data were analyzed using a custom made computer program with a resolution of 2 ms and adjustable gain and time scale. Using lead II, electrophysiological measurements of cycle length of the idioventricular rhythm (CL-IVR) and QT(U) time were performed before and after the administration of *d*-sotalol and MgSO₄. The QT_c time was calculated using the formula of Bazett ($QT_c = QT/CL-IVR$, 24), to correct for rate differences. The action potential duration was measured after total repolarization (=APD₁₀₀) and was determined for both ventricles at the same time points as the other parameters. Interventricular dispersion was calculated as the difference between the APD of the left and right ventricle (Δ APD). Each value represents the mean of 5 consecutive beats. Additionally the incidence of spontaneous beats was scored which occurred during the first 10 minutes of *d*-sotalol.

Statistics

Paired Student's *t*-test was applied to compare data under the different conditions (drugs). To compare differences between inducible and non inducible dogs an unpaired *t*-test was used. P values ≤ 0.05 were considered significant. All data are presented as mean $\pm$ standard deviation (SD).

RESULTS

Electrophysiological effects of d-sotalol

In the twenty dogs tested, *d*-sotalol significantly prolonged QT time from 405 ± 75 to 500 ± 90 ms and the CL-IVR from 1585 ± 335 to 1890 ± 385 ms (both $P < 0.001$), while the QRS duration was not affected. All these measurements were performed at the start of *d*-sotalol (control) and at the end of the observation period of 10 minutes. The APD of the LV (390 ± 65 to 495 ± 90 ms, $P < 0.01$) prolonged more (both absolutely and relatively) than the RV (330 ± 40 to 395 ± 55 ms) leading to a larger interventricular Δ APD after *d*-sotalol (from 60 ± 30 to 100 ± 65 ms, $P < 0.01$).

Only once we saw EADs under control conditions (1/20, see further). Within the 10 minutes observation period after *d*-sotalol spontaneous ectopic beats occurred in 12/20 dogs. The responsible EADs occurred in 14/20 dogs, and were present more predominantly in the LV (12/20) than in the RV (7/20).

Characteristics of APD, interventricular Δ APD and intraventricular Δ APD

Under baseline conditions, the LV APD of these 20 dogs showed a linear relation with QT time ($r = 0.81$, $P < 0.01$) and with CL-IVR ($r = 0.8$, $P < 0.01$).

The APD of both ventricles increased when the PCL was lengthened from 500 to 1000 ms (figure 1). In addition, a clear frequency dependence was seen for the interventricular Δ APD ($P < 0.05$), because the LV APD prolonged more than the RV APD when the PCL increased. In the absence of EADs, similar observations were obtained after *d*-sotalol. The more pronounced increase of the LV APD at the different PCL resulted again in bradycardic lengthening of the interventricular Δ APD. However, *d*-sotalol did not increase the interventricular Δ APD at these specific paced frequencies (figure 1).

In the 7 dogs presented in table 1 (4 I and 3 NI), the APD was measured at several intraventricular sites after *d*-sotalol. The mean interventricular Δ APD at randomly chosen sites was 105 ± 65 ms. The mean intraventricular difference in APD of the left and right ventricle only amounted to maximal 30 ± 25 ms and 20 ± 20 ms respectively. Furthermore the intraventricular differences did not differ between inducible and non inducible dogs.

Table 1. Inter and intraventricular differences in action potential duration after *d*-sotalol

dog	CL-IVR	LV APD	RV APD	Δ APD	LV APD*	RV APD*	LV-LV*	RV-RV*	Δ APD RANGE
1 [#]	2215	425	390	35	435	385	10	5	35-50
2	1335	460	375	85	460	390	0	15	70-85
3 [#]	2295	590	505	85	640	480	50	25	85-160
4 [#]	1830	540	305	235	485	320	55	15	165-235
5 [#]	2600	590	445	145	555-590	495	0-35	50	60-145
6	1350	395	320	75	355-425	325	30-70	5	30-105
7	1660	455	385	70	485	385	30	0	70-100
MEAN 1900		495	390	105			30	20	100
$\pm$ SD	$\pm$ 490	$\pm$ 80	$\pm$ 70	$\pm$ 65			$\pm$ 25	$\pm$ 20	$\pm$ 55

All values are expressed in milliseconds, CL-IVR: cycle length of the idioventricular rhythm, LV APD: action potential duration of the left ventricle, RV APD: action potential duration of the right ventricle, Δ APD: LV APD - RV APD (interventricular difference as found when only 2 MAPs would have been placed), LV APD*, RV APD*, APD of the left and right ventricle at other sites, LV-LV*, RV-RV*: range of intraventricular differences found, Δ APD RANGE, the range of interventricular differences, [#] inducible animals.

Pacing without EADs

Figure 1. Frequency dependent behavior of action potential duration (APD) and interventricular difference in APD (Δ APD) before and after *d*-sotalol.

In this graph the frequency dependent behavior of the APD and the interventricular Δ APD is shown. On the y axis the APD is depicted of the left (LV) and the right ventricle (RV). The dogs (n=7) are paced at steady state through the left epicardial electrode. The x axis represents the paced cycle length (PCL). At 500 ms the interventricular Δ APD amounts 35 ms during baseline. With an increase in cycle length to 1000 ms the APD of both ventricles prolong significantly. The LV APD increases relatively more, leading to a significantly higher interventricular Δ APD at the longer cycle length ($P<0.05$). *d*-Sotalol (dS) increases the absolute value of both APDs. Interventricular Δ APD displays bradycardia dependence although the amount of interventricular Δ APD is not increased at a specific PCL in the absence of EADs.

Torsade de Pointes arrhythmias

When we divided the dogs in two groups (inducible and non inducible), we found that under baseline conditions the electrophysiological values (CL-IVR and QT) of the susceptible dogs were longer than those of the non inducible dogs (reference 18 and table 2). This was also the case for the LV, RV APD and interventricular Δ APD (75 ± 30 vs 40 ± 30 ms, $p \leq 0.01$), while the QT_c was similar.

d-Sotalol further enhanced the length of the repolarization, as illustrated in figure 2. With the exception of interventricular Δ APD in the non inducible group, all parameters

Table 2. Electrophysiological changes after *d*-sotalol(2mg/kg)

Inducible dogs (N=11)	Control		D-SOT (10')		%
CL-IVR (ms)	1810 ±	245	2115 ±	320 [#]	+17
QT(U)	440 ±	75	540 ±	80 [#]	+22
QT _c	325 ±	45	370 ±	50 [#]	+14
LV APD	425 ±	60	550 ±	80 [#]	+29
RV APD	345 ±	35	410 ±	60 [#]	+19
ΔAPD	75 ±	30	135 ±	55 [#]	+80
EADs	1/22		14/22 [#]		

Non inducible dogs (N=9)	Control		D-SOT (10')		%
CL-IVR (ms)	1310 ±	210 [*]	1615 ±	265 ^{*#}	+23
QT(U)	360 ±	40 [*]	450 ±	70 ^{*#}	+25
QT _c	320 ±	45	355 ±	65 [#]	+11
LV APD	345 ±	30 [*]	430 ±	50 ^{*#}	+25
RV APD	305 ±	35 [*]	375 ±	45 [#]	+23
ΔAPD	40 ±	30 [*]	60 ±	40 [*]	+38
EADs	0/18		5/18		

* P<0.05 vs inducible, [#] P<0.05 vs control, D-SOT (10'): 10 minutes *d*-sotalol, CL-IVR: cycle length idioventricular rhythm, QT_c: corrected QT time according to Bazett, LV APD: action potential duration of the left ventricle, RV APD: action potential duration of the right ventricle, ΔAPD: LV APD - RV APD, EADs: early afterdepolarizations observed on the MAP in both the right and left ventricle i.e. in total 20*2=40 MAP recordings.

demonstrated a significant increase (table 2), resulting in a large difference in inter-ventricular ΔAPD (135 ± 55 ms vs 60 ± 40 ms, p<0.01) between the inducible and non inducible group.

The development of EADs after *d*-sotalol was noticed in almost all inducible (10/11, figure 2) as well as in half of the non inducible dogs (4/9). The ectopic beats did not differ in both groups (8/11 vs 4/9).

With the exception of one dog (see below) pacing under control conditions did not lead to TdP (figure 2, panel 1). Following *d*-sotalol, induction of TdP occurred often during the pacing train, because pacing exaggerated the occurrence of EADs and spontaneous beats (figure 2, panel 2).

One of the susceptible dogs showed already prominent U waves when the dog was tested consciously prior to the experiment. When anesthetized, this dog possessed EADs and a large interventricular dispersion under baseline conditions (figure 3, panel 1) and pacing led to TdP. After treatment with MgSO₄ the ECG normalized and the induction

Figure 2. Initiation of a "pacing induced" Torsade de Pointes arrhythmia after *d*-sotalol.

A three lead ECG and two monophasic action potential (MAP) signals recorded endocardially in the right (RV) and left ventricle (LV) are shown at a paper speed of 10 mm/s. A pacing train of 8x600 ms followed by an extrastimulus during control conditions does not induce any arrhythmic event (panel 1). Stimulation is shown by a S. Interventricular Δ APD amounts to 50 ms. In panel 2, administration of *d*-sotalol leads to an increase in cycle length of the idioventricular rhythm, the QT time, and both the RV MAP (350 to 460 ms) and the LV MAP (400 to 600 ms). Because the increase in LV APD is more pronounced, the interventricular Δ APD is increased to 140 ms. Note the occurrence of EADs on both MAPs (see arrows). Repetition of the pacing train results in a TdP. The arrhythmia starts already during the pacing train by beats that are triggered by EADs (marked by an asterisk). Also subthreshold EADs can be clearly seen, especially in LV MAP (arrows).

Figure 3. Spontaneous TdP after *d*-sotalol.

In this figure a six lead ECG is shown together with MAP recordings in the right (RV MAP) and in the left ventricle (LV MAP) recorded at a paper speed of 10 mm/s. Already under control conditions this dog had a pronounced interventricular dispersion of APD (Δ APD) of 115 ms and EADs can be seen on the LV MAP (arrow, panel 1). Administration of *d*-sotalol (panel 2) increases the CL-IVR, QT time, RV APD to 460 ms and LV APD to 640 ms resulting in a dispersion of 180 ms. Also negative T waves develop and the EADs became more pronounced (see arrows), leading to ectopic beats (asterisk) and spontaneous TdP. This self terminating TdP starts in the classical way of a short long short sequence triggered by the EADs occurring on the LV MAP.

of TdP by pacing was prevented. Thirtyfive minutes after MgSO_4 , *d*-sotalol induced spontaneously triggered beats resulting several times in episodes of spontaneous TdP (figure 3, panel 2). For a second time MgSO_4 was administered to this dog, to abolish the effect of *d*-sotalol (figure 4).

Effects of MgSO_4

MgSO_4 was administered at 35 ± 8 minutes after *d*-sotalol to 7 animals (4 I and 3 NI). Because the electrophysiological effects of *d*-sotalol decrease in time (18), the value “*d*-sotalol 35’” was introduced representing the electrophysiologic measurements at the start of MgSO_4 administration. No significant differences were found between *d*-sotalol 10’ and “*d*-sotalol 35’”. In 6/7 animals EADs (both inducible and non inducible for

Figure 4. Effect of MgSO_4 .

Same dog and composition as figure 3. At the start of MgSO_4 (20 minutes after *d*-sotalol) a bigemic rhythm exist. The LV MAP is markedly prolonged compared with the RV APD and has very pronounced EADs, that trigger ectopic beats (panel 1). Three minutes after start of MgSO_4 the repolarization process has changed (disappearance of the negative U waves), the ectopic beats are suppressed and the QT time, RV APD and the LV APD markedly shortened resulting in less dispersion. All this takes place while the CL-IVR increases (!) to 4180 ms. The EADs of LV MAP have disappeared completely, while the EADs in RV MAP remained present but appear with less amplitude.

TdP) and in 2/7 animals ectopic beats were present at the moment of MgSO_4 administration. The effect of MgSO_4 was most pronounced at 3 minutes after the start of the injection. Although the CL-IVR increased, MgSO_4 1) diminished QT time (table 3), 2) suppressed ectopic beats (2/2) (figure 4, panels 1-2), and 3) either suppressed (3/6) or diminished the amplitude of EADs (3/6, eg figure 4, panel 2), and 4) shortened RV and LV APD. Because of the more overt decrease of the LV APD, the interventricular ΔAPD (55 ± 60 ms) was significantly decreased to values comparable with baseline (60 ± 35 ms, table 3). MgSO_4 prevented TdP induction (4/4). The magnitude of the electrophysiological effects of MgSO_4 was more pronounced for the CL-IVR, LV APD and RV APD in the inducible dogs, while interventricular ΔAPD was halved in both groups.

Table 3. Electrophysiologic effects of MgSO₄ (100mg/kg) in 7 dogs (4 inducible and 3 non inducible)

	CONTROL	D-SOT (35')	MgSO ₄ (3')
CL-IVR (ms)	1625 ± 320	1885 ± 320*	2400 ± 755*
QT(U)	410 ± 50	530 ± 85*	470 ± 65*
QT _c	325 ± 50	390 ± 70*	310 ± 60*
LV APD	390 ± 70	510 ± 100*	435 ± 85*
RV APD	330 ± 40	400 ± 60*	380 ± 40*
ΔAPD	60 ± 35	110 ± 45*	55 ± 60*

MEAN ± SD; * P < 0.05 vs control, # P < 0.05 vs D-SOT (35'); D-SOT (35'): 35 minutes *d*-sotalol, MgSO₄ (3'): 3 minutes MgSO₄, CL-IVR: cycle length idioventricular rhythm, QT_c corrected QT time, LV APD: action potential duration of the left ventricle, RV APD: action potential duration of the right ventricle, ΔAPD: interventricular dispersion of APD calculated as LV APD – RV APD.

DISCUSSION

In our opinion, this study presents new information regarding the relevance of interventricular ΔAPD for the initiation of acquired TdP arrhythmias in our animal model. Although this has been suggested by others (13,17) we have made the following important observations: 1) In the absence of EADs, interventricular ΔAPD demonstrates bradycardia dependence: there is an increase in interventricular ΔAPD when the heart rate slows; 2) After *d*-sotalol, interventricular ΔAPD is increased because of slowing of the IVR and site specific occurrence of EADs; 3) endocardially measured interventricular ΔAPD is always greater than intraventricular differences in APD; 4) Both the presence of EADs and interventricular ΔAPD are greater in the inducible dogs, while the incidence of ectopic beats does not differ; and 5) suppression or prevention of TdP by MgSO₄ is related to disappearance of EADs and diminishment of interventricular ΔAPD.

Interventricular dispersion in APD

Since the description of TdP arrhythmias more than 25 years ago (25), several mechanisms have been proposed to explain its occurrence, like EAD dependent triggered activity (ectopic beats) and reentry based on dispersion. In the intact heart, both parameters can be measured by the use of MAP catheters, which provide information on regional repolarization (22,26). In patient studies, both intra- and interventricular differences in APD have been indicated to be related to TdP (14-17). When EADs originate at specific sites, APD will prolong regionally and by definition dispersion will increase. Recent studies have also indicated an increased dispersion of repolarization in acquired TdP by measuring QT intervals in multiple ECG leads (27-29).

Rate dependent behavior of repolarization parameters and EADs is widely acknowledged (see e.g. 1,4,9,10). Data concerning the frequency dependence of APD dispersion are scarce. Still, there is some evidence that differences in APD are smaller at faster rates (30,31). In the present study ≥ 2 MAPs were recorded simultaneously. For the first time, we present evidence that the sensitivity of the APD to prolong when the heart frequency decreases is different for the LV than the RV, resulting in an increase in interventricular Δ APD (bradycardia dependence). These observations are obtained in the absence of EADs on the registered MAPs so that the increase cannot be explained by the contribution of EADs to interventricular Δ APD. Similar information can be obtained from table 2, but now in the presence of EADs. Comparing the different cycle lengths in the groups with and without *d*-sotalol, a clear lengthening of the interventricular Δ APD is seen as the CL-IVR between groups increases. To what extent EADs exactly contribute to this interventricular Δ APD is not known, but a contribution will exist when EADs are induced at selected sites as is the case with *d*-sotalol for the LV.

A possible explanation for the higher sensitivity of the LV to prolong after a frequency change might be the presence of M cells (32). The canine myocardium is said to exist of circa 40% of M cells. Under baseline conditions the M cells of the LV show more prolongation at slower cycle lengths than the RV M cells, leading to an interventricular Δ APD of approximately 100 msec at 2000 msec (figure 4 of reference 12). Regional development of EADs by class III drugs will attribute to the dispersion and it is known that M cells are also more sensitive than endo- and epicardial cells to develop EADs after drugs such as quinidine, *dl*-sotalol, and Bay K 8644 (12).

Although *d*-sotalol will not increase interventricular Δ APD at steady state CL in the absence of EADs (figure 1), administration of the drug will influence interventricular Δ APD in several ways: 1) increase in CL-IVR, and 2) occurrence of (site-specific) EADs. A second indication that EADs and interventricular Δ APD are two independent phenomena is indicated by the effects of $MgSO_4$. The induced increase in CL-IVR would normally be associated with an increase in APD and interventricular Δ APD. When extrapolation of the APD to a CL of 2400 ms is allowed (using table 2, baseline values, no EADs), the calculated LV APD would be approximately 510 ms, while the RV has a length around 380 ms. The mean values that were observed after $MgSO_4$ are however much less: LV APD of 435 ms and RV APD of 380 ms, resulting in a interventricular Δ APD of 55 ms similar to baseline (table 3). The shorter LV APD is partially caused by the disappearance of the EAD but to a larger extent to homogenization of repolarization by a more pronounced shortening of the LV APD.

Whether the pronounced interventricular Δ APD is related to the presence of chronic complete AV block seems indicated by the results of a recent study (33).

To determine the exact contribution of EADs to interventricular Δ APD further studies need to be performed.

Induction of TdP

Based on our data, we cannot assess the exact contribution of EADs for interventricular Δ APD. But we do believe that we provide evidence that both EADs and interventricular Δ APD, as suggested by others (1,13,17), have to be present in a certain magnitude to induce TdP. Inducible dogs have a higher incidence of EADs, a similar incidence of ectopic beats as non inducible dogs but possess a more pronounced interventricular Δ APD. Because EADs and ectopic beats are also present in the non inducible group, their appearance alone seems not sufficient to induce TdP. The fact that the presence of ectopic beats does not lead to spontaneous TdP nor seems related to pacing induced TdP could be explained by 1) the differences in coupling interval, 2) the number of beats in the pacing train, 3) to the lack of interventricular Δ APD, or 4) combination of these factors.

Secondly, prevention of TdP by MgSO_4 is achieved through a more pronounced reduction of repolarization leading to less interventricular Δ APD. Similar data that we obtained in a different study confirm this observation: in the same dog the higher occurrence of (spontaneous) TdP after almokalant was associated with more EADs and a higher interventricular Δ APD compared with *d*-sotalol (34).

This study again emphasizes the role for bradycardia in the genesis of TdP. EADs, APD and interventricular Δ APD all show bradycardia dependence. Acceleration of the rate either by isoprenaline or ventricular pacing prevents the occurrence of TdP (18). And a further slowing of the rate by a second bolus of *d*-sotalol in the non inducible dogs, increases inducibility to 70-80% (18).

Intraventricular differences in APD

Next to interventricular Δ APD, intraventricular differences in APD could also contribute to dispersion of repolarization. Although only in part of the dogs the APD was measured at multiple sites in the same ventricle, these data show that in our animals (table 1) the observed intraventricular differences are much less than interventricular Δ APD. So it seems that the ventricles themselves behave more uniformly, although potentially large intraventricular differences can never be excluded, because of the regional information provided by the MAP catheter which was placed randomly.

Possible consequences of dispersion for the mechanism of TdP

A non uniform repolarization of the heart (dispersion) has already for a long time been acknowledged as a substrate for lethal ventricular arrhythmias (35). Differences in repolarization will favor reentry which could perpetuate the ectopic impulse formation after the first triggered beat(s) and so initiate TdP. One of the possibilities is spiral wave reentry which causes polymorphic patterns in epicardial sheets. The spiral wave reentry

is due to a gradient in dispersion or conduction velocity (36). Another possibility is intramural reentry caused by transmural dispersion due to the M cells (12).

However a role of reentry in the mechanism of continuation of TdP has never been proven. Recent research using three dimensional mapping suggested the presence of different foci (37,38), originating from the endocardium as the perpetuation substrate of the arrhythmia. The present study is not able to discriminate between triggered activity or reentry for the mechanism of continuation of TdP.

Limitations

Our data point to the involvement of interventricular Δ APD and EADs in the initiation of TdP. However neither the presence of EADs nor an absolute amount of interventricular Δ APD can predict the occurrence of TdP, because a small overlap in the values of the interventricular Δ APD between inducible and non inducible dogs is present.

It may be important to note that all rate dependent effects of the differences in APD are reported during steady state paced cycle lengths or during a relatively stable CL-IVR (30,31). A sudden rate change after *d*-sotalolol i.e. by programmed electrical stimulation or by triggered beats may increase interventricular Δ APD, due to a (temporal) accentuation in EADs (see e.g. figure 2). This can provide the trigger for the initiation of TdP, either due to the triggering of the accentuated EADs or due to the prolonged interventricular Δ APD. The dynamic behavior of EADs and interventricular Δ APD in relation to frequency needs therefore further investigation.

During spontaneous IVR the activation times will differ depending on the origin of the focus. This may influence differences in APD when we consider a relation between the time of activation and AP duration (39,40). There are numerical places for impulse formation in our dogs. Therefore, we do not believe that specific activation site will markedly influence dispersion in APD.

Conclusions

Like EADs, interventricular Δ APD independently demonstrates frequency dependence during steady state rhythms. An increased interventricular Δ APD with or without the contribution of local EADs is an important factor in the initiation of TdP.

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Chapter 4

Further observations to confirm the importance of interventricular dispersion of repolarization and early afterdepolarizations in the genesis of acquired Torsade de Pointes arrhythmias: a comparison between almokalant and *d*-sotalol using the dog as its own control

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ABSTRACT

Objectives: Further investigation of the initiating mechanism of acquired Torsade de Pointes (TdP) arrhythmias.

Background: Administration of class III agents can be associated with TdP. We have developed a dog model in which after *d*-sotalol TdP can be reproducibly induced by pacing. This model shows reproducible results over weeks.

Methods: In 14 anesthetized dogs with chronic complete AV block, two separate experiments were performed in which *d*-sotalol (2 mg/kg) or almokalant (0.12 mg/kg) were administered. Monophasic action potentials were simultaneously recorded from the endocardium of the right and the left ventricle to register early afterdepolarizations (EADs) and to measure the action potential duration (APD). Interventricular dispersion of repolarization (Δ APD) was defined as the APD of the left ventricle (LV) minus that of the right ventricle (RV). **Results:** Baseline conditions were identical in the serial performed experiments (cycle length of the idioventricular rhythm (CL-IVR): 1645 ± 325 ms, QT time: 400 ± 55 ms, LV APD: 375 ± 45 ms, RV APD: 330 ± 35 ms, and interventricular Δ APD: 45 ± 25 ms (mean $\pm$ SD). The CL-IVR and QT time increased with 16% and 26% after *d*-sotalol and with 15% and 31% after almokalant. Similar to EADs (18/22 vs 11/24, $P < 0.05$), single or multiple ectopic beats were more frequently observed after almokalant than after *d*-sotalol. After both drugs the LV APD prolonged more than that of the right ventricle, thereby increasing interventricular Δ APD. This was more pronounced after almokalant (110 ± 60 ms) than for *d*-sotalol (80 ± 45 ms, $P < 0.05$). Spontaneous TdP was observed in 10/14 dogs after almokalant, and the incidence could be increased to 12/14 after programmed electrical stimulation (PES). After *d*-sotalol, TdP could only be induced by PES (5/14, $P < 0.05$).

Conclusions: In the same dog, almokalant induced more delay in repolarization, more EADs, and more ventricular inhomogeneity in APD than *d*-sotalol. These changes were related to a higher incidence of TdP, and thereby confirm the importance of EADs and interventricular Δ APD in the genesis of TdP. The findings also show the possible value of our model to evaluate proarrhythmic potential of different drugs.

INTRODUCTION

Antiarrhythmic drugs which prolong repolarization without affecting conduction have attracted interest, because of their possible value in prevention and suppression of reentrant tachycardias (1-3). However class III drugs do produce Torsade de Pointes (TdP) arrhythmias in about 1-5% of patients (1,2,4-9).

Many articles have pointed to the relevance of early afterdepolarizations (EADs) and EAD dependent ectopic beats (EBs) for the initiation of TdP arrhythmias (9-12). Whether they can be solely responsible for the initiation and/or whether other factors

like ventricular dispersion of repolarization (Δ APD) do contribute is still a matter of discussion (13-15). Regional differences in the duration of the action potential (APD) can be present within the ventricular wall (transmural), within one ventricle (intraventricular) and/or between ventricles (interventricular).

Comparing inducible vs non inducible animals in our canine model of TdP, we have demonstrated that the number of EADs is higher and the amount of interventricular Δ APD larger, using endocardially placed monophasic action potential (MAP) catheters (16). In anesthetized dogs with chronic complete AV block, TdP can be reproducibly initiated using the combination of d-sotalol and pacing in half of the experiments (17). Because the response is maintained over weeks (17), we compared the effect of two antiarrhythmic drugs, d-sotalol and almokalant in their ability to induce TdP arrhythmias in the same dog. In this way we could assess whether differences in the occurrence of EADs, EAD dependent EBs and/or interventricular Δ APD could explain initiation of TdP.

METHODS

The study protocol was approved by the Committee for Experiments on Animals of the University of Limburg, Maastricht, the Netherlands and conducted in accordance with the guidelines of the American Physiological Society.

General

The experiments were performed on anesthetized adult mongrel dogs of either sex with a body weight between 20 and 31 kg. In preliminary surgery, a right thoracotomy was performed to induce a permanent complete AV block by injection of 37% formaldehyde into the AV junction (18). During the same session, a pacing electrode (Bakken Research Center, Medtronic) was inserted at the apex of the left ventricle. The wire was exteriorized through the back of the neck of the dog. Six surface electrocardiographic leads and 2 endocardial MAP were simultaneously registered and stored on optical disc. All drugs were administered through a canula in the cephalic vein.

Anaesthesia was induced by 1) premedication i.m. (1 ml/5 kg: 10 mg oxycodon, 1 mg acepromazine and 0.5 mg atropine) and 2) sodium pentobarbital (20 mg/kg i.v.). The dogs were artificially ventilated through a cuffed endotracheal tube using a mixture of oxygen, nitrous oxide and halothane (vapor concentration 0.5%-1%) by a respirator. Ventilation was controlled by continuous reading of the carbon dioxide concentration in the expired air. A thermal mattress was used to maintain adequate body temperature. Proper care was taken before and after the experiments, including antibiotics (1000 mg ampicillin) and analgesics (0.03 mg/7 kg i.m. buprenorfine). A temporary ventricular

pacemaker (VVI) was sometimes given after the AV block operation and after the experiments. Pacing was switched off after a maximum of 24 hours.

Determination of dose almokalant

In sinus rhythm dogs, other investigators have shown that the effect of 0.35 mg/kg almokalant on electrophysiological parameters as APD was about two times as strong as 3 mg/kg *d*-sotalol (19). Therefore we chose a dose of 0.12 mg/kg almokalant, to create similar effects as 2 mg/kg *d*-sotalol. This is the dose that we have used in the past (16-18) to induce TdP.

In preliminary experiments two dogs were tested with this dose of almokalant. These dogs showed repetitive episodes of spontaneous TdP. This is in contrast with the low incidence ($\pm 8\%$) of spontaneous TdP with *d*-sotalol in our model (17). Administration of almokalant also resulted in the death of an animal during the infusion of almokalant (0.06 mg/kg): an episode of TdP deteriorated into ventricular fibrillation which could not be cardioverted. Therefore, we started to administer almokalant (0.12 mg/kg/5 min) first to selected dogs ($n=3$) in whom no TdP could be induced by *d*-sotalol. In these experiments the arrhythmogenic potential of almokalant was well controlled so that we than administered almokalant to eleven successive dogs. In the 14 dogs tested, *d*-sotalol was given first (4 ± 2.2 weeks after creation of complete AV block) in eleven dogs, followed by almokalant 3 ± 2.1 weeks later. In three dogs the order was reversed; almokalant at 5 ± 2.6 weeks and *d*-sotalol at 9 ± 3.1 weeks of chronic complete AV block. The time of administration of almokalant was doubled from 5 to 10 minutes in the last 10 animals following a publication on the possible relation between (high) infusion rate and the initiation of TdP by almokalant (11).

Induction of Torsade de Pointes arrhythmias

A detailed description of the TdP protocol is described elsewhere (17). In short, at least 2 weeks after creation of AV block anesthetized animals received two defibrillation patches that were attached to both sides of the chest and connected with a defibrillator. At least half an hour after the onset of anaesthesia programmed electrical stimulation (PES) was performed from the epicardial electrode. Stimulation was done with a programmable stimulator capable of pacing synchronously to the QRS complexes. Unipolar stimuli were given using a pulse of 2 ms and a stimulus strength of twice diastolic threshold. As indifferent electrode a needle was placed through the skin. PES consisted of two different pacing protocols: 1) A short long short sequence (400 800 + extrastimulus, or 4×600 1200 + extrastimulus) and 2) 8 basic stimuli followed by an extrastimulus. The interstimulus intervals were 600 or 1200 ms. The extrastimulus interval in both pacing protocols was shortened from 500 ms using steps of 50 ms till

300 ms. After completing the basic pacing protocol, *d*-sotalol (2 mg/kg/5 min) or almokalant (0.12 mg/kg/5 or 10 min) were administered.

Pacing was resumed 10 minutes after the start of the infusion unless spontaneous TdP had occurred during the observation period. When TdP occurred, we tried to perform the pacing protocol at 15 minutes. Pacing was always performed in a random order. A TdP arrhythmia was defined as a polymorphic ventricular tachycardia consisting of 5 beats or more twisting around the baseline in the setting of a prolonged QT(U) duration. TdP was terminated using cardioversion (60-70 J) when it lasted longer than 10 seconds. A dog was called inducible when TdP was induced 3 times or more using the same pacing mode and/or when spontaneous initiation of TdP occurred (≥ 3 times). In one experiment problems with cardioversion urged us to stop prematurely, so that reproducibility could not be assessed.

Monophasic Action Potentials

MAPs were recorded to observe the occurrence of EADs and to measure the APD of the left (LV) and right ventricle (RV). Quadripolar contact electrodes (Franz combination catheter, EPT # 1650), that provide both pacing and MAP recording capabilities were placed endocardially in the right and in the left ventricle via the external jugular vein and the carotid artery under fluoroscopic guidance. MAP phases were defined according to the definitions used for transmembrane potentials (20). Amplitude was defined between phase 4 and 2 of the signal. Besides a minimal amplitude of 15 mV the MAP had to have a constant configuration and a smooth shape during control circumstances.

The MAP catheters were randomly placed in the ventricle and accepted for analysis if all these conditions were fulfilled. Good quality MAPs in both ventricles were present in 11/14 experiments with almokalant and in 12/14 experiments with *d*-sotalol. EADs were defined as an interruption of the smooth contour of phase 2 and/or 3 of the action potential (10). The presence of EADs was examined in both MAPs.

Data analysis

With the use of a custom made computer program with a resolution of 2 ms and adjustable gain and time scale, the following parameters were measured: cycle length of the idioventricular rhythm (CL-IVR), QT time and APD. All electrophysiological data reported are the mean of 5 consecutive (single) beats, and were checked by an independent blinded observer.

Interventricular Δ APD was defined as the difference between the LV APD and RV APD measured after total repolarization ($=APD_{100}$).

Statistics

Multiple measurements ANOVA, followed by a Bonferroni *t*-test was used to compare the data between the two treatments and a χ^2 test was used when the data were presented as percentages. *P* values ≤ 0.05 were considered significant. All data are presented as mean $\pm$ standard deviation (SD), unless otherwise specified.

RESULTS

Slow and fast infusion of almokalant

The change in infusion rate (5 vs 10 minutes) of almokalant did not alter the electrophysiological effects nor the (time of) occurrence of EBs and/or incidence of TdP. Therefore, we will present the data as a whole.

Electrophysiological effects

Baseline conditions were similar in each of the 14 serially tested animals. Values for CL-IVR, QT time, APD of both ventricles and the interventricular Δ APD are summarized in table 1. At 10 minutes, *d*-sotalol had significantly prolonged the CL-IVR with 16%, and the QT time with 26%. It also significantly increased the LV APD and RV APD. Because prolongation of the LV APD was more pronounced (27% vs 21%), interventricular Δ APD increased significantly (table 1).

Also almokalant significantly increased the CL-IVR and all the repolarization parameters. Again interventricular Δ APD increased ($p \leq 0.05$) due to a more pronounced increase in the LV APD compared to the RV APD (45% vs 29%, table 1).

Table 1. Electrophysiologic effects of *d*-sotalol and almokalant

	<i>d</i> -sotalol			almokalant		
	0'	10'	%	0'	10'	%
CL-IVR	1620 $\pm$ 300	1880 $\pm$ 370*	+16	1670 $\pm$ 360	1910 $\pm$ 425*	+15
QT	390 $\pm$ 50	495 $\pm$ 80*	+26	415 $\pm$ 55	545 $\pm$ 115*	+31
RV APD	320 $\pm$ 30	390 $\pm$ 45*	+21	335 $\pm$ 35	440 $\pm$ 60*#	+29#
LV APD	370 $\pm$ 45	470 $\pm$ 60*	+27	375 $\pm$ 45	535 $\pm$ 110*#	+45#
Δ APD	50 $\pm$ 25	80 $\pm$ 45*	+75	40 $\pm$ 20	110 $\pm$ 60#	+330

* $P < 0.05$ $t=10$ vs $t=0$, # $P < 0.05$ almokalant vs *d*-sotalol, CL-IVR: cycle length of the idioventricular rhythm, APD: action potential duration, LV: left ventricle, RV: right ventricle, Δ APD: LV APD - RV APD. Data are in milliseconds (mean $\pm$ SD) measured at the start and after 10 minutes of drug administration.

Figure 1. Occurrence of EADs, triggered beats and spontaneous TdP-arrhythmia after almokalant in the canine heart.

Three simultaneously recorded surface electrocardiographic leads and two endocardial monophasic action potentials (one from the left: LV MAP and one from the right ventricle: RV MAP) are presented. Paper speed is 10 mm/sec. Panel 1: Baseline conditions with a spontaneous idioventricular rhythm having a cycle length of 1670 ms (IVR), and a QT time of 390 ms. The difference between the left and the right action potential duration (Δ APD) is 65 ms. Panel 2: 7 minutes after almokalant CL-IVR (2170 ms) and QT-time (625 ms) have increased and the positive T wave (lead II and III) has changed into a negative TU wave. Dispersion in repolarization (Δ APD) has increased to 200 ms. In both MAPs, early afterdepolarizations (EADs) are present (arrows). Spontaneous ectopic beats occur in the repolarization phase. Some of them clearly seemed to be triggered by an EAD that has reached threshold (asterisk). This is followed by a short period of TdP.

When comparing *d*-sotalol with almokalant, no differences were found in the amount of lengthening of the CL-IVR. However the effect on the parameters of repolarization was greater after almokalant (table 1).

Presence of early afterdepolarizations, ectopic beats, and spontaneous initiation of Torsade de Pointes

In the first 10 minutes, administration of the drugs resulted in the occurrence of EADs (figure 1, panel 2) in 18/22 MAPs with almokalant and in 11/24 MAPs after *d*-sotalol (table 2). With *d*-sotalol EADs tended to appear more frequent in the left ventricle ($P < 0.1$, table 2), whereas no difference was seen after almokalant. Single EBs developed

Table 2. Incidence of early afterdepolarizations and Torsade de Pointes arrhythmias (TdP) after *d*-sotalol (dS) and almokalant (A)

dog	LV/RV	EAD dS	EAD A	INDU TdP dS	INDU TdP A
1	LV	+	+	no	no
	RV	+	+		
2	LV	+	+	PES	Spon [#]
	RV	—	+		
3	LV	+	+	no	Spon
	RV	—	+		+ PES
4	LV	—	+	PES	PES
	RV	—	+		
5	LV	—	+	no	Spon [#]
	RV	—	+		
6	LV	+	+	PES	Spon
	RV	+	—		+ PES
7	LV	+	+	PES	Spon [#]
	RV	—	+		
8	LV	+	+	no	Spon
	RV	—	+		+ PES
9	LV	+	—	PES	PES
	RV	—	—		
10	LV	+	+	no	Spon
	RV	+	—		+ PES
11	LV	—	+	no	
	RV	—	+		PES
12	LV	—	ND	no	Spon [#]
	RV	—			
13	ND		ND	no	Spon
14	ND		ND	no	no
Total		11/24	18/22*	5/14	12/14*

LV: left ventricle, RV: right ventricle, EADs: early after depolarizations present in RV or LV, ND: not determined, INDU: inducibility; no: no TdP, PES: induction by programmed electrical stimulation, Spon: spontaneous initiation of TdP. *P<0.05 vs dS. [#] Due to spontaneous activity no PES could be performed.

in almost all dogs with almokalant (13/14) and in less than half of the dogs after *d*-sotalol (5/14). Moreover, multiple EBs were observed after almokalant. In some experiments these spontaneous beats could be associated with triggering of EADs recorded with the MAP catheter (figure 1, panel 2).

Despite the occurrence of EBs after *d*-sotalol, none of these dogs developed spontaneous TdP. Almokalant induced spontaneous TdP in 9 dogs (figures 1 and 2), which was

Figure 2. Continuous ectopic activity and TdP after almokalant.

Recordings are shown at a paper speed of 10 mm/s. Before the administration of almokalant (CONTROL) a stable cycle length of the idioventricular rhythm (CL-IVR) (1510 ms) with a QT time of 430 ms is present. Administration of almokalant (0.12 mg/kg/10 min) led to the occurrence of ectopic beats at 3 minutes. From that time on to 30 minutes after the start of the infusion, episodes of ectopic beats and TdP alternated with the IVR. In this figure a continuous recording of 225 sec starting 5 minutes after start of almokalant is shown. Almokalant has increased the CL-IVR to 1745 ms and the QT time to 680 ms. Furthermore the T wave has become more negative. At the end of this T wave an ectopic beat starts a period of TdP. After cessation another period of TdP evolves. After a change in the site of origin of the IVR (different QRS configurations) a new episode of TdP starts now after a short long short sequence.

Figure 3. Incidence of spontaneous Torsade de Pointes related to interventricular dispersion.

This graph shows the mean values ($\pm$ SEM) of interventricular dispersion in APD (Δ APD) in the animals with MAPs at both experiments. Dispersion has been divided in three groups 1) baseline, 2) after class III without spontaneous TdP (class III, d-sotalol and almokalant; $n=15$), and 3) after class III drugs in those experiments with spontaneous occurrence of TdP (sp TdP, almokalant; $n=7$). The last group has the largest interventricular Δ APD ($P<0.05$) compared with baseline (*) and class III (#). Interventricular Δ APD of 95 ms has a specificity of 73% and a sensitivity of 85% to predict TdP

Figure 4. Induction of TdP by almokalant.

Same layout, abbreviations, and animal as in figure 1. At 43 minutes after the start of the almokalant administration, all values still are shorter as compared with figure 1, but still longer than after *d*-sotalol (figure 5). Also negative T waves are present and the MAP recording clearly demonstrates the presence of EADs (arrows). The Δ APD is 125 ms. A pacing train of 400/800+1 extrastimulus initiates a self terminating TdP. Note the shortening of the APD and suppression of EADs after termination of the TdP with a diminution of Δ APD to 60 ms. Thereafter the EADs reoccur and APD starts to increase.

associated with a significantly larger interventricular dipersion (figure 3) and a higher incidence and number of the EBs.

Induction of Torsade de Pointes after programmed electrical stimulation

After *d*-sotalol, PES resulted in the induction of TdP in 5 dogs.

PES could not be performed in 4/9 spontaneously inducible dogs because ectopic activity and periods of TdP prohibited this (see example in figure 2). In the other 5 dogs pacing induced TdP in 4 animals (table 2). In the dogs without spontaneous arrhythmias ($n=5$), pacing resulted in 3 dogs in TdP. Therefore the total incidence of TdP after almokalant was 12/14 dogs.

An representative illustration of the difference in response to *d*-sotalol and almokalant in the same animal is shown in figures 4 and 5. Comparing *d*-sotalol with almokalant

Figure 5. No TdP with pacing after *d*-sotalol.

Same animal as in figures 1 and 4. After *d*-sotalol a slow CL-IVR (1645 ms) is seen accompanied by a prolonged QT time (490 ms). In both ventricles no EADs are present, and the interventricular Δ APD measures 90 ms. Performance of the same pacing train as used with almokalant did not initiate a TdP after *d*-sotalol, although pacing seemed to evoke an EAD in the RV (arrow).

showed: 1) a higher incidence of TdP by almokalant independent of the order of the experiments, and 2) all inducible dogs with *d*-sotalol were also inducible with almokalant (table 2).

In two dogs, *d*-sotalol was administered in a third experiment. The response was similar to the first experiment thereby confirming reproducibility.

DISCUSSION

The exact contribution of the different factors leading to initiation of TdP is still a matter of discussion. Besides the accepted parameters: bradycardia and (class III induced) prolonged QT time, EADs and Δ APD have been indicated in the genesis of TdP. By

using MAP catheters, the in vivo appearance of EADs have been associated with EAD triggered EBs, negative T or TU waves. Similarly, an increased intraventricular Δ APD has been found in patients with congenital or acquired TdP (13). However, only one case report has shown a causal relation between the occurrence of EAD triggered EBs, interventricular Δ APD and initiation of spontaneous acquired TdP (15). In this study we have tried to confirm the importance of both parameters in the genesis of acquired spontaneous and pacing induced TdP by comparing the proarrhythmic effects of almokalant with *d*-sotalol.

Interventricular Δ APD in relation to spontaneous and pacing induced Torsade de Pointes

Spontaneous occurrence of TdP after almokalant was preceded by 1) an increase in the LV APD and RV APD, 2) occurrence of subthreshold EADs, 3) an increase in interventricular Δ APD (figure 3), and 4) spontaneous EBs. In comparison with the other almokalant and *d*-sotalol experiments not showing spontaneous TdP, we found that all the above mentioned parameters were significantly increased. This confirms and extends the importance of EADs (and their triggered responses) and interventricular Δ APD in the genesis of spontaneous TdP. In a previous study we have shown that these parameters also discriminate between pacing inducible and non inducible dogs after *d*-sotalol in this model (16). Prevention and/or suppression of TdP by $MgSO_4$ resulted from disappearance of EADs (13,17) and diminishment of interventricular Δ APD (16).

Using PES, we demonstrated that the incidence of TdP could be increased markedly. Also in this study pacing inducible dogs showed a larger interventricular Δ APD, than the non susceptible ones.

In dogs with chronic complete AV block, we have shown that interventricular Δ APD is present under baseline conditions and is bradycardia dependent, with a decrease in heart rate an increase in interventricular Δ APD is seen (16). In vitro studies using isolated canine myocytes showed that under baseline conditions the APD of the left endocardial (and epicardial) cells is longer than that of the right ventricle (21). This difference has been described to be even more pronounced for the M cell, located within the myocardium.

Like the Purkinje fiber, the M cell is much more sensitive for class III agents in its ability to prolong the APD and to develop EADs (21). The increase in interventricular Δ APD in our experiments after class III drugs, is primarily due to a larger decrease of the LV APD compared to the RV APD. By decreasing heart rate, class III agents will increase the interventricular differences in APD. Therefore the effect of almokalant (or *d*-sotalol) could be different for the various tissues involved in the heart explaining interventricular Δ APD. The M cells are not only described in dogs (21), but recent studies also showed the existence of these M cells in normal human myocardium (22). It is not known to

what extent the M cell contribute to the APD as recorded by the endocardially placed MAP catheter.

Regional appearances of EADs can also explain the increase in interventricular Δ APD. *d*-Sotalol predominantly induced EADs in the left ventricle, while almokalant produced them in both ventricles. Because administration of almokalant led to a more pronounced increase in interventricular Δ APD, the relative contribution of the amplitude of the EADs might be important.

Difference between spontaneously occurring and pacing induced TdP

When spontaneous episodes of TdP occur, pacing will normally also lead to the reproducible induction of these arrhythmias (see e.g. figures 1 and 4 and reference 17). Dogs with spontaneous TdP have more EBs (both single and multiple EBs) than dogs without. For the induction of pacing induced TdP, the presence of single EBs during idioventricular rhythm does not seem to be essential (23), indicating that either the number of EBs and/or their respective coupling interval is not sufficient to start spontaneous TdP. Our pacing modes do consist of several beats with multiple frequency changes (17) and can lead to the occurrence of EADs and triggered EBs within the pacing train. Therefore, application of PES can lead to TdP in those dogs that do not show spontaneous TdP. When considering that the respective contributions of the different parameters is equal, pacing can only lead to TdP when interventricular Δ APD is or becomes present. Until now we have not been able to determine the dynamic changes within the pacing train.

Almokalant and d-sotalol: proarrhythmic and electrophysiological effects

The proarrhythmic potential of class III agents is well known (1-9). Prolongation of repolarization is the mechanism of the antiarrhythmic effect of class III drugs, but prolongation is also associated with the risk of TdP development. Measurement of the absolute QT interval does not predict proarrhythmic potential both in clinical conditions (5) as in our animal model (24). Currently QT dispersion (as a parameter of non homogenous repolarization) is frequently mentioned as a possible tool to predict proarrhythmic risk as well as antiarrhythmic efficacy (25). The QT dispersion measured in multiple ECG leads correlates with the dispersion in regional repolarization measured by epicardial MAPs (26). Because QT dispersion shows a large variation, due to different methodologies and different patient characteristics, the question to its use as a general arrhythmic marker has still not been resolved (27).

This makes it important to screen for the proarrhythmic potential of a new class III agents using an animal model. Most animal TdP models have used non clinically relevant drugs like cesium (10,13,28) or Bay K8644 (29). The only exceptions are an awake AV block dog model with hypokalemia and β blockade followed by antiarrhythmic drugs

(30) and a rabbit model (31). In the awake dog it is not possible to measure MAPs and thereby correlate proarrhythmic findings to EADs and APD. In the rabbit, TdP develops at relatively fast heart rates after administration of class III agents and concomitant α adrenergic stimulation. Comparison between different agents in the same animal concomitant with MAP recordings have not been described until this study (30-32). Because the response of the dog is maintained over weeks, serial comparison for screening of proarrhythmic effects of (antiarrhythmic) drugs is feasible. Although this information cannot be translated into the exact incidence of TdP in the patient population, it allows an estimation of the relative risk of drugs and their risk/benefit ratio.

Almokalant and *d*-sotalol both prolong the APD by blockade of K^+ currents (33-35). While almokalant is a selective blocker of I_{Kr} (33,35), the data from the literature are not consistent for *d*-sotalol. It might also affect other K^+ channels like I_{K1} , I_{Ks} and I_{to} (35-37). In addition, *d*-sotalol has some β blocking effects, (less than 5% of *dl*-sotalol (6)) whereas almokalant is devoid of β blocking activity (34).

The difference in prolongation of the APD and occurrence of EADs after *d*-sotalol compared to almokalant may be explained by 1) differences in dose and/or kinetics, or 2) administration time. In the preparation of this study we calculated that a dose of 0.12 mg/kg almokalant should give approximately the same effect on repolarization as 2 mg/kg *d*-sotalol (13). The former is comparable with the dose given to patients to treat ventricular tachycardias (38). The effect on CL-IVR and QT was about equal for both drugs (table 1), although it should be mentioned that in the experiments with almokalant, the electrophysiological measurements were often hampered due to recurrent presence of EBs and episodes of TdP. This could have led to an underestimation of the electrophysiological effects of almokalant. The kinetics between the two drugs could also be different, *in vitro* data show that the effect of *d*-sotalol has a slower onset than almokalant (35).

Doubling the time of administration of almokalant did not alter the response, which is in contrast to the reported effects in patients (38) and rabbits (11).

Limitations and implications

In this study two MAP catheters were randomly placed, one in the left and the other in the right ventricle. This provides information about the differences in repolarization between the two ventricles (interventricular Δ APD). Possible transmural or intraventricular differences in APD have not been assessed in this study. Placement of multiple MAP catheters with *d*-sotalol revealed that the intraventricular difference amounted only to 20-30% of the interventricular Δ APD (16). We do not know the type and the amount of cells contributing to the MAP. Similarly, whether the presence of EAD on the MAP is real or based on differences in transmural repolarization is not

known. However, interventions specifically directed to EAD formation like pacing and drugs leads to disappearance of these EADs.

Transmural differences have been implicated in the mechanism of TdP (21). Besides their relevance for the occurrence of EADs, differences in APD have been shown to create the substrate for a reentrant tachycardia (39). Whether reentry plays a role in the initiation and continuation of TdP is still unclear. It is conceivable that reentry succeeds triggered activity (40,41), this is certainly the case when TdP progresses into ventricular fibrillation.

Interventricular Δ APD is not likely to play a role in reentrant arrhythmias, because spatial dispersion is located too far apart. Still the polymorphic appearance of TdP can be explained by interventricular Δ APD. Appearance of EBs from single foci can lead to TdP when this "monomorphic tachycardia" encounters continuously shifting areas of repolarization. Things become more complicated when triggered EBs arise from multiple sites in the ventricles.

Conclusions

Our study confirms the relevance of interventricular Δ APD and EADs for the initiation of (spontaneous) acquired TdP.

These findings also show that this canine model of TdP can be used to screen for the proarrhythmic potential of drugs.

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Chapter 5

An essential contribution of early afterdepolarizations to dispersion of repolarization in the mechanism of acquired Torsade de Pointes arrhythmias

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ABSTRACT

Spontaneous termination of pacing induced acquired Torsade de Pointes arrhythmias (TdP) and their reinduction was examined in anesthetized dogs with chronic AV block. Therefore early afterdepolarizations (EADs), action potential duration (APD) and interventricular dispersion (Δ APD), before (B), of the last beat (L) and after TdP, were analyzed in 20 episodes of spontaneous terminating TdP (15 ± 9 beats, cycle length (CL): 240 ± 20 ms). TdP termination was associated with a shortening of the APD, a decrease in EADs (L: 0/40 vs B: 20/40) and interventricular Δ APD (L: 10 ± 15 vs B: 80 ± 55 ms). The first beat after termination again showed EADs and an interventricular Δ APD of 60 ms. Thereafter, with no change in CL, EADs diminished significantly from 23/40 (beat 1) to 9/40 (beat 5), while Δ APD decreased to control values. TdP could only be reinduced by pacing when EADs and Δ APD had completely returned. When measuring interventricular Δ APD in the presence and absence of EADs, the contribution of EADs to interventricular Δ APD was calculated to be around 35%. Therefore an essential contribution of the EAD to the changes in interventricular Δ APD and induction TdP is indicated.

INTRODUCTION

Torsade de Pointes arrhythmias (TdP) are an intriguing polymorphic ventricular tachycardia which can either be congenital or acquired following the administration of certain drugs or electrolyte abnormalities (1). In the presence of a prolonged QT(U) interval, TdP is often initiated by a short long short sequence (SLS, 1,2). The occurrence of these premature ectopic beats has been associated with the development of early afterdepolarizations (EADs, 3-7). However the presence of induced EADs leading to ectopic beats does not always result in the initiation of TdP (6), suggesting that at least one additional parameter is required to initiate TdP. The spontaneously occurring SLS sequence and its consequences can be mimicked by frequency changes induced by programmed electrical stimulation (PES) of the heart. In our animal model of acquired TdP, PES is able to reproducibly induce TdP after administration of a therapeutic dose of the class III drug *d*-sotalol (7). We have recently shown, using endocardial monophasic action potential (MAP) recordings, that induction of TdP in this model is associated with bradycardia, prolonged QT duration, EADs, and interventricular dispersion in the duration of ventricular repolarization (Δ APD,7,8). The presence of EADs, and the length of the action potential duration (APD) are positively correlated to the cycle length (9-14). Interventricular Δ APD is also frequency dependent: in the absence of EADs the interventricular dispersion increases when the cycle length prolongs (8,15). It seems plausible that EADs and interventricular Δ APD are related, but it is unknown to what extent EADs contribute to interventricular Δ APD. It is also not clear whether

EADs and interventricular Δ APD play a role in the mechanism of continuation of TdP. Because part of the TdP episodes terminate spontaneously (1,2,7), we used these episodes to gain more insight the possible contribution of EADs to interventricular Δ APD and their role in the mechanism of TdP.

METHODS

All experiments were performed according to "Guiding principles in the care and use of animals" of the American Physiological Society and approved by the Committee for Experiments on Animals of the University of Limburg, Maastricht, the Netherlands.

General

The experiments were performed on 20 anesthetized adult dogs with chronic AV block (6 ± 3.5 , range 2-11 weeks) of either sex with a body weight between 20 and 30 kg. Each dog had one epicardial electrode (Bakken Research Center, Medtronic, Maastricht, The Netherlands) implanted at the apex of the left ventricle, after a right sided thoracotomy was performed to produce chronic complete AV block (for details see reference #16).

Pacing was done with a programmable stimulator capable of pacing synchronously to the QRS complexes. Unipolar stimuli were given using a pulse width of 2 ms and a stimulus strength of twice the diastolic threshold. As indifferent electrode a needle was placed through the skin. Six surface electrocardiographic leads and 2 MAP signals were simultaneously registered, digitized, visualized on a monitor and stored on hard disc. All drugs were administered through a canula in the cephalic vein.

Anaesthesia was induced by 1) premedication i.m. (1 ml/5 kg consisting of 10 mg oxycodon, 1 mg acepromazine and 0.5 mg atropine) and 2) sodium pentobarbital (20 mg/kg i.v.). The dogs were artificially ventilated through a cuffed endotracheal tube using a mixture of oxygen, nitrous oxide and halothane (vapor concentration 0.5%-1%) by a respirator. Ventilation was adjusted on the basis of continuous reading of the carbon dioxide concentration in the expired air. A thermal mattress was used to maintain adequate body temperature.

Proper care was taken after the experiments, including antibiotics (1000 mg ampicillin) and analgetics (0.1 mg i.m. buprenorfine). Temporary ventricular pacing (for a maximum of 24 hours) was performed after the AV block operation and after the experiments.

Induction of Torsade de Pointes arrhythmias

A detailed description of the Torsade de Pointes protocol is given elsewhere (7). In short, anesthetized animals received two defibrillation patches that were attached to both sides of the chest and connected with a defibrillator. Half an hour after the onset of

anaesthesia, pacing was done from the epicardial electrode using two different pacing modes: 1) short long short sequence, with intervals of 400 and 800 ms followed by an extrastimulus or intervals of 600, and 1200 ms followed by an extra stimulus and 2) Eight basic stimuli with an interstimulus interval of 600 ms followed by an extra stimulus. During both pacing modes the interval of the extrastimulus was shortened from 500 ms in steps of 50 ms until 300 ms. After completing the pacing protocol, one of the following class III agents was administered intravenously; *d*-sotalol (2 mg/kg/5 min) or almokalant (0.12 mg/kg/10 min). Pacing was resumed 10 to 15 minutes after the start of the injection of the drug. A Torsade de Pointes arrhythmia was defined as a polymorphic ventricular tachycardia consisting of ≥ 5 beats, which twisted around the baseline and occurring in the presence of a prolonged QT(U) duration. A second dose of *d*-sotalol (2 mg/kg) was given when no TdP could be induced after the first dose. This was followed by repetition of the pacing protocol.

Monophasic Action Potentials

Monophasic action potentials were recorded to observe the occurrence of EADs and to measure the APD in the left and right ventricle before initiation, at the time of and after the termination of the TdP. The method used to record MAPs has been described in detail in an earlier report (8). Briefly, endocardial quadripolar contact electrodes (Franz combination catheter, EPT # 1650), with pacing and MAP recording capabilities were placed at random in the right and the left ventricle via the external jugular vein and the carotid artery.

MAP phases were defined according to the definitions used for transmembrane action potentials (17). Amplitude was defined as the distance between phase 4 and 2 of the signal. Under control circumstances the MAP was required to have a minimal amplitude of 15 mV, an identical configuration in successive beats and a smooth shape.

EADs were defined as an interruption of the smooth contour of phase 2 or 3 of the action potential (5). The presence of EADs was examined in the LV as well as in the RV MAP.

Inclusion criteria of TdP episodes

We looked at 27 consecutive experiments in which either *d*-sotalol (n=22) or almokalant (n=5) were administered in combination with two simultaneous recorded MAP's in both ventricles. All spontaneously terminating TdP that were induced by the pacing protocol showing stable MAP recordings were included. Spontaneous initiation of TdP or TdP lasting longer than 10 seconds requiring cardioversion were excluded.

Analysis to estimate the contribution of EADs to interventricular Δ APD

Both the development of EADs and interventricular Δ APD is bradycardia dependent. It is known that *d*-sotalol will increase the development of EADs by increasing APD. Because *d*-sotalol also lengthens CL-IVR, contribution of EADs to interventricular Δ APD can only be assessed at the moment EADs appear and/or disappear when CL is kept constant. In 6 additional experiments, we therefore continuously paced the ventricle during administration of *d*-sotalol leading to development of EADs. Paced CL was chosen just beneath IVR at baseline.

Secondly we performed a further subgroup analysis in the TdP group in which the disappearance and reappearance of EADs after termination was related to changes in interventricular Δ APD.

Data analysis

All data were analyzed by two independent observers using a custom made computer program with a resolution of 2 ms and adjustable gain and time scale.

Electrophysiological measurements of cycle length of the idioventricular rhythm (CL-IVR) and APD were performed before, at the last beat of the TdP and after the spontaneous termination of the TdP. From each episode of TdP, the number of TdP beats was counted and the mean cycle length of the whole arrhythmia was determined. The first 5 beats after the TdP were individually measured. Thereafter every fifth beat was measured until the pacing train was repeated.

Interventricular dispersion of repolarization (Δ APD) was calculated as the difference between the left and right APD using the total repolarization duration ($=APD_{100}$) in each ventricle.

Statistics

Comparisons were made using a paired or unpaired Student *t*-test when appropriate. *P* values ≤ 0.05 were considered significant. All data are presented as mean $\pm$ standard deviation (SD).

RESULTS

In 15/27 experiments, episodes of TdP could be reproducibly (≥ 3) induced by PES. In the other experiments no TdP could be induced ($n=10$), or the occurrence of numerous spontaneous TdP episodes prevented the performance of a pacing protocol ($n=2$). In 10/15 experiments spontaneous terminating TdP occurred. In these 10 experiments (10 dogs) a total of 39 (mean 3.9 ± 1.5) pacing induced episodes were seen: 12 had to be

Figure 1. Repolarization parameters during baseline conditions and after administration of two dosages of *d*-sotalol.

Three ECG leads are recorded simultaneously with two monophasic action potential recordings in the right (RV) and in the left ventricle (LV) at a paper speed of 10 mm/s. Before pacing, a stable idioventricular rhythm (IVR) is seen with a cycle length (CL) of 1150 ms and a QT time of 340 ms (panel 1). Both action potentials are smooth with a duration (APD) of 340 ms for the LV and 320 ms for the RV, leading to a dispersion in duration of repolarization (Δ APD) of 20 ms. The pacing train consisting of two stimuli of 400 and 800 ms followed by an extra after 400 ms is not able to induce a TdP under baseline conditions. In the second panel the effect of the first dose of *d*-sotalol is seen. The CL-IVR and QT time prolong to 1570 ms and 475 ms, while both APD's increase to 420 and 440 ms respectively. No TdP could be induced by the pacing protocol. Therefore a second dose of *d*-sotalol (panel 3) was given resulting in a further increase in CL-IVR to 1800 ms. Also the duration of all repolarization parameters prolonged further. Now the development of early afterdepolarizations (EADs, arrows) can clearly be seen in both MAPs. The Δ APD has increased to 100 ms, due to a more pronounced increase in the LV APD compared to the RV APD.

cardioverted and 27 ended spontaneously. Of the spontaneously terminating TdP, 20 episodes fulfilled the criteria in regard to the MAP signal. These 20 episodes occurred in 6 dogs after the first injection of *d*-sotalol, in 2 dogs after the second dose, and in 2 dogs after almokalant.

Table 1. Electrophysiological parameters (mean±SD) before drug administration, before Torsade de Pointes (TdP), the last beat of TdP and after a spontaneously ending episode of TdP (TdP)

	control	before TdP	last beat of TdP	1 st beat after TdP	5 th beat after TdP
CL-IVR (ms)	1645 ± 295	1870 ± 255	250 ± 30	1920 ± 420	1935 ± 315
LV APD (ms)	365 ± 45	460 ± 80	250 ± 25*	430 ± 55*	390 ± 55 [#]
RV APD (ms)	325 ± 30	380 ± 50	235 ± 15*	370 ± 50*	345 ± 40 [#]
ΔAPD (ms)	40 ± 25	80 ± 55	10 ± 15*	60 ± 50*	45 ± 30
EAD	0	20/40	0/40*	23/40	9/40 [#]

* P<0.05 vs before TdP, [#] P<0.05 vs 1st beat after TdP. CL-IVR: cycle length of the idioventricular rhythm, LV: left ventricle, RV: right ventricle, APD: action potential duration, ΔAPD: difference between LV and RV action potential duration, EAD: early afterdepolarization.

Induction of TdP by PES

In the control situation, programmed electrical stimulation did not result in TdP, nor were any EADs registered (figure 1, panel 1). Ten minutes after the administration of the class III agents, *d*-sotalol or almokalant, the CL-IVR increased significantly from 1645 ± 295 to 1995 ± 270 ms, the LV APD from 365 ± 45 to 500 ± 110 ms, the RV APD from 325 ± 30 to 400 ± 70 ms and interventricular ΔAPD from 40 ± 25 to 100 ± 75 ms (all P<0.01). In addition, development of EADs after administration of the drugs (figure 1, panel 3) was observed in 8/10 experiments prior to TdP induction.

Characteristics of the TdP

The 20 analyzed periods of TdP lasted 15 ± 9 beats (range 5-35) with a mean cycle length of 240 ± 20 ms and occurred 22 ± 9 minutes after the start of the drug injection. The electrophysiological parameters are summarized in table 1. All parameters were somewhat decreased compared with 10 minutes after the start of the injection. Prior to TdP induction the mean LV APD amounted to 460 ± 80 ms and the RV APD 380 ± 50 ms. Therefore the interventricular ΔAPD prepacing was 80 ± 55 ms. EADs were present in 20/40 MAP recordings. These EADs were more frequently seen in the LV MAP (n=14) than in the RV MAP (n=6, P<0.05). A representative example of a spontaneously terminating TdP can be seen in figure 2. The high rate of the TdP resulted in a significantly shorter APD and almost no dispersion in the last beat of the TdP before spontaneous termination. Furthermore no (sub-threshold) EADs were seen in any of the terminations (table 1, third column and figure 2, panel 2).

Figure 2. Initiation and spontaneous termination of a Torsade de Pointes arrhythmia (TdP) following *d*-sotalol administration.

This is the same experiment as in figure 1. Prior to TdP induction by pacing the CL-IVR amounts to 1855 ms with a QT time of 550 ms. The LV APD measures 615 ms and the RV APD 515 ms resulting in a Δ APD of 100 ms. In both the LV APD and the RV APD a clear EAD (arrow) is seen. The same pacing train (400/800/400 ms, indicated by S) is now interrupted by an ectopic beat and after the extrastimulus the TdP starts. In panel 2 the MAP recordings during initiation and spontaneous termination have been enlarged. The last beat of the TdP has a Δ APD of 25 ms and the EADs have disappeared, although a small deviation of the MAP is still present. This was not considered to be an EAD. In the first beat after the TdP the EAD is back in both MAPs (panel 2). In subsequent beats the EAD gradually decreases. In the 5th beat the EAD from the RV has disappeared and the EAD in the LV has clearly diminished. In the 10th beat post termination the EAD on the LV has also disappeared (shown enlarged in panel 3). In line with the disappearance of the EADs the Δ APD decreases (90 ms in the 5th to 45 ms in the 10th beat).

Behavior of electrophysiological parameters after spontaneous termination

The first ventricular beat after the termination of the TdP occurred after 1920 ± 550 ms, and had a shorter APD compared with the prepacing beat. However EADs were again present (figure 2, panel 2) and interventricular Δ APD was 60 ± 50 ms. In subsequent beats the APD shortened with a concomitant decrease in interventricular Δ APD (figures 2-3). This decrease took place without a change in CL-IVR, and seemed to be related to a decrease in EADs (23/40 at the first beat to 9/40 at the 5th beat,

Figure 3. Return of EADs and dispersion leading to reinduction of TdP.

This figure is a continuous registration after figure 2 and has the same composition. It starts 17 beats after spontaneous termination of the TdP. An EAD is only minimally present in the left MAP and becomes more pronounced in the following beats (see the enlargement in panel 2). With the increase in height of the EAD in the LV MAP, the Δ APD also gradually starts to increase from 60 (beat 17) to 110 ms (beat 30, see enlargement in panel 3), just before the repetition of the pacing train (panel 1). The EAD in the right MAP returns around the 25th beat (panel 3). The same pacing train performed after 32 beats again induces a TdP.

$P < 0.05$). At the fifth beat, interventricular Δ APD (45 ± 30 ms) is comparable to the control value (40 ± 25 ms) for this CL-IVR (table 1).

After a short stable level of the APD between the 5th and the 10th beat post termination, the APD of both ventricles started again to increase (figures 2–4). This was accompanied by the reappearance of EADs and augmentation of interventricular Δ APD (figure 3). The behavior of the LV and RV APD was not always synchronously, for example in figures 2 and 3, the RV EAD disappears around beat 5 whereas the LV APD only disappears around beat 10. Also in the reappearance there are differences, the LV EAD is the first to return followed by the RV EAD. This incongruent behavior of the LV and RV EAD influences APD and interventricular Δ APD. The smallest interventricular Δ APD is found when EADs are absent in both ventricles (figure 2, panel 3), and the reappearance of LV EADs increases the interventricular Δ APD again to 120 ms at beat 25 (see further, figure 3).

Figure 4. Time course of APD adaptation after spontaneous termination of TdP.

In this figure the time course of the APD of the right (●) and left ventricle (○) is shown as the mean $\pm$ SD before and after 12 episodes of TdP. The length of the APDs are shown on the vertical axis, and the number of beats before and after the TdP on the horizontal axis. The first value is the APD of the last beat before (B) the start of the pacing train leading to induction of TdP. Beat L is the last beat of the TdP. Due to the fast rate of the TdP the APD of the last beat is decreased. The first beat (1) after termination of the TdP has an APD significantly shorter than beat B. The APD decreases further till a minimum value is reached after 5 beats. Thereafter the APD increases slowly. At 30 beats after TdP the APD is still shorter than before the start of the TdP. The Δ APD is shown between the lines of the RV and LV APD. The same pattern of shortening and subsequent increase can be observed.

The course of the APD could be followed in 12 episodes for 30 beats after termination (figure 4). At the end of these 30 beats the APD had not yet completely returned to its prepacing value (see below). Again the difference in APD behavior can be observed, the curve of the LV APD seem to differ from the RV APD. In this subgroup of TdP, the CL-IVR ranged from 1825 ± 235 ms at the prepacing beat to 1860 ± 665 , 1935 ± 300 and 1815 ± 235 ms at the first, fifth and 30th beat after termination.

Reinduction of TdP

Repetition of the pacing train after 32 ± 9 beats was performed in 15/20 TdP. A positive response i.e. reinitiation of TdP was evoked in 8/15 of the attempts (figure 3). In 5 other attempts a maximum of 4 ectopic beats were evoked, which did not meet our definition

Table 2. Electrophysiologic parameters in relation to either successful reinduction or failure to reinduce TdP

	CL-IVR (ms)	LV APD (ms)	RV APD (ms)	Δ APD (ms)	EADs
First episode n=8	1810 $\pm$ 295	455 $\pm$ 90	380 $\pm$ 70	70 $\pm$ 30	8/16
Reinducible	1790 $\pm$ 290	455 $\pm$ 75	385 $\pm$ 60	70 $\pm$ 35	7/16
First episode n=7	1965 $\pm$ 220	445 $\pm$ 85	370 $\pm$ 40	75 $\pm$ 65	5/14
Not reinducible	1995 $\pm$ 150	420 $\pm$ 70*	370 $\pm$ 45	50 $\pm$ 40*	2/14

* P<0.05 vs TdP first episode, same abbreviations as table 1.

of TdP. In table 2, the electrophysiological findings are separated in two groups: 1) Those showing reinduction of TdP and 2) those without reinduction of TdP. Reinduction of TdP was characterized by values of LV APD, RV APD, interventricular Δ APD and presence of EADs similar to those of the first episode of TdP. The non reinducible group had a shorter LV APD (445 $\pm$ 85 vs 420 $\pm$ 70 ms, P $\leq$ 0.05), similar RV APD, a lower incidence of EADs and a significantly smaller interventricular Δ APD than when the TdP could be induced.

Short and long episodes of TdP

The influence of the duration of the TdP was studied with a comparison between short episodes ($\leq$ 10 beats) and long episodes ($>$ 10 beats). The CL-IVR, LV APD and interventricular Δ APD before TdP were similar. Termination of TdP occurred when the LV APD was $\pm$ 250 ms irrespective of duration. Also the reoccurrence of EADs in the first beats after termination and their subsequent disappearance were similar in both groups.

Estimate of the contribution of EADs to interventricular Δ APD

Disappearance of EADs: In general after spontaneous termination all EADs disappeared. However the temporal behavior for their disappearance in the dogs was different. Moreover within dogs the response of the LV and RV is different in time (see e.g figure 2-3). The EAD of the left ventricle disappeared after a mean of 5 beats (range 2-9) and that of the right ventricle after 3 beats (range 2-5).

The presence of EADs was diminished from 23/40 (LV:12, RV:11) in beat 1 to 9/40 (6 LV, 3 RV) in beat 5. In table 3, four different situations are compared between beat 1 and beat 5: 1) Total absence of EADs in the first and 5th beat (n=3), 2) Total disappearance of the EAD in the left and/or right ventricle (n=10). Irrespective of the

Table 3. Relation between the presence and or disappearance of EADs with the amount of Δ APD in beat 1 to 5 after spontaneous TdP termination

	Δ APD beat 1	Δ APD beat 5	difference (ms)
1) Absence of EADs (n=3)	30 $\pm$ 50 ms	30 $\pm$ 25 ms	0 (0%)
2) Total disappearance of all EADs (n=10)	50 $\pm$ 50 ms	35 $\pm$ 15 ms	15 (30%)
3) Disappearance of LV EADs (n=7)	80 $\pm$ 45 ms	40 $\pm$ 10 ms	40 (50%)
4) Continued presence of LV EADs (n=6)	80 $\pm$ 45 ms	80 $\pm$ 45 ms	0 (0%)

For abbreviations see table 1.

behavior of the EAD in the right ventricle we scored 3) Disappearance of the EAD in the left ventricle (n=7), or 4) Persistence of LV EADs (n=6).

When the EADs disappeared (group 2 and 3) we calculated that EADs contribute 30 to 50% to the interventricular Δ APD (table 3). In both other instances no change in interventricular Δ APD was observed. In the 15 episodes of table 2, EADs reappeared in 9 MAPs. The reappearance of the LV EAD increased the interventricular Δ APD from 75 $\pm$ 55 to 120 $\pm$ 55 ms (45/120= 35%).

Occurrence of EADs: Paced CL was 1750 $\pm$ 390 ms. During the administration of *d*-sotalol the APD of both ventricles significantly increased from 420 $\pm$ 45 to 495 $\pm$ 65 ms (LV) and 355 $\pm$ 30 to 420 $\pm$ 35 ms (RV). In 4/6 experiments EADs developed (LV EADs: 4 and RV EADs: 1). In the experiments with appearance of EADs, the interventricular Δ APD increased significantly with approximately 40% from 65 $\pm$ 40 to 90 $\pm$ 40 ms, whereas in the 2 experiments without appearance of EADs no change in interventricular Δ APD occurred.

DISCUSSION

In this study self terminating TdP was analyzed to 1) obtain further knowledge about the mechanism of termination and thereby also of the mechanism of continuation of TdP, And 2) to address the relationship between EADs and interventricular Δ APD. Termination of acquired TdP is associated with a decreased incidence of EADs and a decreased interventricular Δ APD, while (re)induction is associated with the presence of EADs and an increased interventricular Δ APD. Although EADs and interventricular Δ APD are bradycardia dependent, EADs contribute significantly (35%) to interventricular Δ APD.

Mechanism of initiation of TdP

Triggered activity based on EADs is generally considered to be the initiating mechanism of TdP (4,5,7-10,14,17). In our animal model of TdP the occurrence of EADs or triggered ectopic beats after *d*-sotalol administration occur also in non inducible experiments and seems therefore not to be sufficient to initiate TdP (6). We and others suggested that besides EADs, TdP induction requires the presence of a critical amount of interventricular Δ APD (8,18,19), caused by regional differences in repolarization, which are possibly accentuated by abrupt frequency changes.

At baseline, EADs are absent, while interventricular Δ APD is relatively small. Abrupt frequency changes (by using PES) do not result in TdP. Class III agents induce EADs, increase interventricular Δ APD and TdP can now be induced by sudden frequency changes, which may either occur spontaneously (SLS sequence) or can be induced by the use of PES. Occurrence of (spontaneous) TdP is associated with a large interventricular Δ APD and presence of EADs and ectopic beats (8,20).

In this study, confirmation of the involvement of both parameters came in the attempts to reinduce TdP. Reinduction was only possible when EADs and interventricular Δ APD had returned to their original values. In addition, suppression of EADs and diminishment of interventricular Δ APD by drugs (i.e. $MgSO_4$ or flunarizine) prevents spontaneous and/or pacing induced TdP (8,21).

Mechanism of termination of the TdP

By definition a decrease in cycle length (e.g. by a tachycardia), will decrease EADs, APD (5,7-10,12,13) and the interventricular Δ APD (8). The disappearance of EADs and the marked shortening of the (Δ)APD at spontaneous termination are therefore in line with the effect of the increased rate. In this animal model around 60% of the total shortening of the APD takes place in the first 20 beats after an increase in rate (22).

Spontaneous termination indicates the impossibility to continue the arrhythmia. The mechanism of termination might give more insight about the electrophysiological changes responsible for continuation. The last beat of the TdP is devoid of EADs and the interventricular Δ APD is small. The time of disappearance is not known because the MAP recording does not allow analysis during the TdP. Because the duration of TdP episodes varies (in clinical circumstances TdP can last over 1 minute (23,24), other factors than EADs and Δ APD can be involved in the continuation and eventual termination of TdP. Also the time point at which TdP deteriorated into ventricular fibrillation is different. Because VF is based on a reentrant mechanism (25) there is a strong indication that other mechanisms can be responsible for continuation.

This is also suggested by in vitro and computer models proofing the existence of a reentrant mechanism (e.g. 26,27). However mapping data obtained in vivo demonstrate focal activity (28,29) as possible mechanism of continuation. This point is not yet settled

and additional experiments should be performed e.g. using multiple transmural MAP electrodes. This would enable to investigate transmural, inter- and intraventricular differences in repolarization and the formation (and triggering) of local EADs.

EADs and Δ APD are both present in the first beat post termination. The (re)appearance of EAD might be caused by the sudden deceleration of the rate. As we and other have shown sudden rate changes can lead to pronounciation of (the amplitude of) EADs (7,30). In the following beats the EAD disappears concomitantly with the Δ APD. The APD (independent of EADs) shortens, which might be a delayed compensation for the fast rate of the TdP. Thereafter APD starts to increase to pre TdP levels. A similar APD behavior has been described in humans after an abrupt deceleration in rate (11).

Presence of EADs and Δ APD

Discrimination between EADs and Δ APD in the genesis of TdP is difficult because both are bradycardia dependent (8,13,17,31). Already at baseline without EADs, at a CL-IVR of 1650 ms, there is a difference between LV and RV APD of approximately 40 ms. At longer CL this Δ APD increases (8,15). This frequency dependence of interventricular dispersion is also observed in vitro: left ventricular cells demonstrate a longer APD than the right ventricle at slow heart rates. This not only the case for endocardial (and epicardial) cells but also and even more pronounced for the recently described M cells (32). At 2000 ms this leads to a Δ APD of about 100 ms (figure 4 of reference 32) without the presence of action potential prolonging drugs. The presence of EADs can further increase Δ APD. The M cell behaves like a Purkinje fiber in the aspects of rate dependent APD behavior and their sensitivity to develop EADs (14). However Purkinje fibers do not contribute much to the MAP signal due to their small mass (30-33). Because M cells are found throughout the left, right and in the ventricle septum (34), their mass could be sufficient to be registered by the MAPs. In the intact canine heart, EADs arising from the M cells have been also described (35). The combination of EADs and increase in CL contribute both to the observed increase in Δ APD after class III agents.

In the literature several arguments are in favor of a contribution of the EAD to the Δ APD. 1) The longest APD is often the one with the most pronounced EAD (18,19). Site specific EADs could therefore contribute to inter- but also to intraventricular Δ APD. 2) Magnesium suppresses the EADs and decreases Δ APD despite an increase in CL-IVR (8).

In this study the disappearance of EADs after spontaneous termination is associated with a decrease in Δ APD while (re)appearance of EADs corresponds to an increase of Δ APD (figures 2,3,4). An estimation of the contribution of EADs to Δ APD is only possible without changes in cycle length. We calculated the contribution of the EAD to the Δ APD to be between 25 to 50%.

There are some limitations to this estimate: 1) random placement of the MAPs might fail to detect all EADs, 2) the LV and RV do not seem to behave synchronously (figure

2-4). This dynamicity alters Δ APD from beat to beat, and 3) the role of the RV EAD in Δ APD is not clear. Presence of EADs in both ventricles is not associated with a smaller Δ APD (see e.g. figure 2-3). A possible explanation for this apparent contradiction could be the difference in baseline values. It is likely that EADs will develop earlier in longer APDs. The RV EAD development will therefore lag behind the LV EAD. If the LV EAD still grows when the RV EAD occurs the value of the Δ APD will be maintained, only when the LV EAD has reached its maximum value the contribution of the RV EAD to APD will alter Δ APD.

A fourth point are the small variations in CL-IVR and QRS complexes after termination, which might have influenced the behavior of EADs and Δ APD by different activation times and rate. However the contribution of EADs to Δ APD was confirmed in the steady state pacing experiments with *d*-sotalol, where both activation time and rate are constant. Another important fact is that the experiments are performed in dogs with chronic AV block. Chronic AV block leads to biventricular hypertrophy due to volume overload (15) and may play a role in the occurrence of EADs and the magnitude of Δ APD. It has been described that ventricular hypertrophy facilitates the induction of EADs (9). The effect of hypertrophy on EADs and Δ APD requires further investigation.

In conclusion the fast rate of the TdP reduces EADs and interventricular Δ APD, concomitant with termination of the TdP. Besides their role in the initiation this indicates an important role for as well EADs as Δ APD in the mechanism of continuation of TdP. EADs provide an essential contribution (25-50%) to the changes in inter-ventricular Δ APD, as evidenced by the behavior of EADs and Δ APD after spontaneous termination and after administration of class III agents at paced cycle lengths.

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Chapter 6

The effect of flunarizine and ryanodine on acquired Torsade de Pointes arrhythmias in the intact canine heart.

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ABSTRACT

Ryanodine, a specific blocker of the Ca^{2+} release channel of the sarcoplasmic reticulum, and flunarizine, a $[\text{Ca}^{2+}]_i$ overload blocker, possess antiarrhythmic effects against delayed afterdepolarizations (DADs) and DAD dependent arrhythmias. In vitro controversy exists about their effect against early afterdepolarizations (EADs): no effect was reported against cesium induced EADs, while ryanodine did prevent EADs induced by isoproterenol.

Objectives: To study the possible role of intracellular Ca^{2+} overload in acquired EAD dependent Torsade de Pointes arrhythmias (TdP), we tested the effects of flunarizine and ryanodine in our animal model of TdP. **Methods:** Anaesthetized dogs with chronic AV block received *d*-sotalol or almokalant followed by pacing. A subset of dogs with reproducible TdP (≥ 3 times) were selected to receive flunarizine (2 mg/kg/ 2 min) or ryanodine (10 $\mu\text{g/kg/10 min}$). **Results:** After *d*-sotalol, TdP was induced at a mean cycle length of the idioventricular rhythm (CL-IVR) of 2070 ± 635 ms and a QT(U) time of 535 ± 65 ms. Induction of TdP was prevented by flunarizine in all experiments (8/8), electrophysiologically this was associated with a decrease in CL-IVR, QT(U) and QT_c time (390 ± 100 to 320 ± 45 , $P < 0.05$). Ryanodine prevented TdP induction in 4/5 experiments, and decreased the CL-IVR, QT(U) and the QT_c time from 385 ± 75 to 320 ± 20 ms ($P < 0.05$). Both drugs also suppressed the almokalant induced EADs and related ectopic activity. This antiarrhythmic action corresponded with the inability to reinduce TdP by pacing. **Conclusions:** Blockade of the Ca^{2+} release channel of the sarcoplasmic reticulum by ryanodine or the reduction of $[\text{Ca}^{2+}]_i$ overload by flunarizine prevents induction of EAD dependent acquired TdP arrhythmias, suggesting a role for $[\text{Ca}^{2+}]_i$ overload in acquired Torsade de Pointes.

INTRODUCTION

Over the years, Torsade de Pointes arrhythmias (TdP) have attracted the interest of many investigators. However, several questions are still unresolved or a matter of controversy, including 1) the mechanism(s) responsible for the initiation and perpetuation of TdP, for which dispersion of repolarization and/or early afterdepolarizations (EADs) have been suggested (1). 2) The transmembrane currents by which TdP can be generated. It has been shown that TdP (or EADs) can be evoked under different circumstances and by several drugs (2-11), all prolonging action potential duration. 3) The involvement of intracellular Ca^{2+} transients (9,12,13). It was first suggested by Capogrossi and Lakatta (12), that spontaneous Ca^{2+} oscillations are the common basis of EADs and delayed afterdepolarizations (DADs). Experimentally this has been confirmed in situations of $[\text{Ca}^{2+}]_i$ overload (9,13,14). Pharmaceutical interventions directed

to reduce the $[Ca^{2+}]_i$ -overload will suppress DADs and perhaps EADs and their arrhythmias.

Ryanodine, a blocker of the Ca^{2+} release channel of the sarcoplasmic reticulum (SR) is known to suppress DAD dependent tachyarrhythmias both in vivo (15,16,17) and in vitro (18). Its effects on EADs is somewhat controversial: in vitro no effect was seen in cesium induced EADs (18) whereas it suppressed isoproterenol induced EADs (9). Similar results have been documented for the $[Ca^{2+}]_i$ overload blocker flunarizine. Flunarizine prevented the development of DADs caused by ouabain and/or catecholamines in vitro (19) and stopped and prevented ouabain induced tachycardia in the intact dog heart (17). Flunarizine has been reported not to influence cesium induced EADs in Purkinje fibers (19).

No studies are available on the antiarrhythmic effect of ryanodine and flunarizine on EADs and EAD dependent arrhythmias in the intact heart. Therefore we tested these drugs in our animal model of acquired EAD dependent Torsade de Pointes arrhythmias (20). Secondly, because the exact mechanism of action of flunarizine is still unknown, this study also allowed a comparison between the mode of action of flunarizine and ryanodine.

METHODS

All experiments were performed according to the principles of the American Physiological Society and approved by the Committee for Experiments on Animals of the University of Limburg, Maastricht, the Netherlands.

The experiments ($n=17$) were performed on 11 anaesthetized adult mongrel dogs with chronic AV block (9 ± 6 weeks) of either sex with a body weight between 18 and 32 kg. In between the AV block operation and the experiment, dogs were daily checked whether their idioventricular rate was sufficient to obtain adequate cardiac output.

Each dog had two epicardial electrodes (Bakken Research Center, Medtronic, Maastricht, The Netherlands) implanted at the apex of the left ventricle and at the base of the right ventricle, when a right sided thoracotomy was performed to produce a chronic AV block (21).

Pacing was done with a programmable stimulator capable of pacing synchronously to the QRS complexes. Unipolar stimuli were given using a pulse width of 2 ms and a stimulus strength of twice diastolic threshold. As indifferent electrode a needle was placed through the skin. Six surface electrocardiographic leads were registered and stored on optical disc. All drugs were administered through a canula in the cephalic vein.

Anaesthesia was induced by 1) premedication i.m. (1 ml/5 kg: 10 mg oxycodon, 1 mg acepromazine and 0.5 mg atropine) and 2) sodium pentobarbital (20 mg/kg i.v.) The dogs were artificially ventilated through a cuffed endotracheal tube using a mixture of oxygen, nitrous oxide and halothane (vapor concentration 0.5%) by a respirator.

Ventilation was controlled by continuous reading of the carbon dioxide concentration in the expired air. A thermal mattress was used to maintain adequate body temperature. Proper care was taken before and after the experiments, including antibiotics (1000 mg ampicillin) and analgesics (0.1 mg i.m. buprenorfine). A temporary ventricular pacemaker (maximum of 24 hours) was given after the AV block operation and after the experiments.

Induction of Torsade de Pointes arrhythmias

A detailed description of the Torsade de Pointes protocol is given elsewhere (20). In short, anesthetized animals with chronic AV block received two defibrillation patches that were attached to both sides of the chest and connected with a defibrillator. Half an hour after onset of anaesthesia, pacing was done from the epicardial electrode and consisted of three different pacing modes: 1) short long short sequences and 2) 8 basic stimuli followed by an extrastimulus and 3) 3 basic stimuli. The interstimulus interval was 400, 600 or 1200 ms, the extrastimulus interval shortened from 500 ms in steps of 40 ms until the ventricular effective refractory period was reached. After completing the pacing protocol, *d*-sotalol (2 mg/kg/5 min) or almokalant (0.12 mg/kg/10 min) was administered. Pacing was resumed 10 minutes after the start of the injection. A Torsade de Pointes arrhythmia was defined as a polymorphic ventricular tachycardia consisting of ≥ 5 beats, which twisted around its baseline in the presence of a prolonged QT(U) duration. When TdP did not stop spontaneously within 10 seconds or deteriorated into ventricular fibrillation, cardioversion (60-70 J) was performed.

Inducibility was defined as the initiation of TdP 3 or more times using the same pacing mode. Earlier experiments have proven that the electrophysiological and pro-arrhythmic effects of a single intravenous bolus of *d*-sotalol and almokalant last for at least 60 minutes (20,22). In 3 experiments (flunarizine $n=2$, ryanodine $n=1$) a second bolus of *d*-sotalol or *dl*-sotalol was needed to provide reproducible inducible TdP. In subsequent experiments, in two of these three dogs induction was repeated consistently after the second bolus to also test this reproducibility in time.

Flunarizine and ryanodine administration

Reproducible inducible TdP experiments were selected to test the preventive effect of flunarizine (2 mg/kg/2 min) and ryanodine (10 μ g/kg/10 min). Both drugs were administered within 45 minutes after the last bolus of *d*-sotalol, and within 20 minutes after almokalant. Five minutes (flunarizine) and 10 minutes (ryanodine) after the start of the injection the pacing train(s) which initiated the TdP was (were) repeated.

Both flunarizine and ryanodine are known to decrease blood pressure (16,23,24), due to their vasodilatory effects and/or negative inotropic effects. This blood pressure drop may lead to a reflex tachycardia. Because an increased ventricular rate may protect against

TdP induction (20), we performed for both drugs experiments in which the basic heart rate was kept constant by pacing. In case of ryanodine, TdP induction was attempted with the use of a basic ventricular paced rhythm of 2000 ms during two entire experiments (*d*-sotalol and almokalant), because the CL-IVR of the dogs was very slow (± 2200 ms). With flunarizine, TdP induction was attempted for *d*-sotalol both at IVR (1320 ms) and at the “expected” CL after flunarizine (1100 ms), and at a basic ventricular rhythm of 2000 ms for almokalant. In one experiment TdP was induced by administration of *dl*-sotalol instead of *d*-sotalol, so that the acceleration of ventricular rate by flunarizine would be prevented by β blockade.

Electrolytes

In 8 of these animals electrolyte levels were measured after complete anesthesia was installed. To exclude changes in electrolytes by drugs, or due to cardioversion, we measured electrolytes in 2/8 dogs at two other time points: namely after induction of TdP (with and without the necessity to cardiovert) and after administration of flunarizine or ryanodine.

Electrophysiology

Electrophysiological measurements of cycle length of the idioventricular rhythm (CL-IVR) and QT(U) time were performed before and after the administration of *d*-sotalol, almokalant and the two drugs. The QT_c time was calculated using the formula of Bazett (25).

Early afterdepolarizations

To review the direct effects of ryanodine and flunarizine on the presence of EADs, a monophasic action potential catheter (MAP) was placed in the right ventricle to visualize action potential duration (APD) and EADs in 5 experiments after *d*-sotalol. The MAP (Franz combination catheter, EPT # 1650) was placed under fluoroscopy through the jugular vein. The MAP was amplified by a DC-coupled differential amplifier, which is provided with a 20 mV calibration pulse. The MAP signals were sampled at a rate of 1 KHz. The recording sites were chosen based on stability, quality of the signal and an amplitude which had to exceed 15 mV (20).

In the first experiments (n=3), we had difficulties to maintain a stable MAP throughout the duration of the experiment (± 120 minutes). Although this problem is now resolved, it is often difficult to visualize clearly distinguishable EADs after *d*-sotalol in the right ventricle (see also ref #20). Therefore we used a second class III drug: almokalant of which the prevalence of endocardial EADs is higher than after *d*-sotalol (22).

Statistical significant differences ($P \leq 0.05$), were calculated with the use of a paired student *t*-test. All values are given as mean $\pm$ SD.

RESULTS

Induction TdP

In our animal model of TdP, the combination of bradycardia, *d*-sotalol and pacing results in reproducible TdP in about 50% of the experiments (20). A second bolus of *d*-sotalol increases this figure to almost 90%. Administration of almokalant results in $\pm$ 80% of the animals in reproducible TdP (22).

Fifteen inducible experiments ($n=13$ with *d*-sotalol) in 11 dogs were selected to test the preventive antiarrhythmic activity of ryanodine ($n=6$) and flunarizine ($n=9$). Animals tested twice had 1) different pacing modes tested in the subsequent experiments ($n=2$), or 2) dl-sotalol instead of *d*-sotalol to provide β blockade ($n=1$). Identical responses to *d*-sotalol were observed in these dogs indicating a high reproducibility of this animal model.

d-Sotalol prolonged the CL-IVR from 1830 ± 510 to 2070 ± 635 ms ($P < 0.01$) and the QT(U) time from 445 ± 60 to 535 ± 65 ms ($P < 0.01$). *d*-Sotalol administration was often accompanied by a change in the TU wave configuration. In one experiment next to pacing induced TdP, some episodes of spontaneous TdP were seen. In figure 1, a representative example of pacing induced TdP is presented.

Administration of almokalant ($n=2$) resulted in the presence of stable EADs (figure 2, panel 2). Moreover, spontaneous and pacing induced TdP were seen. TdP induction by almokalant is alike *d*-sotalol characterized by an increased CL-IVR, QT time and change in QT morphology (22).

Reproduction of TdP in time after second bolus of d-sotalol

Application of cardioversion in order to terminate the TdP did not influence the reproducible induction of the TdP. Also after a second bolus of *d*-sotalol, TdP induction remained reproducible until 60 minutes after administration.

Flunarizine

Administration of flunarizine did not alter CO₂ values of the capnograph indicative that no major hemodynamic changes occurred by flunarizine. In table 1, the time dependent effects of *d*-sotalol and flunarizine ($n=7$) are summarized. Following the bolus of

Figure 1. Induction of a Torsade de Pointes arrhythmia by *d*-sotalol and pacing. A six lead ECG at a paper speed of 25 mm/s is shown. In the upper panel two QRS morphologies can be seen, one before and one complex after pacing. In control conditions (panel 1) pacing (400/800 and an extra of 340) ms does not result in ectopic activity (stimulated beats are indicated by an S). In this animal the stimuli were given via a bipolar endocardially located catheter in the right ventricle and not by the epicardial electrode as done in all other experiments. *d*-Sotalol (panel 2) increases the CL-IVR and the QT time. The same pacing train results in a self terminating TdP.

d-sotalol, its electrophysiologic effects diminished over time. Therefore we added a second 'control' measurement (C2), just before the start of flunarizine (29 ± 12 min after *d*-sotalol). There were no differences in the electrophysiological values at 10 minutes after *d*-sotalol compared to C2. Flunarizine decreased the CL-IVR (18%) and

Table 1. Effects of *d*-sotalol and flunarizine on electrophysiological parameters (n=7)

	C1	dS	C2	F2	F10
CL-IVR	1715 ± 595	2030 ± 780*	1940 ± 580	1760 ± 485	1595 ± 380**
QT	435 ± 60	540 ± 50*	525 ± 75	440 ± 65	395 ± 55**
QT _c	345 ± 70	395 ± 55*	390 ± 100	340 ± 55	320 ± 45**

* P<0.05 vs C1, ** P<0.05 vs C2; C1: control values before administration of *d*-sotalol, C2: value before the administration of flunarizine (29±12 minutes after *d*-sotalol), dS: values at 10 minutes after the start of *d*-sotalol administration, F2: values at the end of the bolus flunarizine, F10: 10 minutes after flunarizine administration.

QT(U) time (25%). The latter was more pronounced than could be expected from the decrease in CL-IVR, as expressed by the decrease in QT_c from 390 ± 100 to 320 ± 45 (P<0.05). Width of the QRS complex did not change after flunarizine.

Administration of flunarizine after almokalant had similar effects: shortening of the QT(U) time, normalization of the TU wave and furthermore suppression of the EADs (figure 2). All parameters at 5 minutes were again comparable with the control situation before almokalant (figure 2). Flunarizine prevented pacing induced episodes of Torsade de Pointes in all experiments (9/9, *d*-sotalol and almokalant), as can be seen in figure 3. Flunarizine also suppressed runs of ectopic beats in the two animals with spontaneous TdP (n=1 *d*-sotalol, n=1 almokalant, data not shown).

The preventive effect of flunarizine was independent of the decrease in CL-IVR. This could be seen in the experiments where a paced basic rhythm was used, and further when we analyzed the individual data: 2 experiments did not show rate acceleration after flunarizine. In one of these two experiments this was due to β blockade by *dl*-sotalol. The antiarrhythmic effect of flunarizine lasted at least 30 minutes. Thereafter TdP could be reinduced in 2 experiments. Besides these electrophysiological effects, flunarizine had also distinct effects on the configuration of the TU wave in all experiments; after flunarizine administration the U wave disappeared or the T wave became more peaked suggesting a more homogeneous repolarization of the ventricles. This was also present in a dog that showed repolarization disturbances already during and directly after pacing under control conditions (figures 4-5).

Ryanodine

Ryanodine had no effect on the CO₂ values of the capnograph. Ryanodine was administered at 36 ± 19 minutes after *d*-sotalol (C2). Again there was no difference in electrophysiologic parameters between C2 and 10 minutes after *d*-sotalol. Ryanodine decreased the QT(U) time and did not significantly affect the CL-IVR at the end of the

Figure 2. Development of EADs after almokalant and suppression of EADs by flunarizine.

Lead II of the normal surface ECG and a monophasic action potential recording (MAP) at a paper speed of 25 mm/s are shown. In panel 1, the control situation is depicted: a ventricular paced rhythm of 2010 ms and a QT time of 455 ms can be seen. Just before the administration of flunarizine (2 mg/kg/2'), almokalant (0.12 mg/kg) has increased the QT time and APD, has changed the QT morphology and has led to EADs (arrows, panel 2). At the end of the bolus flunarizine (panel 3), the QT and the APD has been shortened to 505 ms and 540 ms but an EAD is still present. At 5 minutes after flunarizine the EAD has completely disappeared and the QT and the APD have been further reduced (panel 4).

Figure 3. Induction of TdP after almolokant and prevention by flunarizine.

Same experiment and abbreviations as figure 2. A three lead ECG is depicted with a MAP recording. There is still a constant paced ventricular rhythm of 2010 ms. Almolokant has led to a prolonged QT time of 600 ms and appearance of EADs (indicated by arrow). Pacing (a short cycle of 600, followed by a pause of 1200 and an extrastimulus 500 ms) results in the induction of a TdP (panel 1). Note that the last extrastimulus does not capture and that an ectopic beat initiates the TdP. Administration of flunarizine shortens the QT, normalizes TU morphology and causes disappearance of the EADs. Repetition of the same pacing train now does not lead to induction of TdP.

infusion (10 minutes). At 15 minutes both QT(U) time (23%) and CL-IVR (20%) were however significantly reduced (table 2).

Ryanodine prevented pacing dependent TdP successfully in 4/5 dogs after *d*-sotalol. In the one experiment with almolokant ryanodine prevented spontaneous TdP by a decrease in APD and a suppression of EADs, starting from the 8th minute after start of the infusion.

The effect of ryanodine cannot only be attributed to the acceleration in rhythm, as seen from the QT_c and in the experiments with a constant paced rhythm in which it was also effective. The effect of both ryanodine and flunarizine seems therefore to be dependent on the (more pronounced) decrease in QT_c time. No reoccurrences of the TdP were observed after the preventive effect of ryanodine.

In one dog ryanodine administration after *d*-sotalol did not prevent TdP. In that experiment even a spontaneous TdP occurred during administration of ryanodine

Figure 4. Occurrence of pacing induced negative T waves under baseline conditions. One ECG lead at a paper speed of 25 mm/s is shown. Pacing under control conditions reveals repolarization disturbances as seen by: 1) the development of a deep TU wave during and after pacing; and 2) the occurrence of two spontaneous ectopic beats in the pacing train. The altered TU wave morphology postpacing is disappearing in time (continuous tracing).

(figure 6). During *d*-sotalol the use of a pacing train was always necessary to initiate the TdP. The spontaneous TdP at 7 minutes after the start of the bolus points to a possible agonistic effect of ryanodine at low concentrations. Repetition of the pacing protocol after 10, 30 and 40 minutes however still resulted in the induction of TdP. With the exception of a longer CI-IVR, there were no electrophysiological differences found in this animal in comparison with the other animals.

Figure 5. Homogenization of repolarization under baseline conditions by flunarizine. Administration of flunarizine (panel 1) in the experiment of figure 4 prevents the development of the deep TU wave during and after pacing. Furthermore no spontaneous activity arises during the pacing train. At 65 minutes after flunarizine (panel 2) the effect of flunarizine is diminished. The pacing train again leads to the development of negative TU waves post pacing, although they are present for a shorter time post pacing as at control (see figure 4). No ectopic activity is seen.

Electrolytes

All tested animals had normal electrolyte levels, mean potassium and magnesium level were 4.2 ± 0.54 mmol/L and 0.81 ± 0.05 mmol/L respectively. Administration of *d*-sotalol/ almokalant, flunarizine/ryanodine or cardioversion did not change the level of any of the electrolytes.

Table 2. Effects of *d*-sotalol and ryanodine on electrophysiological parameters (n=5)

	C1	dS	C2	R10	R15
CL-IVR	1965 ± 400	2175 ± 485 [*]	2015 ± 345	1930 ± 430	1620 ± 220 ^{**}
QT	455 ± 65	530 ± 80 [*]	540 ± 90	490 ± 55	415 ± 20 ^{**}
QT _c	320 ± 30	365 ± 50 [*]	385 ± 75	360 ± 60	320 ± 20 ^{**}

^{*} P<0.05 vs C1, ^{**} P<0.05 vs C2; C1: control values before administration of *d*-sotalol, C2: value before the administration of ryanodine (36 ± 19 min after *d*-sotalol), dS: values at 10 minutes after the start of *d*-sotalol administration, R10: values at the end of the bolus ryanodine, R15: 15 minutes after ryanodine.

Figure 6. Development of a spontaneous TdP after ryanodine. Three ECG leads at a paper speed of 10mm/s are shown. Just before administration of ryanodine a slow rhythm (CL-IVR: 2345 ms) with a prolonged QT interval of 635 ms and very deep T waves during *d*-sotalol (panel 1). Ryanodine (10µg/kg) is administered in 10 minutes. At 7 minutes after the start of ryanodine infusion (panel 2) the CL-IVR increases to 2510 ms, the T wave becomes less negative although the QT time is not altered. Interruption of the IVR by a spontaneous beat initiates a TdP. This episode of TdP requires cardioversion because it did not stop spontaneously within 10 seconds. After this episode of TdP spontaneous ectopic activity remains present in the form of single ectopic beats or short runs (panels 3 and 4). Note that the T wave changes after ryanodine and is completely positive at 9 minutes.

DISCUSSION

Induction of EAD dependent acquired Torsade de Pointes arrhythmias was prevented by administration of ryanodine and flunarizine. The prevention of TdP was associated with a normalization of repolarization as evidenced by a decrease in QT(U)_(c) time following both drugs. Because both drugs decrease intracellular Ca²⁺, their inhibition of EADs and preventive action against TdP points to the involvement of [Ca²⁺]_i overload and/or intracellular Ca²⁺ dyshandling in the initiating mechanism of TdP.

Animal model

Our animal model of acquired pacing dependent Torsade de Pointes arrhythmias has a high and reproducible incidence of TdP (20). In half of the experiments *d*-sotalol administration gives rise to spontaneous ectopic beats, whereas “spontaneous” TdP occurs in approximately 10% of the experiments. Abrupt frequency changes induced by pacing, result in about 50% of the experiments in reproducible TdP. The incidence can be increased to 90% with a second dose. Dogs which show spontaneous TdP always had inducible TdP by pacing (20). The ability to induce TdP reproducibly is present for about 60 minutes (20,22), which allows testing of (pharmaceutical) interventions aimed to prevent the arrhythmia. Further proof for this observation comes from the reinduction of TdP in two experiments after flunarizine.

The TdP periods are related to bradycardia, a prolonged QT(U) time and the presence of EADs (figures 4-5, 20). These EADs are accentuated by pacing and their ectopic beats often interrupt the pacing train.

Our experience with *d*-sotalol to record EADs using the MAP at the time of these experiments prohibited visualization of the effects of flunarizine and ryanodine on EADs. Because we know that almokalant, also a I_{Kr} blocker, leads to more pro arrhythmogenic responses and shows (at random placement of the MAP) more (pronounced) EADs than *d*-sotalol (22), we added this drug. Unfortunately we could not establish in these experiments a direct link between the EAD and the start of the TdP (figure 3) as we have done before. The ability to record triggering EADs depends on the good fortune to place the MAP catheter (already during control) close to the site of origin of the (later developing) EADs (as e.g. in the observations during the development of this model, figure 4 of ref # 20). Replacing the catheter is time consuming and was not judged to fall within the scope of this investigation. New experiments are currently performed in which we study the site specificity of EADs in the right and also in the left ventricle.

None of the interventions performed during the study led to a change in electrolyte levels. Also there were no major hemodynamic changes during the experiment place as evidenced by the stable CO₂ values registered by the capnograph. Although we are aware

that this is a crude measurement. Therefore we do not consider it likely that electrolyte or hemodynamic changes contribute to the prevention of TdP.

Ryanodine

Ryanodine inhibits the Ca^{2+} release channel of the sarcoplasmic reticulum (26,27). A dose of 10 $\mu\text{g/kg}$ was effective to suppress ouabain induced tachycardias without reaching toxic levels (15,16). Toxicity of ryanodine is dependent on the dose and the infusion rate, that has to be slow enough to prevent death of the animals (15). During a slow rate of infusion there will be a relatively long time with a low plasma concentration, that may exert an agonistic effect on the Ca^{2+} release (28) instead of an antagonistic effect. A low dose of ryanodine have been reported to enhance cesium induced ectopic activity (29). Therefore possible proarrhythmic responses can be anticipated during the infusion time.

Flunarizine

Flunarizine has proven to be effective both in vitro (19,30,31) and in vivo (17) against DAD dependent arrhythmias caused by ouabain intoxication. Flunarizine is a very specific drug to suppress triggered arrhythmias without affecting arrhythmias based on abnormal automaticity or reentry (19,23,32).

The mechanism of flunarizine is not clear. A broad scala of mechanisms have been proposed, which all involve regulation of intracellular Ca^{2+} . A possible mechanism could be blockade of sodium entry (30), although flunarizine does not interfere with the TTX sensitive Na^+ channels. The effect on sarcolemmal Ca^{2+} channels is low (33), and only detected at high concentrations. Under physiologic conditions, ECG parameters representing different electrophysiological parameters did not show any changes after flunarizine (23). Another possible mechanism of action is a direct effect on the SR, e.g. the ryanodine sensitive Ca^{2+} release channel or SR Ca^{2+} ATPase.

Mechanisms of EADs and $[\text{Ca}^{2+}]_i$ overload

EADs can be caused by several interventions that prolong the action potential duration. These include clinical drugs such as class Ia and class III drugs (2-5,11,20) and experimental drugs such as cesium (6,7,19,34), anthopleurine (8), and Bay K 8644 (10). More recently isoprenaline has been found to induce EADs (9). The exact mechanism of the EAD is unclear and can be different for the different substances.

EADs can be differentiated in early (phase 2) EADs and late (phase 3) EADs (6). Early EADs are probably based on the Ca^{2+} entry through the L-type Ca^{2+} channel (10,18), because they are suppressed by the Ca^{2+} antagonist nitrendipine and potentiated by the Ca^{2+} agonist Bay K 8644 (18). On the other hand late EADs, seem to depend on an

increased intracellular Ca^{2+} overload (9,13,14). That can be suppressed by ryanodine, benzanil and low extra cellular Na^+ (9,34).

$[\text{Ca}^{2+}]_i$ overload is an established cause of triggered arrhythmias based on DADs. It has been suggested that spontaneous Ca^{2+} oscillations are the common basis of both DADs and specific (phase 3) EADs (9,12-14). The Ca^{2+} -overload related afterdepolarizations could be induced by an inward Na^+ current e.g. via the $\text{Na}^+/\text{Ca}^{2+}$ exchange (9,34), and/or non inactivated Na^+ channels (3,7,35). Recent computer simulation confirmed the possible role of both Na^+ current and $\text{Na}^+/\text{Ca}^{2+}$ exchange (36) in the formation of EADs. Therefore we used two drugs known to suppress DADs, to evaluate an effect of diminished Ca^{2+} overload in TdP.

Prevention of TdP by flunarizine and ryanodine

The normalization of the repolarization, and the preventive effect of flunarizine and ryanodine was independent of the increase in heart rate. The latter can be associated with a reflex mechanism due to the negative hemodynamic or inotropic effect of these drugs (16,23,24). When we corrected the increase by the use of a paced rhythm ($n=4$), or by β blockade using *dl*-sotalol ($n=1$), we saw similar preventive effects. Therefore it seems that these drugs exert their antiarrhythmic effect by a direct effect on EADs and APD. This is in contrast with the observation of others who describe that ryanodine (18) and flunarizine (19) had no effect against cesium induced EADs in vitro. From these findings Marban et al. draw the conclusion that involvement of $[\text{Ca}^{2+}]_i$ overload was less likely, and he emphasized the involvement of the Ca^{2+} L type channel in the genesis of EADs (18). However recent data with isoprenaline induced EADs and DADs showed that they are caused by complex Ca^{2+} transients which can be abolished by the use of ryanodine (13). Therefore, it seems logic that the different ways to initiate EADs also require different treatments. The EADs observed in our animal model (figures 2-3, 20) occur most frequently during phase 3 of the action potential similar to isoproterenol induced EADs. These EADs were diminished and/or suppressed by flunarizine and ryanodine, explaining the preventive action of flunarizine and ryanodine on pacing induced TdP.

The occurrence of a spontaneous TdP during the bolus of ryanodine may point to the enhancement of the SR Ca^{2+} channel by a low concentration of ryanodine (29). This is an extra argument for a role of $[\text{Ca}^{2+}]_i$ overload and or Ca^{2+} dyshandling in the mechanism of TdP.

Comparison flunarizine and ryanodine

A way to assess the mechanism of a drug is to compare it with drugs of known action. Prevention of Torsade de Pointes arrhythmias, either induced by *d*-sotalol or almokalant, was achieved by both drugs. Electrophysiologically the effects were similar,

although flunarizine decreased the QT_c time more than ryanodine. The fact that flunarizine was effective in all dogs within 2 minutes, whereas ryanodine took longer to be effective and was ineffective in one experiment might point to an important difference. This difference can be related to the possible agonistic effect of ryanodine at low concentrations (28) as seen in these experiments and by others (29). Another confirmative observation was made recently in dogs, ryanodine caused an acceleration of ouabain ventricular tachycardia before termination occurred (17). Although the data of the present study gave a similar result of ryanodine and flunarizine in suppressing EADs and prevention of TdP, this does not imply that flunarizine blocks the ryanodine sensitive Ca^{2+} release of the SR.

Limitations of the study

In this study we report that $[Ca^{2+}]_i$ overload could play an important role in the mechanism of acquired EAD dependent TdP. We come to this conclusion based on the indirect effects of ryanodine and flunarizine. And although these drugs are very specific to prevent $[Ca^{2+}]_i$ overload and restore a normal Ca^{2+} condition in the cell (19,26,27), we did not measure Ca^{2+} transients to prove $[Ca^{2+}]_i$ overload in the generation of TdP.

General conclusions

The results of these animal experiments point to $[Ca^{2+}]_i$ overload as playing a role in the development of acquired TdP. This is suggested by the preventive effect of ryanodine and flunarizine as well as by the occurrence of a spontaneous TdP after ryanodine.

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Chapter 7

Electrophysiologic adaptations induced by chronic complete atrioventricular block predispose the canine heart for the induction of acquired Torsade de Pointes arrhythmias

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ABSTRACT

We have reported that the combination of 2 mg/kg *d*-sotalol (dS) and pacing results in the reproducible induction of early afterdepolarization (EAD) dependent Torsade de Pointes arrhythmias in 50% of anesthetized dogs with chronic complete AV block. The AV block induced bradycardia accompanied by a volume overload causes biventricular hypertrophy: left ventricle/body weight (LV/BW) increases from 4.3 ± 0.9 to 5.8 ± 0.9 g/kg and the RV/BW from 1.4 ± 0.4 to 2.6 ± 0.9 g/kg ($P < 0.05$). Ventricular hypertrophy is associated with prolongation of the action potential duration (APD) and sudden death. What the contribution of the changes due to AV block are for the occurrence of acquired TdP is not known. In this model the effect of pacing and dS was therefore tested twice: at the moment of AV block ($n=9$) and after 5 weeks of complete AV block ($n=10$). Five dogs were tested at both time points. Cycle length (CL) was kept constant in both experiments. Monophasic actionpotential catheters were placed in the right (RV) and the left ventricle (LV) to record EADs, to measure actionpotential duration (APD), dispersion of repolarization (Δ APD: LV APD-RV APD), and the incidence of TdP.

Results: At baseline: CL (1600 ± 280 ms), both the LV APD (295 ± 20 vs 390 ± 60 ms) and RV APD (260 ± 20 vs 315 ± 10 ms) significantly increased after 5 weeks of AV block ($P < 0.05$). EADs were not seen and TdP was not inducible at baseline. dS administration increased APD significantly in both groups, but to a different degree. There was a more pronounced increase in Δ APD (40 ± 35 to 45 ± 30 vs 70 ± 30 to 125 ± 65 ms) and more EADs at 5 weeks. No TdP could be induced after dS at 0 weeks, whereas TdP occurred in 6/10 dogs at 5 weeks AV block.

Conclusions: The changes occurring after chronic complete AV block increase the arrhythmogenic effects of dS resulting in a higher incidence of TdP. This study also emphasizes again the role of Δ APD and EADs in the genesis of TdP.

INTRODUCTION

Left ventricular hypertrophy is a well known risk factor for sudden (arrhythmic) death (1-4). This higher incidence of ventricular arrhythmias is independent of other factors like coexisting coronary disease and/or heart failure (4,5). Cardiac hypertrophy can have different etiologies, like pressure or volume overload (6,7) and may involve the left, the right or both ventricles. Many studies have described the mechanical, structural and biochemical alterations occurring in ventricular hypertrophy (e.g. 6-10). Much less attention has been given, to the electrophysiological changes that occur in the hypertrophied myocardium (11). Independent of its cause, ventricular hypertrophy is associated with a lengthening of the transmembrane action potential duration (8). It has been suggested that due to this lengthening of repolarization, early afterdepolarizations (EADs) and triggered activity may occur in hypertrophy (2,11), and one may assume

that EAD related Torsade de Pointes arrhythmias with the possibility of deterioration into ventricular fibrillation could be a possible cause for sudden death (12). Other pathological conditions such as fibrosis or ischemia, could also lead to arrhythmogenic death based on other mechanisms like reentry or abnormal impulse formation.

In our animal model of acquired Torsade de Pointes arrhythmias (TdP), we have reported the reproducible development of pacing induced TdP in 50% of the dogs after administering a class III agent, d-sotalol (2 mg/kg, 13). These dogs were tested minimally 2 weeks after creation of complete atrioventricular (AV) block. Other investigators using a similar or higher dosage of (d)-sotalol (14,15) did not report the occurrence of spontaneous TdP in dogs, when the experiments were carried out in the acute phase of AV block.

Similar to clinical TdP (16), induction of TdP in our canine model depends on bradycardia, prolonged APD and the development of EADs (13). Furthermore, we have described that interventricular dispersion plays an essential role in the induction of TdP (17).

Our first goal was to assess the structural and electrophysiologic processes, which take place after the creation of complete AV block. Comparing dogs with normally conducted sinus rhythm (SR) with chronic AV block (see results), we noticed that the AV block dogs had 1) biventricular hypertrophy, and 2) prolonged repolarization parameters. The role of these in the development of acquired TdP in our animal model is unknown and was the subject of this study.

METHODS

The study protocol was approved by the Committee for Experiments on Animals of the University of Limburg, Maastricht, the Netherlands and conducted in accordance with the guidelines of the American Physiological Society.

General

The experiments were performed on 15 anesthetized adult mongrel dogs of either sex with a mean body weight of 25 ± 4 kg (range 20-41 kg). Anesthesia was induced by 1) premedication i.m. (1 ml/5 kg: 10 mg oxycodone, 1 mg acepromazine and 0.5 mg atropine) and 2) sodium pentobarbital (20 mg/kg i.v.). The dogs were artificially ventilated through a cuffed endotracheal tube using a mixture of oxygen, nitrous oxide and halothane (vapor concentration 0.5%-1%) by a respirator. Ventilation was controlled by continuous reading of the carbon dioxide concentration in the expired air. A thermal mattress was used to maintain adequate body temperature.

A right sided thoracotomy was performed 1) to implant an epicardial electrode (Bakken Research Center, Medtronic, Maastricht, The Netherlands) at the apex of the left

ventricle, and 2) to create complete AV block by injection of formaldehyde (37%) in the AV node (18). Proper care was taken after the experiments, including antibiotics (1000 mg ampicillin, pre and post operatively) and analgesics (0.1 cc/7 kg i.m. buprenorfine).

Pacing

All electrical stimulation was done from the left epicardial electrode with a custom build programmable stimulator (University of Limburg), that delivers unipolar stimuli synchronous to the QRS complexes, with a pulse of 2 ms and a stimulus strength of twice the diastolic threshold. As indifferent electrode a needle was placed through the skin.

Measurement of electrophysiological parameters

Retrospective analysis consisted of a comparison between electrophysiological studies obtained in dogs with normal conducted SR (n=18, 18-20) and in dogs with chronic AV block (n=9, 13). In these experiments the ventricular effective refractory period (VERP) was determined using the extrastimulus technique, after a basic train of 8 paced stimuli with an interstimulus interval ranging between 250 and 600 ms. The coupling interval of the extrastimulus was shortened with 10 ms steps till refractoriness was reached. The VERP was defined as the longest interval that did not capture. QT time was measured in lead II of the ECG from the last five ventricular paced beats after 30 seconds of continuous pacing at a cycle length of 300 and 400 ms.

Heart weight

To assess the amount of hypertrophy due to the volume overload, the heart weight was determined in twenty of the dogs used for the above described electrophysiologic studies (SR: n=11, AV block: n=9). After a final experiment the hearts were excised, rinsed with water and stored in a formaldehyde solution for at least 2 weeks. The weight of the total heart, right and left ventricle (including septum) was assessed and related to the body weight. In addition, the heart weight was assessed in five dogs used in the prospective limb of this study.

Evaluation of the electrophysiologic and proarrhythmic effects of d-sotalol

Animals were instrumented to investigate the response to *d*-sotalol during spontaneous idioventricular rhythm (IVR) at both time points: acute AV block and at 5 weeks of chronic complete AV block. Directly after closure of the chest following creation of AV block, MAP catheters were placed in both ventricles of 7 dogs. Thereafter the TdP

induction protocol was performed (see further). To avoid possible influences of the preceding thoracotomy, four dogs received a ventricular (VVI) pacemaker immediately after AV block. The pacemaker was programmed at a rate comparable to the former SR and the dogs were scheduled to be tested three days later. Each day an electrocardiogram was registered to check pacemaker function. These dogs were again anesthetized, three days after creation of AV block and the endocardial MAP catheters were placed and the VVI pacemaker was switched off. In the presence of a stable IVR (≥ 5 minutes), the TdP protocol (see further) was performed. Unfortunately one of these four dogs died before the experiment could be carried out, and in another dog the AV node conduction partially returned (2:1 AV block). These dogs were excluded, so that 9 dogs were evaluated for TdP induction in the acute phase of AV block.

In these experiments 7 dogs met the standards in respect to the quality of both MAPs to ensure appropriate data sampling ($7 \times 2 = 14$ MAPs). These dogs were planned to be retested 4-6 weeks later. Unfortunately one of them died, and in one dog we could not record adequate MAP signals during the second experiment, i.e. five dogs could be analyzed at both time points (table 3). To increase the number of dogs with chronic AV block, we added 4 consecutive dogs that were only tested at 5 weeks of AV block. So during chronic AV block 10 dogs were given *d*-sotalol, of which 9 had adequate MAPs for electrophysiological analysis ($9 \times 2 = 18$ MAPs).

Torsade de Pointes induction protocol

A detailed description of the TdP induction protocol is described elsewhere (13). In short, anesthetized animals received two defibrillation patches that were attached to both sides of the chest and connected with a defibrillator. Two different pacing modes were used 1) short long short sequence, A) an interstimulus interval of 400 ms followed by one of 800 ms and by an extrastimulus or B) four intervals of 600, followed by one of 1200 ms and an extra stimulus and 2) An 8+1 protocol with an interstimulus interval of 600 ms followed by one extra stimulus (13). During both pacing modes the coupling interval of the extrastimulus was shortened from 500 to 300 ms in 50 ms steps. After completing the pacing protocol, *d*-sotalol (2 mg/kg/5 min i.v.) was administered. The pacing protocol was resumed 10 minutes after the start of the drug injection. A Torsade de Pointes arrhythmia was defined as a polymorphic ventricular tachycardia consisting of ≥ 5 beats, which twisted around the baseline and occurring in the presence of a prolonged QT(U) duration. A dog was called inducible when TdP could be induced ≥ 3 times with the same pacing mode. When TdP did not terminate spontaneously within 10 seconds or when it deteriorated into ventricular fibrillation, cardioversion (60-70 J) was performed.

Because pacing is necessary to induce TdP, we also paid careful attention in those dogs tested twice to the development of EADs and ectopic beats within the pacing mode.

Variables of ventricular repolarization are heart rate dependent and it was previously reported that inducible dogs have a longer cycle length of the IVR than the non inducible ones (13). Therefore, in those dogs studied both during acute and chronic AV block, we performed when necessary the TdP induction protocol after *d*-sotalol during AV block at two basic cycle lengths: during spontaneous IVR and at a paced cycle length identical to the cycle length of the first experiment. Continuous pacing was started 20 minutes after *d*-sotalol.

Monophasic Action Potentials

MAPs were recorded to observe the occurrence of EADs and to measure the duration of the action potential (APD) of the left and right ventricular endocardium at 100% of repolarization. Quadripolar contact electrodes (Franz combination catheter, EPT # 1650), that provide both pacing and MAP recording capabilities were randomly placed endocardially in the left and the right ventricle via the carotid artery and the external jugular vein. MAP phases were defined according to the definitions used for transmembrane potentials (21). Amplitude was defined as the voltage difference between phase 4 and 2 of the signal. Besides a minimal MAP amplitude of 15 mV, a stable configuration and a smooth shape had to be present under control circumstances. EADs were defined as an interruption of the smooth contour of phase 2 and/or 3 of the action potential (22), and were examined in both MAPs.

Data acquisition and analysis

Six surface electrocardiographic leads and 2 MAP signals were simultaneously registered and stored on hard disc during the experiment. Both MAPs and ECG leads were sampled with at rate of 1 kHz. Applying a custom made computer program (ECG View; University of Limburg, Maastricht, the Netherlands) with a resolution of 2 ms and adjustable gain and time scale, the following parameters were measured: CL-IVR, QT time (lead II) and LV and RV APD₁₀₀ at baseline and at 10 minutes after *d*-sotalol. In the 5 dogs tested twice we also measured these parameters at 20 minutes after *d*-sotalol when the cycle length was corrected. All electrophysiological data reported are the mean of 5 consecutive beats. Interventricular dispersion (Δ APD) was defined as the difference between the left and right ventricular APD at a certain time point.

Statistics

Paired and unpaired Student's *t*-test, were applied to compare data obtained in pre drug condition and after *d*-sotalol and between 0 and 5 weeks of AV block. The χ^2 test was used when the data were presented as a proportion. P values ≤ 0.05 were considered significant. All data are presented as mean $\pm$ standard deviation (SD).

RESULTS

Effects of chronic AV block: heart weight and electrophysiologic findings

Assessment of the heart weight in dogs with chronic AV block (20 ± 11 weeks) revealed a significant increase in mass when compared to the hearts of dogs in SR with comparable body weight (table 1). This increase is present in the left and right ventricle (table 1), leading to biventricular hypertrophy.

Anesthetized dogs with chronic AV block (CL-IVR: ± 1670 ms) had significantly longer repolarization parameters than dogs in SR (SR-CL: ± 520 ms). This remained present after 30 seconds of continuous ventricular pacing at 300 ms: the QT time amounted 205 ± 15 ms in dogs with SR, vs 275 ± 20 ms in the chronic AV block dogs ($P < 0.05$). A similar pattern was found with 400 ms pacing (225 ± 15 vs 300 ± 20 ms). At these cycle lengths the VERP was also significantly increased when chronic AV block dogs were compared with dogs in sinus rhythm (figure 1).

Prospective study

Heart weight

The amount of biventricular hypertrophy in the subgroup of 5 dogs studied prospectively was: LV/BW of 5.9 ± 0.8 and a RV/BW of 2.6 ± 0.3 g/kg. The dogs had chronic

Table 1. Heart weights of dogs in sinus rhythm (SR) compared with dogs with chronic complete AV block (20 weeks, range 10-34)

	SR (n=11)	AV (n=9)
Heart weight (g)	225 ± 34	$280 \pm 64^*$
H/BW (g/kg)	7.7 ± 1.2	$11.2 \pm 1.9^*$
LV (g)	125 ± 26	145 ± 32
RV (g)	40 ± 8	$65 \pm 22^*$
LV/BW (g/kg)	4.3 ± 0.9	$5.8 \pm 0.9^*$
RV/BW (g/kg)	1.4 ± 0.4	$2.6 \pm 0.9^*$
BW (kg)	29.6 ± 5.6	25.1 ± 5.6

H/BW: Heart/bodyweight (g/kg), LV: left ventricle weight (g), RV: right ventricle weight (g), LV/BW: left ventricle/body weight (g/kg), RV/BW: right ventricle/bodyweight; $P < 0.05$ vs SR

Figure 1. Increase in ventricular effective refractory period after chronic AV block.

Graph showing the results of a retrospective study performed in anesthetized dogs in which we compare the ventricular effective refractory period (VERP) in sinus rhythm (■, n=18) with dogs in chronic AV block ($\pm$ 9 weeks, □, n=9). The VERP was determined by one single extrastimulus following 8 basic stimuli at different basic cycle lengths. The ventricular cycle length during sinus rhythm (SR) and the mean cycle length of the idioventricular rhythm (CI-IVR) are indicated on the horizontal axis. The AV blocked dogs show a significantly longer VERP than the dogs in SR at those cycle lengths that can be compared (400–300 ms).

AV block for 9 weeks and the data were comparable with the retrospective data shown in table 1.

Behavior of APD and interventricular Δ APD at IVR during baseline

After creation of complete block or after switching off the pacemaker a stable idioventricular rhythm evolved with a cycle length of $\pm$ 1600 ms. No differences were found in the electrophysiological parameters of the dogs in which the experiment was performed right after closure of the chest or with a delay of three days. Therefore we combined the results of the two groups (table 2). At baseline all dogs had smooth MAP signals; no EADs were present. At this time point TdP could not be induced by pacing (n=9).

Figure 2. Differences in baseline repolarization values in acute and chronic AV block.

Two panels with a lead II ECG and a recording of the left and right ventricular (LV and RV) monophasic action potential (MAP) are shown at a paper speed of 25 ms. Panel 1 shows the baseline situation, directly after creation of AV block. The cycle length of the idioventricular rhythm (CL-IVR) is 1840 ms with a QT time of 360 ms. The action potential duration (APD) of the LV is 330 ms whereas the RV APD is 280 ms resulting in an interventricular dispersion of repolarization (Δ APD) of 50 ms. In this dog, 6 weeks of chronic complete AV block led to a prolonged QT time (panel 2) while the CL-IVR and QRS duration are similar. The LV APD and RV APD are also prolonged. Because this prolongation is more pronounced for the LV, the Δ APD has increased to 80 ms.

Five weeks of chronic AV block led to a significant increase in all repolarization parameters (table 2) while the cycle length of IVR was similar. The number of EADs was not increased, but the interventricular Δ APD augmented from 40 ± 35 to 70 ± 30 ms due to the absolute and relative larger increase of the LV APD compared with the RV APD. A representative example of the differences in QT, LV and RV APD measured in the acute and chronic phase of complete AV block is shown in figure 2. In one dog very outspoken repolarization disorders were present in the form of EADs in the left ventricle, resulting in a larger Δ APD (130 ms). TdP could be induced by pacing.

Effect of *d*-sotalol

In the acute phase of complete AV block (after ± 30 minutes), *d*-sotalol increased the CL-IVR, the QT time and APD in both ventricles (table 2), to an extent that was less

Table 2. Electrophysiologic effects of chronic complete AV block and effect of *d*-sotalol

	baseline		<i>d</i> -sotalol			
	0 wks	5 wks	0 wks [#]		5 wks	
CL-IVR	1600 ± 280	1615 ± 280	1720 ± 465		1935 ± 425	
QT	315 ± 25	390 ± 65 [*]	385 ± 40 [#]	+22%	485 ± 85 ^{*+}	+25%
LV APD	295 ± 20	390 ± 60 [*]	340 ± 40 [#]	+15%	495 ± 95 ^{*+}	+28%
RV APD	260 ± 20	315 ± 40 [*]	295 ± 20 [#]	+15%	370 ± 55 ^{*+}	+18%
ΔAPD	40 ± 35	70 ± 30	45 ± 30	+12%	125 ± 65 ^{*+}	+80%
EADs	0/14	1/18	4/14		9/18 [*]	
TdP	0/9	1/10	0/9		6/10 [*]	

Mean ± SD and percent increase after *d*-sotalol; [#]: 5 dogs with a paced rhythm, ^{*} P<0.05 vs 0 wks, ⁺ P<0.05 baseline vs *d*-sotalol, CL-IVR: cycle length of the idioventricular rhythm, APD: action potential duration, LV: left ventricle, RV: right ventricle, ΔAPD: LV APD - RV APD.

Table 3. Incidence of early afterdepolarizations (EAD), amount of interventricular dispersion of repolarization (ΔAPD) and incidence of Torsade de Pointes arrhythmias (TdP) at 0 and 5 weeks after *d*-sotalol (dS) in the same dog

dog	LV/RV	EAD 0	ΔAPD	TdP 0	EAD 5	ΔAPD	TdP 5
1	LV RV	+ +	75	—	+ +	180	+
2	LV RV	— —	35	—	— —	70	—
3	LV RV	— —	80	—	+ +	150	+
4	LV RV	— —	25	—	+ +	80	+
5	LV RV	+ +	40	—	+ +	125	+
Total		4/10	50 ± 25	0/5	8/10	120 ± 45 [*]	4/5

^{*}: P<0.01 0 vs 5 weeks, LV: left ventricle, RV: right ventricle.

D-SOTALOL

Figure 3. Difference in arrhythmogenic response after d-sotalol.

This figure (for abbreviations see figure 2) consists of three ECG leads and two MAP recordings, one in the RV and one in the LV at a paper speed of 10 mm/s. After d-sotalol, during a paced rhythm of 1730 ms the LV APD is 380 and the RV APD 300 ms resulting in a Δ APD of 80 ms. Both MAP's are smooth: no early afterdepolarizations (EADs) are present. Performance of a pacing protocol consisting of 4 beats with a cycle length of 600 followed by beat after 1200 ms and an extra stimulus after 350 ms does not result in an arrhythmia. Six weeks later (panel 2), administration of 2 mg/kg d-sotalol results in a LV APD of 500 and a RV APD of 350 ms causing a Δ APD of 150 ms. Note that both MAPs now show EADs (arrows). Performance of the same pacing mode results in a self terminating TdP.

in comparison to the effect of the chronic adaptations present after AV block. The latter occurred uniformly so that no effect on interventricular Δ APD was seen. In five dogs, the prolongation of the CL-IVR by d-sotalol, led to hyperventilation as evidenced by a fall in CO₂ values. We choose to temporarily (20 min) pace these dogs at a CL comparable with baseline IVR, which resulted in restoration of the functional status. The value found after at 10 minutes d-sotalol is therefore an underestimation of the CL-IVR prolonging effect of d-sotalol. After 20 minutes of d-sotalol, the dogs could tolerate the slow rate and back up pacing was stopped, at spontaneous IVR the TdP induction protocol was repeated for a second time.

The effect of d-sotalol on repolarization was much more pronounced in the dogs with chronic AV block. This was the case using a group comparison (table 2) as well as when the dogs were studied twice (table 3). Especially the prolongation of the LV APD (+28% vs 15%) was larger in the hypertrophied group. EADs also developed more frequently after d-sotalol during chronic AV block (4/14 vs 9/18). All these changes led to an increased Δ APD.

At 20 minutes the CL was similar at both groups (due to the rate correction) the difference in QT, LV APD, RV APD and Δ APD remained significantly higher after chronic AV block.

Proarrhythmic response to d-sotalol

Following acute AV block, pacing after administration of d-sotalol never resulted in induction of TdP (0/9, figure 3, panel 1). Repetition of PES at spontaneous IVR after d-sotalol also did not induce TdP. In contrast, TdP was induced in 6/10 dogs with chronic AV block (table 2, figure 3, panel 2).

When using the dog as its own control, TdP induction was associated with a longer APD, an increased Δ APD and more frequent development of EADs after d-sotalol during spontaneous IVR (table 3). The non inducible dog had the smallest Δ APD and no EADs (table 3). PES was repeatedly interrupted by ectopic beats in 3/5 dogs after chronic AV block while in acute AV block this was never the case. After correcting for the slower heart rate in the inducible animals, pacing did not lead to a different response. They remained inducible.

DISCUSSION

Amount of hypertrophy

Chronic complete AV block is not a frequently used method to induce cardiac hypertrophy, although such a model was already described in the beginning of the century

(23). The increase in heart weight is due to the adaptations that take place to compensate for the volume overload that occurs when the heart rate slows (24). Hypertrophy has been described to appear in both the right as well as in the left ventricle (24,25). These authors also describe the occurrence of cardiac failure in a number of dogs (7/11) when the animals are regularly exercised (25). Later reports in a larger series of dogs, showed compensated hypertrophy on as evidenced by the findings of normal LV hemodynamics (26). It is also our experience that compensated biventricular hypertrophy occurs when the dogs are kept in their cages without regular exercise. In our series of approximately 150 dogs with complete AV block, the incidence of overt heart failure is low ($\pm 10\%$). None of the dogs used in the present study showed any signs of cardiac failure as assessed by body weight measurements, overall impression (ascites), and auscultation of the lungs.

In our model the LV mass to body weight ratio has increased from 4.3 g/kg during sinus rhythm to 5.8 g/kg during chronic AV block. Other canine studies using volume overload due to mitral valve regurgitation or arteriovenous fistula (6,7) or pressure overload by clamping the renal artery (27,28) show similar values of left ventricular hypertrophy. In contrast, aortic banding of puppies results in higher values: from 6.9 to 8.8 g/kg (7,9). In this model, the authors have suggested to differentiate in two subgroups of hypertrophy: mild or severe depending on percent increase of the LV mass and diminished subendocardial blood flow (9).

As far as the degree of RV hypertrophy is concerned, the increase in RV to body weight ratio is more pronounced than that of the left ventricle. In dogs with arteriovenous shunt (6) and pulmonary banding (10) a similar amount of RV hypertrophy (± 2.6 g/kg) has been found.

Hypertrophy and electrophysiological changes

Ventricular hypertrophy results in lengthening of the repolarization parameters, like QT time and APD. It has been suggested that this prolongation predisposes the heart for EADs and triggered arrhythmias (11).

Mild hypertrophy caused by pressure overload in dogs has been shown to prolong LV APD by 14% both in situ (28), and in vitro (29). The present study shows that chronic AV block leads to a prolongation of LV APD with 32%. This different increase in LV APD, despite a similar amount of LV hypertrophy, could be due to differences in cycle length between the two groups: 800 ms in the pressure vs 1600 ms in our dogs (28). APD is frequency dependent and when our dogs are paced at comparable rates the prolongation is less pronounced (30).

Surprisingly, the increase in RV APD is less severe (+21%) than that of the LV APD, despite a similar absolute increase in heart weight and an even higher percentual increase of the right ventricular mass. This could indicate that the changes in the electrophysiological parameters are not only related to hypertrophy and/or bradycardia.

A comparison with other models is not possible because to our knowledge no report has been published describing the electrophysiologic effects in dogs with RV hypertrophy. An explanation for this difference could be the different hemodynamics in the right and left ventricle. Functionally the left ventricle is completely adapted to the volume overload as measured by pressure volume loops (SHM de Groot, personal communication) and also other studies indicate compensation (24,26). The functional status of the right ventricle has not been determined yet, but it is conceivable that a similar volume overload leads to different wall strains in the right compared with the left ventricle. Stretch has the capability to shorten APD (31), and this opposing effect may influence the amount of prolongation.

Also for the comparison of the APD at the two time points, this stretch induced shortening may be a confounding parameter. The measured APD at control could be shorter as expected on the basis of the bradycardia alone.

A second but less likely reason for the difference between the LV and RV APD response, could be a different rate response in M cells of the right and left ventricle. At longer cycle lengths the prolongation of the LV is much longer than the RV (32). Studies in dogs and humans have shown the presence of M cells (32,33).

Consequences of interventricular dispersion

The consequence of the relative smaller increase in the RV APD compared with the LV APD is an increase in the interventricular dispersion under baseline conditions in the chronic AV block phase. Interventricular dispersion is bradycardia dependent, the slower the heart rate the longer the difference between the LV and RV APD (17). In this study, we have controlled for the cycle length, so that the observed increase in dispersion is not based on the frequency. This increased amount of dispersion could have important pro arrhythmic consequences as we have recently described (17), because it is one of the components necessary to induce TdP in our animal model. The occurrence of TdP after administration of class III agents (13) is associated with bradycardia, QT/APD prolongation and the development of EADs (17). Suppression of EADs and a diminished interventricular Δ APD by magnesium prevents TdP (17), whereas the class III agent almokalant, which results in more EADs and a larger Δ APD as *d*-sotalol, has a higher incidence of (spontaneous) TdP occurrence (34).

Similar findings were obtained in this study, both in the serial testing as in the group comparison. TdP induction was related to the presence of EADs and an impressive amount of interventricular dispersion. Following acute AV block no EADs were present under baseline situation and interventricular Δ APD amounted to 40 ms. Administration of *d*-sotalol led to a lengthening of the repolarization parameters, appearance of EADs, but not to an increase in Δ APD, and no TdP could be induced. After 5 weeks of AV block, interventricular Δ APD was increased under baseline situation, while EADs were still not present, with one exception. In that dog TdP could be induced during baseline.

d-Sotalol increased interventricular Δ APD further because of its more pronounced effect on the LV APD and led to a higher incidence of EADs, and to TdP induction in 60% of the dogs. This means that not only the baseline values are higher in hypertrophy but that also the increase in APD and Δ APD after *d*-sotalol is larger. The dogs with a low interventricular Δ APD after *d*-sotalol did not develop TdP (see e.g. table 3).

Role of hypertrophy for the induction of TdP

Hypertrophy and its concomitant increase in the dispersion of intra- and/or inter-ventricular repolarization is also associated with a higher vulnerability to other ventricular arrhythmias in other studies (2,11,27,28,35,36). Moreover, regression of hypertrophy concomitant with a decrease of the electrophysiological parameters results in a marked reduced incidence of arrhythmias to baseline (36).

It would also be interesting to see whether the changes in electrophysiologic parameters in our dogs can be influenced by the prevention or regression of hypertrophy. In this context it is important to mention the study of Kreher et al. (37) suggesting that the adaptations leading to hypertrophy and electrophysiologic changes could be two different processes which can operate independently (37).

Occurrence of TdP in dogs in the acute phase of AV block is not impossible. Two studies have shown the occurrence of spontaneous TdP and polymorphic ventricular arrhythmias after administration of class III agents (38,39). However addition of an α -agonist (39) or a three times higher dose of almokalant was required (34,38). This could imply that the processes which takes places in the weeks following creation of complete AV block are more a facilitating than an absolute requirement for the induction of TdP, especially in regard to the increased APD and dispersion. In this regard it is of interest that in the human heart with complete AV block, TdP is rarely seen during acute ischemic AV block in myocardial infarction, but much more in chronic fibrotic AV block. It is likely that in the latter patient group ventricular hypertrophy is present.

Sensitivity to d-sotalol

Prolonged repolarization facilitates the development of EADs (11). The ionic mechanism of these EADs and of the increase in APD are not completely understood. Several reports show diminishment in outward K^+ currents (2,40). *d*-Sotalol, like most class III agents, blocks the outward K^+ current. Blockade of an already diminished current may explain the higher sensitivity to *d*-sotalol of the hypertrophied muscle in AV block dogs.

Limitations

In this study, we did not assess the time dependent behavior of the anatomical and electrophysiological changes. Because we measured only at two time points, it is possible that the adaptations were not fully completed. Earlier studies, however, showed that there is no relation with duration of AV block and total heart weight when assessed after two weeks (24,25). We had similar findings comparing the obtained heart weights at 20 weeks with 9 weeks AV block. In relation to the electrophysiological effects, the response to *d*-sotalol does not change in time, when we compare the experiments that have been started after 2 weeks AV block (13). This suggests that the alteration responsible for the facilitated TdP induction is already present at two weeks. The time period between 0 and 2 weeks will be the subject of further investigations.

A second limitation could be the random placement of the endocardial MAPs during these experiments. This method ignores possible intraventricular differences in APD. However we have shown that the intraventricular difference is always much smaller (only 20%) than the interventricular dispersion (17).

Finally, we have the difficulty that we are not yet able to measure the changes that occur during the pacing train leading to induction of TdP. We know that changes in frequency lead to augmentation of EADs, and to occurrence of triggered ectopic beats. The dynamicity of the changes in interventricular Δ APD can however not been assessed at this time.

Possible clinical implications

Extrapolation from data derived from experimental animals to the human setting should always be done with great caution. It is known that many different arrhythmic mechanisms may be generated in the hypertrophied myocardium resulted in symptomatic arrhythmias and death (2,3). We can hypothesize that patients with ventricular hypertrophy are more prone to the proarrhythmic effects of the TdP type during treatment with repolarization prolonging agents than patients without hypertrophy. A first step to be better informed about that possibility could be to stratify patients with TdP not only to QT interval, QT dispersion and heart rate but also to their degree amount and/or kind of ventricular hypertrophy.

General conclusions

Chronic complete AV block causes volume overload induced biventricular hypertrophy. Changes after creation of complete AV block do not only results in an increase in APD, they also lead to an increase in EADs and Δ APD after *d*-sotalol. This change is accompanied by the occurrence of TdP arrhythmias, once again confirming the importance of both EADs and Δ APD in the genesis of TdP.

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Chapter 8

General discussion

Since the beginning of this decade, there has been an increase in the use of class III agents instead of class I antiarrhythmic drugs. Class I drugs primarily delay conduction, while class III drugs act by lengthening of repolarization. This change is the result of multi center trials pointing to a higher complication rate when class I drugs are used. 1) The CAST trials (1,2), in which administration of class I drugs (flecainide, encainide, and morizicine) after myocardial infarction led to increased mortality. This adverse effect is observed in patients who had shown a decrease in ventricular arrhythmias on Holter recordings while using these drugs. 2) The ESVEM trial (3), in which various class I agents were found to be less effective than sotalol in treating patients with symptomatic ventricular tachycardia (VT) or those surviving cardiac arrest. In these patients antiarrhythmic treatment was guided by either Programmed Electrical Stimulation (PES) or Holter. 3) The CASCADE trial (4), in which empiric amiodarone therapy was proven to be more effective than class I agents. Again in this study, treatment was guided by PES or Holter.

These findings provided the major motive for the development of new class III agents (5,6). Also, because the existing class III agents (sotalol and amiodarone) 1) had other effects next to their prolongation of the action potential duration, and 2) lacked specific K^+ channel blocking properties, new “pure” class III agents were the targets to develop. Class III agents in general do have the potential to induce proarrhythmic reactions. The best known is the occurrence of Torsade de Pointes arrhythmias (TdP). An increasing clinical use of class III drugs makes it likely that there will be a proportionate increase in TdP. Fear of this problem has already resulted in the discontinuation of the development of a number of pure class III agents (7), as is the case with *d*-sotalol where an increased mortality rate with this drug was reported in the SWORD trial (8), but also in case of almokalant (9) and sematilide.

To develop a safe class III antiarrhythmic drug, it is necessary to define exactly the precise electrophysiologic mechanisms of TdP, the clinical circumstances favoring its occurrence and how these are effected by the antiarrhythmic drug. For that purpose, it becomes mandatory to have an animal model of TdP in which TdP can be reproducibly induced under “clinical” circumstances. Moreover, such a model would allow systematic

investigation of the different parameters involved and would enable comparison of different proarrhythmic and antiarrhythmic effects of drugs.

In our opinion, our recently developed TdP model, which is described in this thesis, meets those requirements. We performed a total of 115 experiments (88 *d*-sotalol, 27 almokalant) to study the different aspects of TdP. We were not only able to reproducibly induce TdP during a single experiment but this TdP induction was maintained over weeks (figure 1).

Like clinical acquired TdP (10), the *initiation of TdP* in our animal model is related to the following parameters 1) heart rate (bradycardia), 2) prolonged QT time, 3) presence of early afterdepolarizations (EADs), and 4) abrupt frequency changes (short long short sequence). In addition, we have confirmed the presumed relevance of another parameter: 5) interventricular dispersion of repolarization (Δ APD) in the genesis of TdP. In the following paragraphs each of these parameters will be discussed in relation to the results presented in this thesis and compared to data from the literature.

Heart rate

The importance of a slow heart rate or long cycle length of the idioventricular rhythm (CL-IVR) is based on the following observations: 1) In the clinical situation TdP often occurs during bradycardia because of sinoatrial or atrioventricular block. In patients with atrial fibrillation TdP typically develops after conversion to (a slower) sinus rhythm; 2) In our own model, non inducible dogs have a faster rhythm; 3) However when the CL-IVR of these non inducible dogs is prolonged with a second bolus of *d*-sotalol, half of them become inducible (chapter 2); 4) Both in the clinical situation and in our dog model, acceleration of the CL-IVR by isoproterenol or ventricular pacing prevents/suppresses TdP induction (chapter 2); 5) CL-IVR is a major determinant of all repolarization parameters: QT time, EADs and interventricular Δ APD.

Although we consider bradycardia to be an important requisite, it has also been shown that other factors need to be concomitantly present like an additional prolonged repolarization time. For example, we demonstrated in inducible dogs that a similar increase in CL-IVR after the β blocker atenolol did not lead to TdP induction (chapter 2). Secondly, a further prolongation of the CL-IVR after magnesium administration was not accompanied by persistence of TdP because the reduction in repolarization prevented occurrence of TdP (chapters 2 and 3).

QT time

In the literature QT values have been mentioned which could possibly predict the risk for the development of TdP (11,12). Therefore we plotted the QT and CL-IVR at baseline, 10 minutes after *d*-sotalol and after prevention by $MgSO_4$, verapamil, flunarizine, ryanodine or levocromakalim (13) to relate these parameters to the induction

Figure 1. Reproducibility of the (in)ability to induce Torsade de Pointes arrhythmias (TdP) after *d*-sotalol over weeks.

The response of the dogs which were tested more than once for the TdP experiments after 2 mg/kg *d*-sotalol is shown: induction of TdP (■) or no induction of TdP (○). On the horizontal axis the number of weeks after the creation of chronic complete atrioventricular block is depicted. Only 4/62 cases showed a different response, leading to a reproducibility of 94% over weeks. Two of these changes could be explained by the use of a diuretic.

of TdP. From figure 2 it is clear that most inducible points fall in the north east quarter of this graph, but no discriminatory line can be established with a sufficient sensitivity and/or specificity to predict TdP occurrence. However, the transition to inducible (*d*-sotalol) or non inducible events (after preventive medication) is always associated with a change in QT. These data confirm clinical findings that the absolute value of QT prolongation is not of great value. In this regard it is of interest to mention that amiodarone while causing a similar or even longer QT time as other class III or class Ia agents, does not result in TdP (14,15).

Lately, articles have appeared that indicate that QT dispersion, that is the difference between the longest and the shortest QT time on the ECG, may be used as a possible predictor for TdP in acquired (16-18) and congenital TdP (19). This work has many limitations, like reproducibility, variation in the number of leads which are measured, differences in methodology of QT time measurements, how to account for T wave

Figure 2. Importance of the QT time and the cycle length of the idioventricular rhythm for the (in)ability to induce TdP.

In this figure, data from 80 experiments with *d*-sotalol are combined. Open circles (○) indicate time points in the experiment in which no TdP could be induced: 1) during baseline, 2) first bolus *d*-sotalol not leading to TdP (non inducible animals) and 3) after anti arrhythmic treatment using MgSO₄ (100 mg/kg), ryanodine (10 μg/kg), verapamil (0.04 mg/kg), flunarizine (2 mg/kg) or levromakalim (0.01 mg/kg). The closed squares (■) are the time points at which TdP could reproducibly be induced after PES (first and second bolus of *d*-sotalol). The specificity and/or sensitivity are low. For example a QT time of 500 ms has a specificity of 0.88, but only a sensitivity of 0.66. When the QT time is lowered to 450 ms, the specificity decreases to 0.61, while the sensitivity increases to 0.8. Therefore no discriminating line can be found using either CL-IVR or QT to predict occurrence of TdP.

morphology, the importance of normal and abnormal spread of ventricular activation, and what to do with the U wave (20). Also these series are small, and follow up time often too short to get an idea about the true value of this approach.

Dispersion of repolarization

Regional differences in the length of repolarization can be subdivided in inter-, intra-ventricular or transmural dispersion, including dispersion between Purkinje and myocardial tissues. Since the introduction of the MAP catheter, many studies have been

performed that measured APD at different sites. Most of them are limited to intraventricular Δ APD. In the normal human heart (see table 5 in chapter 1), the intraventricular difference in MAP duration ranges from 0 to 73 milliseconds (21-23). In patients with congenital long QT values around 125 ms (23) have been described, while there has been found an interventricular Δ APD of over 250 ms (24) in a patient with acquired TdP. Transmural and interventricular dispersion have also been the subject of investigation in vitro. In figure 3, the reaction of 3 different canine ventricular cell types to changes in cycle length of both ventricles are depicted. At longer CL, the APD of the epicardial and endocardial cells are shorter than that of the midmyocardial (M) cell. In addition the APDs of LV endocardial and LV epicardial cells, but especially the M cells are significantly longer than that of the right ventricular cells (25) (compare right and left panels of figure 3).

In the dog heart with chronic complete AV block, baseline values of about 60 ms for interventricular Δ APD are encountered. Interventricular dispersion was shown to be bradycardia dependent (chapter 3), even without the presence of EADs. At a short cycle length (500 ms), there is already an interventricular difference in the APD of around 10-30 ms (chapter 3 and figure 4). The increase in interventricular Δ APD with an increase in CL can be explained by the difference in response of the right versus left ventricular cells.

Administration of *d*-sotalol or almokalant results in a more pronounced interventricular Δ APD. In our dogs the intraventricular difference is only 20 to 30% compared with the interventricular Δ APD (table 1 of chapter 3) after class III drugs. This can not only be explained by the different sensitivity of the LV compared to the RV in regard to changes in frequency or class III drugs, but is in part also related to the development of EADs (see below).

Dispersion of repolarization is not only related to differences in APD but can also be attributed to differences in local activation time (AT) with the beginning of the QRS complex as a reference. It will change when the normal sequence of activation as occurring during conducted sinus rhythm changes to a paced or idioventricular rhythm. In the normal heart there exists an inverse relationship between AT and APD resulting in a relatively homogeneous (endpoint) of repolarization (26). A long repolarization time can therefore to a certain extent be compensated by differences in AT. Although we did not determine AT yet (due to the different activation patterns during the CL-IVR) we do not believe that this is a major contributor to interventricular Δ APD in our experiments.

EADs and ectopic beats

Early afterdepolarizations are defined as an interruption or retardation of the repolarization (27) that prolong the APD. In vitro, not all cells are equally sensitive to develop EADs (28,29). Also in situ, there are findings which suggest a preferential site of EAD

Figure 3. Interventricular and transmural differences in canine ventricular action potential duration (APD) in vitro.

The action potential of the left and right ventricle is shown at different cycle lengths for the individual ventricular cell types: endocardial (Δ), epicardial ($\circ$) and M cells ($\bullet$). Besides larger transmural differences in APD at the longer cycle lengths there is also an appreciable difference in the APD when the left (right part) and right ventricle (left part) are compared. This results in a large interventricular Δ APD of approximately 100 ms at a CL of 2000 ms. Figure adapted from reference 25, published with permission of the American Heart Association and the authors.

development: 1) Endocardial MAP recordings in humans do not show EADs on every site (23,24). 2) Experimental studies in dogs have indicated that a) EADs are more prone to develop on the endocardial than on the epicardial site (29) and b) there exists a differential response of the left and right ventricular EAD amplitude after cesium and left stellate ganglion stimulation (30). We have shown (chapters 2,3 and 5) that after *d*-sotalol the EADs occur more frequently in the left ventricle, whereas with almokalant EADs developed in both ventricles (chapter 4).

The manifestation of EAD dependent triggered activity needs some prerequisites: 1) critical prolongation of the APD by reducing outward current (as after class III agents), or increasing inward current, 2) a net depolarizing current to create the EAD, and 3) propagation of the EAD to other regions of the heart resulting in an extrasystole. The net depolarizing current, causing the EAD, may be secondary to a disbalance in the intracellular calcium, because both ryanodine and flunarizine prevented the EAD and related triggered beats (chapter 6). In our experiments we have found that the coupling interval of such an extrasystole or ectopic beat (EB) is not always within the APD or QT time. This can be explained by the fact that 1) the EB was originating from a cell with a very long APD (e.g. Purkinje or M cell), that is not represented on the MAP or the ECG due to the small mass, or 2) a long conduction time between the triggering cells and the ventricular muscle (29) or both. An alternative explanation that is more controversial, could be the simultaneous presence of EADs and delayed afterdepolarizations (31). When the latter are responsible for the EBs the coupling interval will be beyond the APD.

Frequency changes

In patients a short long short sequence of the rhythm is observed at the start of most episodes of TdP (12,32,33). A recent analysis of Holter recordings in patients with TdP showed that before the development of TdP there was a significant increase in heart rate with the occurrence of short long short sequences in the last minutes preceding the TdP (34).

Frequency changes, either spontaneously occurring (almokalant) or induced by PES (our *d*-sotalol experiments) form the basis for the initiation of the TdP. Without a first ectopic beat no TdP will start. With PES induced TdP, one frequency change can already be sufficient to start EBs and TdP (chapter 2). In the other 50%, more frequency changes are necessary. On the other hand, the occurrence of spontaneous EBs is not always sufficient to start a spontaneous TdP (35) and has no meaning for the inducibility of TdP with PES. The difference could be that 1) the coupling interval of the EB is not appropriately timed, and/or 2) the number of beats is insufficient. The observation that class III agents (chapters 2 and 3) favor the occurrence of (or pronunciation of) the EADs after a rate acceleration was recently confirmed in in vitro studies (36). Rate acceleration

can also affect the interventricular Δ APD due to different dynamic responses of the right and left ventricle to adaptation of the APD.

EADs and interventricular Δ APD

The relation between EADs and Δ APD is not entirely clear. Often the EAD is observed in the MAP with the longest APD (23,24), thereby contributing to the Δ APD. On the other hand, presence of EADs in both ventricles, as with almokalant, is not associated with a smaller interventricular Δ APD (chapter 4, see below). From the behavior after spontaneous termination of TdP and the increase in Δ APD after the development of EADs at a stable paced rhythm we concluded that EADs do contribute to interventricular Δ APD (chapter 5). While it is difficult to quantify their exact contribution, we have hypothesized it to be around 25 to 30%. Furthermore the electrophysiological adaptations that occur after chronic complete AV block seem to facilitate the occurrence of both EADs and interventricular Δ APD (chapter 7).

That both parameters in combination are necessary for TdP induction can be derived from observations like: A1) the significant difference in incidence/magnitude of EADs and interventricular Δ APD between inducible and non inducible dogs. There existed however a significant difference in CL between the two groups (chapter 3); A2) When corrected for this parameter (similar CL), it was noticed that almokalant is associated with more EADs and a larger interventricular Δ APD and a higher incidence of (spontaneous) TdP compared to *d*-sotalol (chapter 4); A3) the significant difference in interventricular Δ APD between acute and chronic AV block experiments with *d*-sotalol and their relevance to the induction of TdP (chapter 7). B1) Independent of the effect on the CL-IVR, suppression/prevention of TdP by magnesium (chapter 3), flunarizine, ryanodine (chapter 6), levromakalim (13), isoprenaline or ventricular pacing, diminishes both the EADs and interventricular Δ APD, at the last beat of the TdP no EADs are present and interventricular Δ APD is small and C) Reinduction of TdP is only possible when EADs and interventricular Δ APD have returned to their original prepacing values, which changed directly after termination of TdP (chapter 5).

In figure 4, we have hypothesized how EADs occurring in both ventricles can lead to a (temporarily present) arrhythmogenic window. Either the EADs occur at different time points in the two ventricles or they have different contribution to the APD or both. When the LV is considered the most sensitive for the development of EADs, both possibilities will lead to a maximum interventricular dispersion. Over time this dispersion will be maintained or reduced, as the RV APD increases.

A limitation of our data is that all measurements concerning interventricular Δ APD were performed during a relative steady state (CL-IVR or paced rhythm) without dynamic changes in CL. However, the alterations during PES in EADs and interventricular Δ APD suggest that a non homogeneous response of the ventricles to an acceleration, may be very important in the continuation of the TdP (37,38).

Figure 4. The relevance of the cycle length and presence of EADs to interventricular dispersion (Δ APD) after *d*-sotalol.

Graph illustrating the possible way in which administration of *d*-sotalol leads to a larger interventricular Δ APD. The solid lines indicate the frequency behavior of the APD and interventricular Δ APD during baseline at paced frequencies. Interventricular Δ APD is bradycardia dependent: from 30 ms at 500 ms (comparable with SR) to 70 ms at idioventricular rhythm (IVR). Administration of *d*-sotalol (interrupted line) prolongs APD homogeneously. Therefore there is no increase in interventricular Δ APD. The second effect of *d*-sotalol, namely the lengthening of the cycle length of the idioventricular rhythm (IVR-CL *d*-sot) does lead to an increased interventricular Δ APD. Finally, administration of *d*-sotalol may lead to EADs. This may further contribute to interventricular Δ APD. As shown by the dotted line 1) the increase in the RV APD lags behind the LV APD due to a slower development of the EAD and/or 2) the RV EADs develop later in time. Both changes will increase the interventricular Δ APD, to arrhythmogenic levels.

In table 1, the individual role of interventricular Δ APD, EB and EADs in the genesis of TdP is summarized as evidenced from the results obtained in our animal model. Without EADs and interventricular Δ APD (baseline or after preventive measures) no TdP can be generated, not even by PES. This suggests that the presence of (triggered) EADs is no guarantee for TdP. PES in the setting of a sufficient interventricular Δ APD may start TdP because PES may mimic and reinforce the occurrence of EADs. Only the combination of EADs, EBs and interventricular Δ APD lead to the spontaneous occurrence of TdP. The occurrence of each of these parameters is favored by bradycardia, administration class III agents and chronic complete AV block (biventricular hypertrophy).

Table 1. Factors related to induction of Torsade de pointes arrhythmias (TdP)

EADs	EBs	Δ APD	$\Rightarrow$	spont TdP	PES TdP
-	-	-	$\Rightarrow$	-	-
+	-	-	$\Rightarrow$	-	-
+	+	-	$\Rightarrow$	-	-
-	-	+	$\Rightarrow$	-	+
+	-	+	$\Rightarrow$	-	+
+	+	+	$\Rightarrow$	+	+

Abbreviations: EADs: early afterdepolarizations, EBs: Ectopic beats, Δ APD: interventricular dispersion; spont TdP: spontaneous TdP, PES TdP: programmed electrical stimulation induced TdP, +: presence of the parameter, -: absence of the parameter.

Consequences of EADs and interventricular Δ APD for the continuation of acquired TdP

It has been suggested that the electrophysiologic mechanism responsible for the initiating beat is triggered activity due to EADs (possibly arising from Purkinje fibers or M cells). In addition the data in this thesis support the hypothesis that a sufficiently long interventricular Δ APD should be present for the initiation of TdP. The electrophysiologic mechanism of the subsequent beats leading to the actual polymorphic oscillatory pattern of TdP is less certain. Preliminary results show that continuation could be due to triggered activity arising from several endocardial sites (39-41), or local reentry (41,42). A reentrant tachycardia requires adjacent zones in which large differences in APD exist. The large difference in APD seems to be the case in our dogs. However, the distance between the left and right ventricle could cause some problems to picture a reentrant circuit between these two ventricles. On the other hand, a possible transmural difference could form the base of the reentrant tachycardia. Another argument against a reentrant circuit is the finding that the first beat of subsequent spontaneous TdP episodes often arises from different places, suggesting different sites of occurrence in the maintenance of TdP.

So far, the mechanism of TdP continuation, being triggered activity, reentrant tachycardia and/or a combination has not been unraveled and will need further study.

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Summary

Disturbances in cardiac rhythm are a common phenomena in humans. These may vary from a single innocent ectopic beat to life threatening arrhythmias. Medical treatment of these arrhythmias is sometimes associated with a worsening of the existing arrhythmia and/or development of a new arrhythmia (proarrhythmic event). In this thesis the mechanism and possible treatments of one of these proarrhythmic events, Torsade de Pointes (TdP) arrhythmias, is studied. TdP is a polymorphic tachycardia, occurring in the setting of an (abnormally) prolonged repolarization in which the QRS complexes twist around the isoelectric axis. TdP can be caused by several antiarrhythmic drugs, but also by other medication which prolong the repolarization.

Chapter 1 is a literature review of the different causes of TdP, with a central role for acquired TdP by antiarrhythmic drugs. Examples are the class Ia drugs: quinidine, procainamide and drugs with a class III effect like (*d*)-sotalol, amiodarone and almokalant. In addition the different experimental models dealing with TdP that were published before 1992 are reviewed. The majority of these models used substances that (in the used dose) are not (or cannot be) used in patients.

In dogs with chronic complete atrioventricular (AV) block, TdP can be reproducibly induced by administration of antiarrhythmic drugs in a clinical relevant dose (chapter 2). In this model the conduction between atria and ventricles is interrupted permanently. This offers the possibility to study ventricular arrhythmias, without interference by normal activation and impulse formation of the atria through the AV node. In addition there is a slow heart rate (a substantial part of acquired TdP).

During the AV block operation an electrode is placed on the heart. Combined with a pacemaker this electrode can be used to change (accelerate) the heart rate. After at least two weeks of AV block, dogs are anesthetized again and monophasic action potential (MAP) catheters are advanced in both heart chambers. The MAP can determine regionally the action potential duration and morphology. The action potential exists of an activation (depolarization) and a deactivation (repolarization) phase.

After this, the TdP induction protocol is performed: a specific pacing protocol after administration of 2 mg/kg *d*-sotalol. With this treatment TdP develops reproducibly in half of the dogs. Acceleration of the basal rhythm with continuous pacing or with isoprenaline prevents TdP, alike the patient situation.

Chapter 3 describes a group comparison in animals with and without TdP. TdP depends on 1) a prolonged repolarization (QT time, APD), 2) presence of early afterdepolarizations (EADs) and 3) a sufficient difference between the APD of the left and right ventricle; the so called interventricular dispersion (Δ APD). EADs are depolarizing

currents that occur before the repolarization is finished. EADs may result in triggered ectopic beats. Both EADs and interventricular Δ APD are increased at a slow heart rate. Magnesium prevents and suppresses TdP, because both the EADs and the Δ APD are diminished.

The response to *d*-sotalol remains similar in the same animal at different experiments. Therefore a comparison can be made to the effect of several interventions, with the animal as its own control like described in chapter 4. In 14 animals *d*-sotalol and almokalant (0.12 mg/kg) were given in consecutive experiments. A higher incidence in the same animal of EADs and Δ APD after almokalant resulted in a higher incidence of (spontaneous) TdP (5/14 vs 12/14). Which confirmed the importance of EADs and Δ APD in the initiation of TdP.

Most episodes of TdP end spontaneously. This fact combined with possibility of reproducible induction of the TdP, allowed study of the behavior of the EADs and interventricular Δ APD. This provides insight in the mechanism of termination and so by thus in the mechanisms of continuation (chapter 5). In the last beat of the TdP, EADs are absent and the difference in APD between the ventricles is minimal. Reoccurrence of EADs coincided with an increase in interventricular Δ APD. Reinduction of TdP is only possible when both the EADs and the interventricular Δ APD are back to their previous level.

Although interventricular Δ APD is an independent parameter, showing a clear frequency dependence, EADs contribute to the interventricular Δ APD. This is supported by the fact that EADs and interventricular Δ APD simultaneously disappear and reappear after spontaneous termination of TdP episodes, and appearance of EADs at a constantly paced CL after sotalol increases the Δ APD (chapter 5).

d-Sotalol and almokalant prolong the APD, induce EADs and lead to TdP. This is prevented by administration of flunarizine and ryanodine (chapter 6). These two drugs affect the $[Ca]_i$. A shortening of the repolarization and a suppression of the EADs. Their prevention points to a disturbed intracellular Ca^{2+} in the genesis of EADs and thereby TdP.

The role of AV block for the TdP model is studied in chapter 7. Dogs ($n=9$) were studied at the moment of the creation of AV block and 4 to 6 weeks after AV block ($n=10$) had evolved. In five of these dogs both time points could be studied. The APD of both ventricles was significantly longer when measured at chronic AV block. In addition the dogs with chronic AV block showed a more pronounced response to *d*-sotalol, this was especially the case for the left ventricle resulting in a significantly larger Δ APD. TdP could never be induced directly after creation of AV block whereas at chronic AV block 6/10 dogs showed reproducible induction of TdP after *d*-sotalol and PES. This was not only the case for the group comparison but also when individual animals were compared. The thesis is concluded by a general discussion which summarizes the results obtained in this model and points to their consequences for the mechanism of TdP.

In short, this animal model of acquired TdP allows 1) reproducible induction of TdP (spontaneous and PES induced), 2) investigation into preventive and suppressive interventions and 3) comparisons between the proarrhythmic effect of different medications. The initiating mechanisms of TdP in this animal model depend on the presence of EADs in combination with interventricular Δ APD. Both parameters are bradycardia dependent. Furthermore, EADs contribute to interventricular Δ APD. The mechanism responsible for the continuation of TdP is not completely clear. Further investigation should be performed to clarify this continuation.

Samenvatting

Verstoringen van het hartritme zijn een normaal verschijnsel in elk mensenleven. Deze kunnen variëren van een onschuldige extra slag tot levensbedreigende ritmestoornissen. Behandeling van deze aritmieën met medicijnen gaat soms (3-5%) gepaard met een verslechtering van een bestaande en/of het ontstaan van een nieuwe ritmestoornis (een proaritmogene reactie). In dit proefschrift wordt het mechanisme en de mogelijke behandeling van een van de proaritmogene responsen de zogenaamde Torsade de Pointes ritmestoornissen (TdP) bestudeerd. TdP is gedefinieerd als een polymorfe kamerritmestoornis waarbij de QRS complexen een rotatie om de iso-elektrische as vertonen op het ECG (vandaar de franse naam Torsade de Pointes). TdP komt voor bij een (abnormaal) verlengde repolarisatie dat onder meer het gevolg kan zijn van het gebruik van bepaalde anti-aritmica, maar ook kan worden veroorzaakt door andere medicamenten die in de geneeskunde worden toegepast.

In hoofdstuk 1 is een overzicht gegeven van de literatuur met betrekking tot de verschillende oorzaken van TdP, waarbij de door anti-aritmica veroorzaakte TdP ("acquired" TdP) centraal staat. Voorbeelden van deze medicijnen zijn klasse I anti-aritmica als: quinidine, procainamide en disopyramide en stoffen met klasse III effecten als *d*-sotalol, amiodarone, en almokalant. Tevens zijn de verschillende (dier)experimentele modellen besproken die zijn gepubliceerd voor 1992. Het merendeel van deze modellen werkte met stoffen die (in die dosering) niet bij patiënten gebruikt (kunnen) worden.

In honden met een chronisch compleet atrioventriculair (AV) blok kan TdP op een reproduceerbare wijze worden geïnduceerd door toediening van anti-aritmica in klinisch relevante doseringen (hoofdstuk 2). Bij dit AV blok model, waarbij een onderbreking in de geleiding tussen de boezem en de kamer wordt geïnduceerd, is het mogelijk kamerritmestoornissen te bestuderen zonder de invloed van de normale kameractivatie door impuls geleiding van de boezem over de AV knoop. Tevens ontstaat er een traag kamerritme (een essentieel onderdeel van "acquired" TdP). Tijdens de AV blok operatie wordt er een elektrode op het hart aangebracht. Die in samenspraak met een pacemaker kan worden gebruikt om het hart een andere (snellere) frequentie op te leggen. Nadat dit complete AV blok minstens 2 weken heeft bestaan worden de honden opnieuw onder narcose gebracht en worden er tijdelijk monofasische actiepotentiaal catheters (MAPs) ingebracht in beide hartkamers. Met deze MAPs kan lokaal de vorm en de duur van de actiepotentiaal van het hart worden bepaald. Deze actiepotentiaal is opgebouwd uit een activatie- (depolarisatie) en een herstel fase (repolarisatie).

Het TdP inductie protocol dat hierna wordt uitgevoerd bestaat uit een specifiek paceprotocol na toediening van 2 mg/kg *d*-sotalol. In de helft van de honden kan op deze manier reproduceerbaar TdP worden geïnduceerd. De TdP episoden zijn net als

bij de humane patiënt te voorkomen door een versnelling van het basale hartritme, met behulp van isoprenaline (β agonist) of elektrisch pacen.

Een groepsvergelijking tussen dieren met en zonder TdP laat zien dat TdP initiatie afhankelijk is van 1) een verlengde repolarisatie (QT tijd en/of actie potentiaal duur (APD) 2) vroege nadepolarisaties (EADs) en 3) een voldoende groot verschil in actiepotentiaal duur tussen de linker en de rechterkamer: de zogenaamde interventriculaire dispersie (Δ APD, hoofdstuk 3). EADs zijn elektrische ontladingen die optreden voordat de repolarisatie fase beëindigd is. EADs kunnen resulteren in het optreden van extra slagen. Zowel het optreden van EADs als interventriculaire Δ APD wordt versterkt door een trager hartritme. Magnesium voorkomt TdP, doordat het zowel de vroege nadepolarisaties onderdrukt als de interventriculaire Δ APD vermindert.

De reactie op *d*-sotalol (dat wil zeggen het wel of niet optreden van TdP) is reproduceerbaar bij hetzelfde dier tijdens verschillende experimenten. Daarom kunnen vergelijkingen gemaakt worden naar het effect van verschillende interventies met het dier als zijn eigen controle zoals beschreven in hoofdstuk 4. In totaal 14 honden kregen zowel *d*-sotalol als almokalant. Almokalant geeft meer EADs en een groter verschil in repolarisatie duur tussen de linker en rechter kamer dan *d*-sotalol. Dit gaat gepaard met een significante toename in incidentie van (spontane) TdP. Dit bevestigt de noodzaak van EADs en interventriculaire dispersie voor het initiëren van TdP.

Omdat TdP in de meeste gevallen spontaan eindigt en reproduceerbaar is op te wekken, kan het gedrag van de actiepotentiaal duur en de EADs na afloop van een TdP worden bestudeerd. Dit is gebruikt om meer inzicht kunnen krijgen in de terminatie en dus de mechanismen verantwoordelijk voor de continuatie van de aritmie (hoofdstuk 5). De laatste slag van de TdP heeft geen EADs en het verschil in APD van de linker en rechterkamer is miniem. Het opnieuw optreden van de EAD na afloop van de TdP ging samen met een toename van de interventriculaire dispersie. Het opnieuw induceren van TdP is alleen mogelijk als zowel de EAD als de interventriculaire Δ APD weer terug zijn op hun oude niveau. Interventriculaire dispersie is een zelfstandig fenomeen dat onafhankelijk van de aanwezigheid van de EADs toeneemt bij een afname van de hart frequentie. De ontwikkeling van EADs na *d*-sotalol bij een gepacete gelijkblijvende hartfrequentie leidt ook tot een toename van de interventriculaire Δ APD. Dit betekent dat de EAD een wezenlijke bijdrage levert aan de interventriculaire dispersie.

Verlenging van de APD door *d*-sotalol en almokalant leidt tot EADs en TdP. Dit kan voorkomen worden door toedienen van ryanodine en flunarizine. Deze twee stoffen beïnvloeden de calcium huishouding in de cel. Flunarizine corrigeert ook repolarisatie stoornissen aanwezig in controle omstandigheden. De preventie van TdP door deze twee stoffen duidt op een belangrijke rol van (intracellulair) calcium in het ontstaan van de ritmestoornis (hoofdstuk 6).

Het chronische complete AV blok vertraagt de kamerfrequentie, waardoor er een volume overbelasting optreedt (hoofdstuk 7). Het hart moet een zelfde hoeveelheid bloed in minder slagen uitpompen. Het hart past zich hieraan aan met een toename van de

spiermassa in beide kamers (biventriculaire hypertrofie). Gecorrigeerd naar het lichaamsgewicht van de hond neemt de rechterkamer meer toe (toename 80%) dan de linkerkamer (toename 40%). Honden met een chronisch compleet AV blok hebben dus niet alleen bradycardie maar ook biventriculaire hypertrofie. De electrofysiologische gevolgen van chronisch compleet AV blok is bestudeerd in 15 honden. Daartoe werden op twee tijdstippen metingen verricht: 1) direct na en 2) 4-6 weken na het maken van AV blok. Na 4-6 weken AV blok is de kamerrepolarisatieduur verlengd en ook het interventriculaire verschil in repolarisatie is toegenomen ten opzichte van metingen direct na het maken van AV blok. Bovendien is bij een zelfde hartfrequentie het effect van *d*-sotalol op de repolarisatieduur en dispersie veel meer uitgesproken. Er ontstaan dan ook meer vroege nadepolarisaties en een grotere interventriculaire Δ APD. In dieren met een chronisch AV blok was TdP aanwezig in 60% van de dieren na 2 mg/kg *d*-sotalol terwijl na acuut AV blok in geen enkel dier TdP kon worden opgewekt met deze dosering *d*-sotalol. Dit maakt duidelijk dat de aanpassingen als gevolg van het chronische compleet AV blok het ontstaan van TdP ritmestoornissen na *d*-sotalol vergemakkelijken of de omstandigheden tijdens acuut AV blok TdP voorkomen.

Samenvattend kan worden gesteld dat in dit diemodel van "acquired" TdP het mogelijk is om 1) reproduceerbaar TdP op te wekken (spontaan en PES-induced), 2) diverse preventieve en suppressieve interventies te onderzoeken en 3) vergelijkingen te maken tussen het proaritmogene effect van verschillende medicamenten. Het initiërend mechanisme van TdP in dit diemodel is afhankelijk van de aanwezigheid van EADs in combinatie met een voldoende grote interventriculaire Δ APD. Beide factoren nemen toe met een vertraging van het hart ritme. Los daarvan dragen de EADs bij aan de interventriculaire Δ APD. Het mechanisme dat verantwoordelijk is voor continuatie van de TdP is nog niet geheel duidelijk. Verder onderzoek zal dit moeten aantonen.

Nawoord

Het "onderzoeksvirus", met als primaire uiting promotie onderzoek, was de afgelopen jaren een zeer besmettelijk fenomeen, terug te vinden in groot deel van mijn studievrienden. Hoewel het "onderzoeksvirus" bij mijzelf gelukkig nog lang niet is genezen, wordt met dit promotieboekje toch een periode afgesloten. Aan het einde van dit proefschrift is dan de gelegenheid om iedereen te bedanken voor zijn of haar bijdragen.

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Curriculum Vitae

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