

Application of Monophasic Action Potential Recording Technique in the Detection of Endothelin-Induced Ventricular Arrhythmias

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Summary

Recently, the development of ventricular tachyarrhythmias caused by intracoronary (i.c.) infusions of low dosages of endothelin-1 (ET-1) was observed in dogs. The presented study was aimed at investigating the pathological mechanism of ET-1-induced ventricular arrhythmias in 42 anesthetized, open-chest, mongrel dogs, which were divided into groups A (n=14) without AV-node ablation and B (n=14), C (n=6), D (n=4), and E (n=4) with AV-node ablation. Groups D and E formed the control groups. The coronary blood flow (CBF) was measured in the left anterior descending (LAD) coronary artery with an electromagnetic flowmeter. Standard ECG, atrial and ventricular electrograms, and, in groups B, C, D, and E, endocardial and epicardial monophasic action potentials (MAP) were recorded. In groups A, B, C, and D, i.c. ET-1 was administered to the LAD in low doses (30 to 60 pmol/min). When the premature beats appeared, the CBF was only slightly decreased. The ventricular effective refractory period (VERP) did not change significantly. Onset of spontaneous polymorphic and monomorphic sustained ventricular tachycardias (sVT) was observed in 5 dogs without bradycardia and in 9 dogs with bradycardia. Ventricular tachycardias in dogs with complete AV block were longer and slower. In most of the cases, ventricular fibrillation (VF) occurred. Treatment with ET-1 resulted in a significant increase in MAPd90 duration at 70 bpm ventricular pacing (values for group B: endocardial MAPd90: 255 ± 9 ms versus 290 ± 8 ms, $p < 0.01$; epicardial MAPd90: 244 ± 10 ms versus 292 ± 12 ms, $p < 0.05$). In 8 cases (group B), third-phase early afterdepolarizations (EAD) could be recorded. According to our results, the mechanism of ET-1-induced arrhythmias seems to be based on MAP-duration prolongation and the development of afterdepolarizations.

Key Words

Ventricular arrhythmia, endothelin, monophasic action potential

Introduction

Endothelins (ET) are potent vasoconstrictor peptides consisting of 21 amino acids. In the heart, ET-1 has a strong coronary constrictor effect which may produce myocardial ischemia. The myocardium also has specific binding sites for ET-1. Via these specific binding sites, ET-1 produces a slight increase in cardiac contractility and also has positive chronotropic effects [1][2].

In a few species, intracoronary (i.c.) administration of an ET-1 bolus has been shown to induce not only vasoconstriction but also arrhythmias, including premature ventricular contraction (PVC) and ventricular fibrilla-

tion (VF) [3]. Apart from its vasoconstrictor effects, ET-1 may also exhibit a primary arrhythmogenic property that is not solely attributable to myocardial ischemia [4][5]. ET-1 increases the action potential duration of ventricular myocytes and causes early afterdepolarizations (EAD) in Purkinje fibers in vitro [6]. This study was aimed at investigating the arrhythmogenic action of low-dose i.c. ET-1 infusions in vivo and the induced pathological mechanism of ventricular arrhythmias in cases with and without permanent bradycardia, using monophasic action potential (MAP) recording. Recording MAP electrograms in vivo is

suitable for studying the characteristics of local myocardial repolarization and identifying the bases for triggered ventricular arrhythmias, thus connecting basic and clinical electrophysiology [6-8].

Materials and Methods

Acute experiments were conducted on 42 open-chest mongrel dogs of either sex weighing 15 to 31 kg. The animals were anesthetized by intravenous pentobarbital sodium (30 mg/kg) and mechanically ventilated with humidified air in the room. Experiments were performed in 5 groups. Group A (n=14) did not undergo AV-node ablation. In groups B (n=14), C (n=6), D (n=4), and E (n=4), AV-node ablation was produced by temperature-controlled radiofrequency ablation (AbControl, BIOTRONIK). Bipolar right ventricular pacing (70 bpm) was performed using pull-out electrodes or quadripolar MAP catheters. The ventricular effective refractory period (VERP) and the inducibility of ventricular arrhythmias were determined by programmed electrical stimulation (PES) (300 ms basic cycle length with 1 and 2 extrastimuli) of the right ventricular anteroseptum or anteroapex. Quadripolar MAP catheters and, in 8 cases, PES of the left ventricular anterior wall with epicardial pull-out electrodes were used.

The arterial blood pressure was recorded in the femoral artery, and coronary blood flow (CBF) was monitored in the left anterior descending (LAD) coronary artery with an electromagnetic flowmeter (Statham SP 2201).

Recordings

A standard ECG was recorded in all cases. In group B, right ventricular endocardial and left ventricular epicardial MAPs were recorded; in group C, right ventricular anteroseptal, right ventricular apical endocardial, and right ventricular lateral and left ventricular anterior epicardial MAPs were used to detect spatial dispersion. The MAP was recorded by a quadripolar endocardial silver/silver chloride (Ag/AgCl) catheter, as well as bipolar or quadripolar fractal iridium(Ir)-coated endocardial and bipolar epicardial leads.

Electrode materials coated with Ir allow MAP recording immediately after a stimulus without disturbing artifacts [6][7][9][10]. An excellent correlation of the monophasic action potential duration at 90 % repolarization (MAPd90) between the Ag/AgCl and the Ir coated electrodes during spontaneous rate and during stimulation was demonstrated using an endocardial catheter with four separate electrodes made of both materials fitted closely together [8].

The four-channel MAP tracings for studying the spatial dispersion were measured and digitized using a DC-coupled isolation amplifier and A/D converter. The signals were then stored on a PC. The surface ECG was printed out simultaneously with the MAP signals, the blood pressure (BP) and the flow of the LAD using a 12-channel chart recorder (Madaus Schwarzer CU 12). MAPs were evaluated using a semiautomatic evaluation program. The cycle length and MAPd90 values were calculated automatically. The correct triggering of the onset of each MAP and the calculating of MAP duration was manually confirmed and corrected.

A 24 G cannula was inserted into the LAD for the administration of ET-1. Intracoronary ET-1 was administered at low doses (30 pmol/min). The dose of ET-1 was increased to 60 pmol/min if spontaneous arrhythmias did not occur after 30 min. Four control dogs received i.c. saline infusions (group D). The reversibility of ET-1-induced arrhythmias was also studied in another control group (group E). In this case, ET-1 infusion was discontinued after the development of non-sustained ventricular tachycardias (VT).

Mean values and the mean standard deviation were calculated for each parameter. Statistical significance was assessed at the 95 % confidence level by using analysis of variance (ANOVA) followed by Bonferroni's t-test.

Statistical analysis

Mean values and the mean standard deviation were calculated for each parameter. Statistical significance was assessed at the 95 % confidence level by using analysis of variance (ANOVA) followed by Bonferroni's t-test.

Results

In group A, ventricular arrhythmias developed in 4 cases after i.c. administration of 30 pmol/min ET-1 and in 10 cases following i.c. administration of 60 pmol/min ET-1. In these dogs without AV block, i.c. ET-1 administration led to the onset of premature ventricular contractions (PVC) and short non-sustained bursts at 20 to 40 min of administration. Later on, the monomorphic and polymorphic non-sustained or sustained tachycardias often accelerated into VF. A VT with a duration of more than 30 s and a rate of more than 100 bpm was considered a sustained VT (sVT) in this study. Onset of

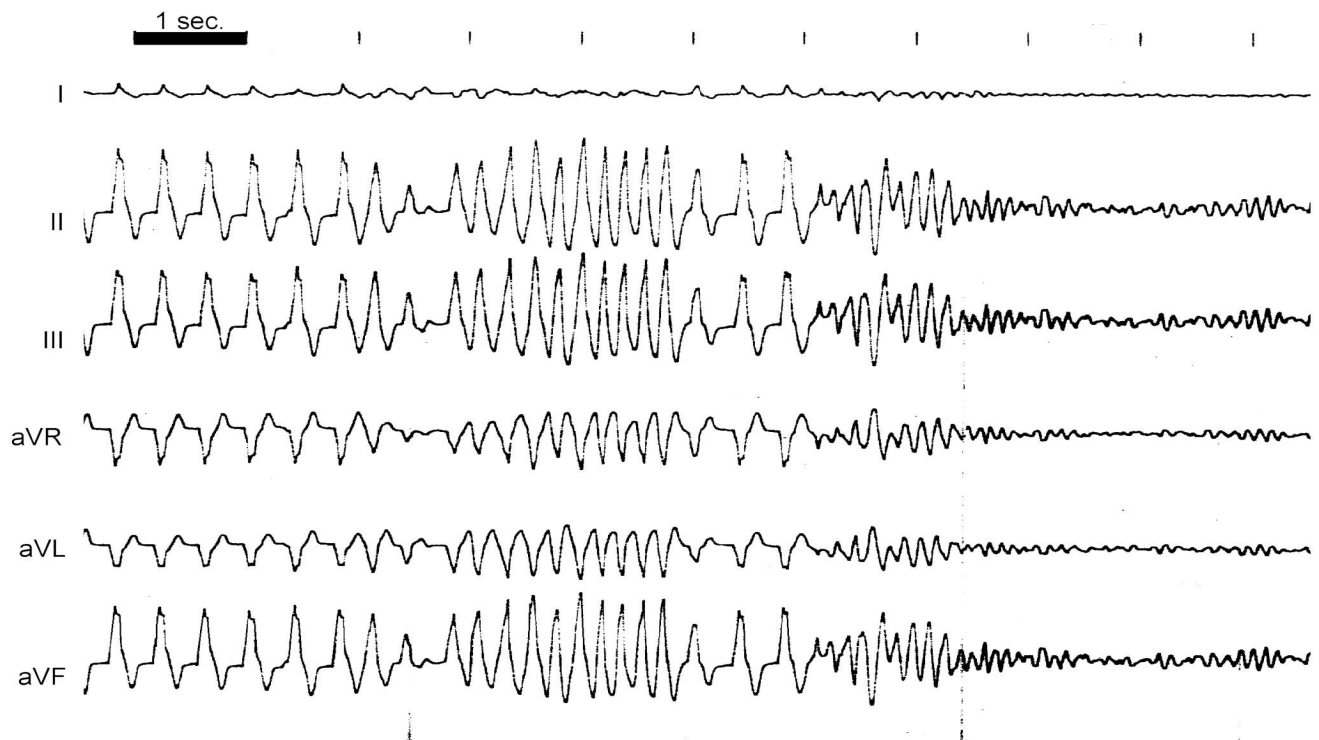


Figure 1. Degeneration of a monomorphic sustained ventricular tachycardia into ventricular fibrillation during intracoronary administration of ET-1 (60 pmol min^{-1}) in dog with complete AV block. After 29 min following the ET-1 administration sustained ventricular tachycardia developed and afterwards the ventricular tachycardia degenerated into ventricular fibrillation.

spontaneous sVTs was observed in 5 dogs. Non-sustained VT developed in 9 dogs. Ventricular fibrillation occurred at termination in 12 cases out of 14 (86 %). In group B, the dogs with AV block, ventricular arrhythmias developed in 6 cases after i.c. administration of 30 pmol/min ET-1 and in 8 cases following i.c. infusion of 60 pmol/min ET-1. In this group, i.c. ET-1 administration led to the onset of PVCs and short non-sustained bursts at 20 to 30 min of administration. Later on, episodes of non-sustained VTs often followed each other in an incessant way. Non-sustained bursts often showed polymorphism. Sustained VTs were observed within 30 to 40 min. In 86 % of the cases, monomorphic and polymorphic VTs occurred alternately. Onset of spontaneous sVTs was observed in 9 dogs. In the other 5 dogs, longer ventricular bursts occurred. Ventricular tachycardia spontaneously accelerated into VF at termination in 11 cases out of 14 (79 %) (figures 1 and 2). Comparing groups A and B, onset of spontaneous sVTs was observed in 36 % of the

cases without AV block and in 64 % of the dogs with AV block. The data are not statistically significant ($p = 0.11$). At termination of the studies, VF occurred in most of the cases. Inducibility is not typical for these ventricular arrhythmias. Despite repeated PES, we were able to induce sVTs only in two cases and VF in another in both groups A and B (table 1).

In contrast to the ET groups, ventricular arrhythmias did not develop in the control group (group D).

ET-1 treatment resulted in a significant increase in QTc and QT duration in groups A and B. When premature beats appeared, no signs of myocardial ischemia were observed in the ECG. Nevertheless, CBF moderately decreased. A considerable worsening of CBF paralleled the progression of ventricular arrhythmias in later experimental phases. Ventricular tachycardias in dogs with complete AV block were longer in duration, and the initial frequency was lower (table 2.)

ET-1 treatment resulted in a significant prolongation of QT duration (in group B: 325 ± 13 versus 358 ± 14 ms;

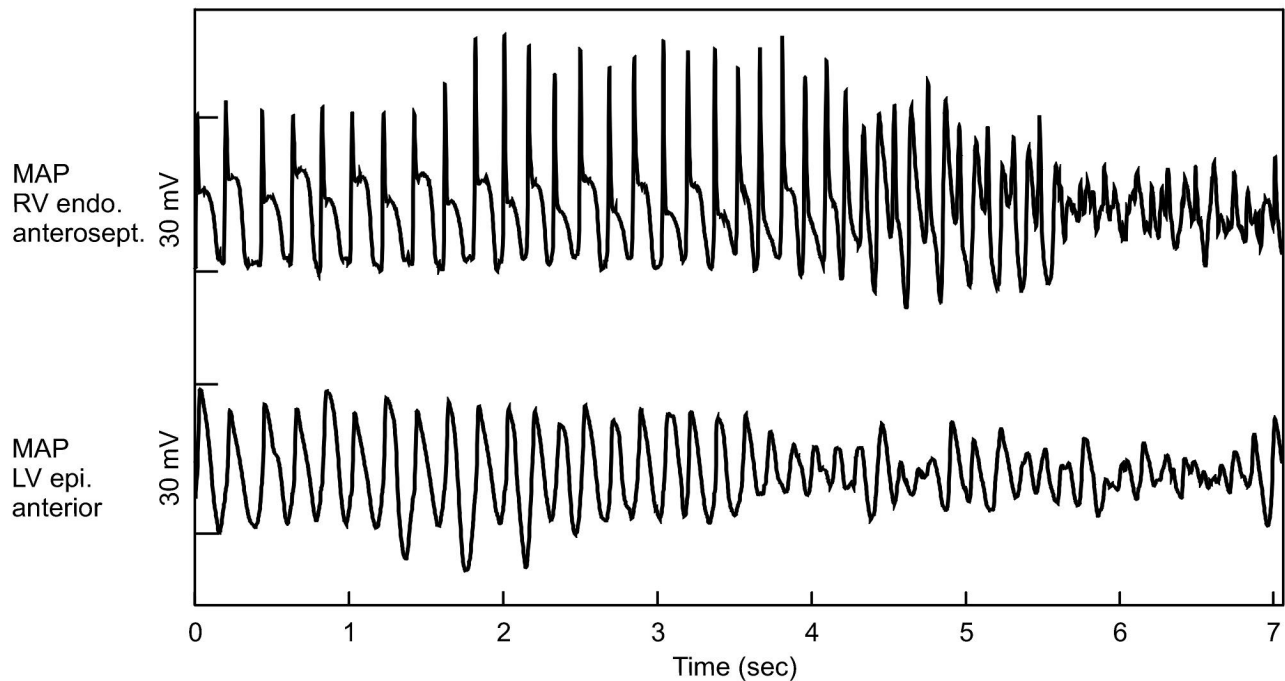


Figure 2. The two-channel digital recording shows the RV endocardial anteroseptal and the LV epicardial anterior monophasic action potentials of a ventricular tachycardia with 290 bpm accelerating to a ventricular fibrillation.

		VPC	nsVT	sVT	VF
Group A, n=14 without AV node ablation	Spontaneous arrhythmias	13	9 (64%)	5 (36%)	12 (86%)
	Induced arrhythmias	7	5	1	0
Group B, n=14 with AV node ablation	Spontaneous arrhythmias	14	13 (93%)	9 (64%)	11 (79%)
	Induced arrhythmias	8	8	1	-

Table 1. Spontaneous and induced arrhythmias during ET-1 administration, only the occurrence of sVT was statistically significant ($p = 0.11$) in group A and B.

	CBF (ml/min)	QT (ms)	sVT rate	sVT duration
Group A, n=14, without AV node ablation after ET-1	34±3	299±6 QTC		
	23±2; $p < 0.01$	345±9; $p < 0.05$	326±17 bpm	84±22 sec
Group B, n=14, with AV node ablation after ET-1	21±2	325±13		
	16±2; $p < 0.01$	358±14; $p < 0.01$	171±14 bpm	180±28 sec

Table 2. Effect of intracoronary ET-1 infusions on basic hemodynamic and electrophysiological parameters of dog hearts (group A without and group B with AV ablation). Values are Mean ± SEM.

Parameters	before ic. ET-1	during ic. ET-1	statistical evaluation
QT (851)	375 ± 13 ms	358 ± 14 ms	p < 0.01
MAPd90 (851) endo	256 ± 9 ms	250 ± 8 ms	p < 0.01
MAPd90 (851) epi	244 ± 10 ms	252 ± 12 ms	p < 0.05
VERP (300)	165 ± 9 ms	153 ± 9 ms	ns
PP interval	371 ± 16 ms	379 ± 17 ms	ns
CBF	21 ± 2 ml/min	18 ± 2 ml/min	p < 0.01
BP	84 ± 12 mmHg	75 ± 13 mmHg	ns

Table 3. Effect of intracoronary ET-1 infusions on basic hemodynamic and electrophysiological parameters of dog hearts (group B with complete AV block). Values are Mean ± SEM.

p < 0.01), endocardial MAPd90 (255 ± 9 ms versus 290 ± 8 ms; p < 0.01), and epicardial MAPd90 (244 ± 10 ms versus 292 ± 12 ms; p < 0.05). Ventricular effective refractory period, PP interval, and BP did not change significantly (table 3).

We also studied the reversibility of ET-1-induced arrhythmias in another four dogs (group E). If ET-1 administration was discontinued after the onset of non-sustained tachycardias, all forms of ventricular arrhythmias-including PVCs-spontaneously disappeared within 30 min.

In 12 dogs, ET-1-induced early afterdepolarizations (EAD) could be recorded using MAP electrodes (endocardially in 8 cases; epicardially in 4 cases; i.e., in half the animals of groups B and C each). Figure 3 shows EADs on the descending part of repolarization in the epicardial and in the right ventricular endocardial MAP recording. Another example of an EAD is pre-

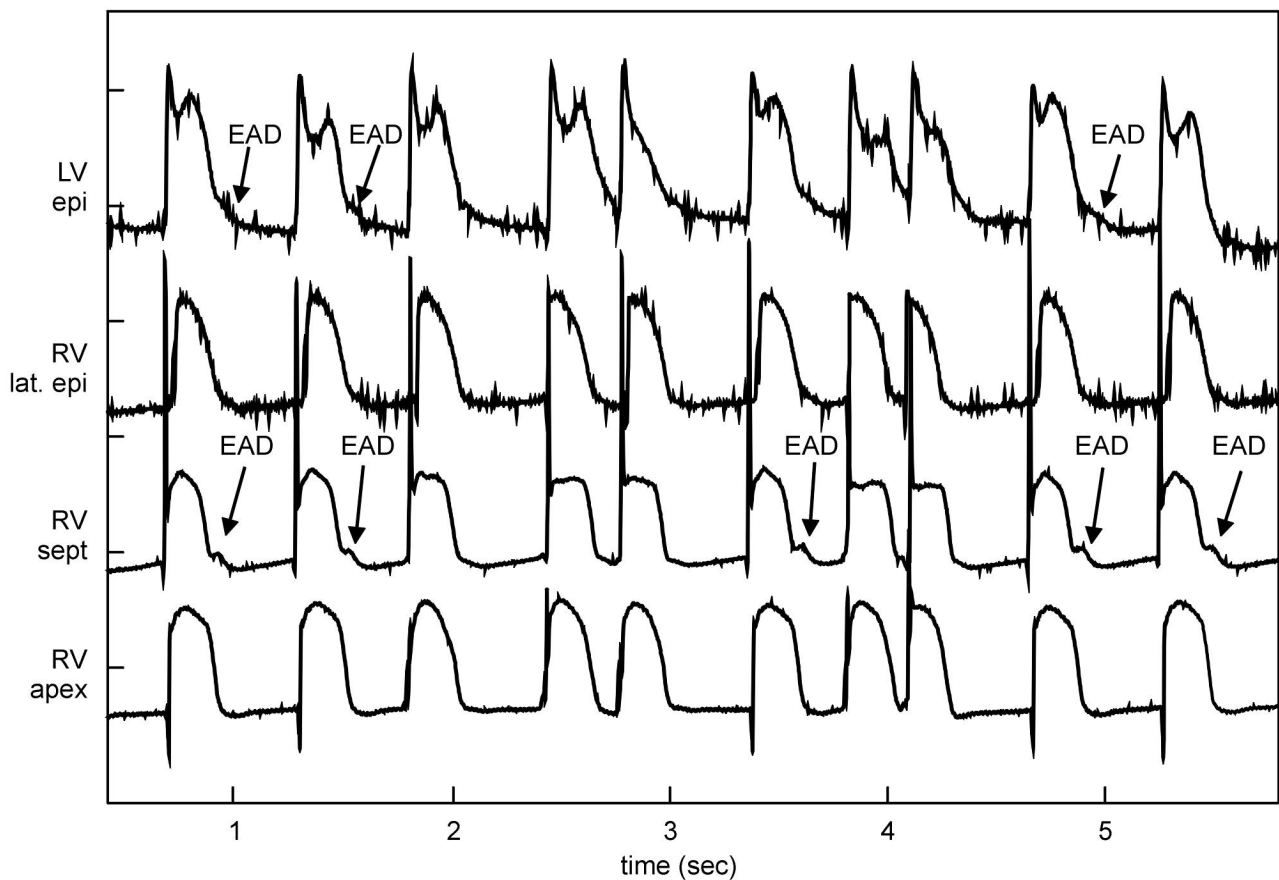


Figure 3. The digital four-channel MAP recording shows a slow polymorphic VT. The origin of ventricular arrhythmias is based on afterdepolarization.

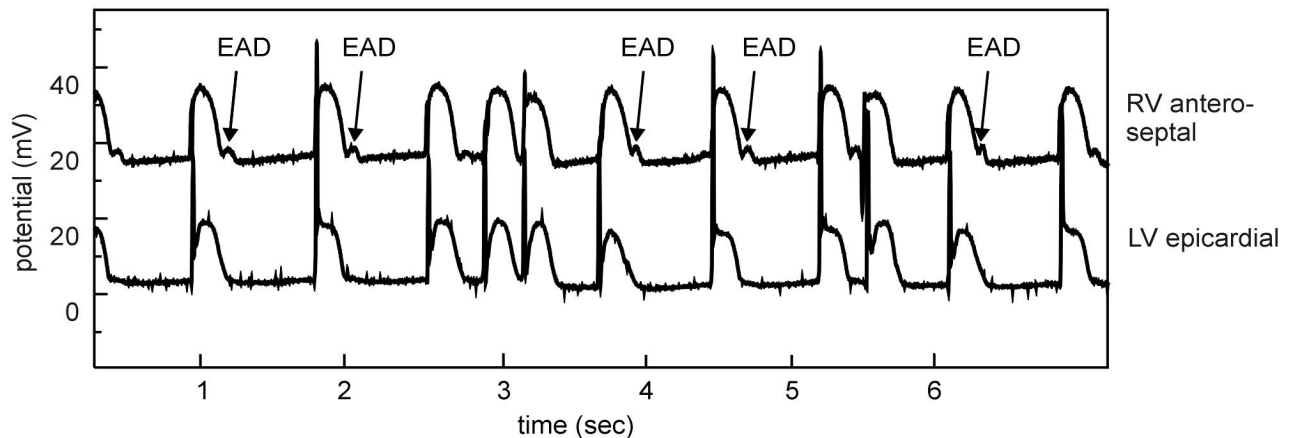


Figure 4. Anteroseptal endocardial MAPs of the right ventricle and anterior epicardial MAPs of the left ventricle are shown on the two channel MAP digital recording. Another example of the early afterdepolarization is presented in the endocardial MAP recording. The triggering of ventricular arrhythmias is related to afterdepolarization.

sented in the two-channel digital recording of the endocardial MAP (figure 4). The triggering of ventricular arrhythmias is related to afterdepolarization.

During the i.c. infusion of ET-1 in group C, the MAP duration of the left ventricular (LV) epicardium and the endocardial right ventricular (RV) apical and septal recordings showed the most pronounced increase: 35 to 60 ms (figure 5). Whereas in the lateral recordings of the MAP from the right ventricle, a slighter increase of 15 ms was observed. This testifies to the spatial dispersion of the MAP between the recordings near and far from the ET-1 infusion site in this dog. Onset of PVCs was observed 20 min after start of the infusion. After 30 min, bursts of non-sustained and sustained VTs were recorded followed by VF after 45 min (figure 6). All dogs of group C show this spatial dispersion of MAP duration during infusion of ET-1. Even before ET-1 treatment, spatial dispersion was seen in all dogs. After 20 min, the dispersion increased with time. The dispersion attained its maximum 30 min after start of the infusion (figure 7). Afterwards, the occurrence of frequent premature contractions and non-sustained VTs did not allow measurement of MAP duration at 70 bpm stimulation due to the appearance of disturbing, frequency-related artifacts.

Discussion

Our in vivo results extend previous reports by showing that low-dose i.c. administration of ET-1 causes spon-

taneous ventricular arrhythmias before signs of myocardial ischemia appear. At the time of the appearance of arrhythmias, no effects on BP, PP interval, and ECG morphology were observed. Coronary blood flow was only decreased about 30 %, but it worsened in parallel to the progression of ventricular arrhythmias.

Endothelin arrhythmias are not readily inducible with PES. These arrhythmias result in VF and death in most of the animals, so ET-1-induced ventricular arrhythmias may serve as a prototype of fatal cardiac arrhythmias [11][12]. Permanent bradycardia promotes the development of sustained VTs.

In our study, low dose i.c. ET-1 infused directly into the LAD resulted in a significant prolongation of QT duration and of epicardial and endocardial MAPd90s. We also observed the spatial dispersion of MAP duration, whereas MAP close to the infusion site showed the most pronounced prolongation. In response to i.c. infusion of ET-1, EADs could be recorded in 50 % of the cases. The results appear to show that ET-1 has a direct arrhythmogenic effect that is based on action potential duration prolongation and on the development of EADs.

This experimental setup can serve as a pathophysiologic model for inducing triggered-activity VTs. This model is also suitable for antiarrhythmic drug testing. The direct arrhythmogenic action of ET-1 does not diminish the role of the indirect action of ET-1 in arrhythmia formation due to its vasoconstrictory effects [11]. Direct and indirect mechanisms can simul-

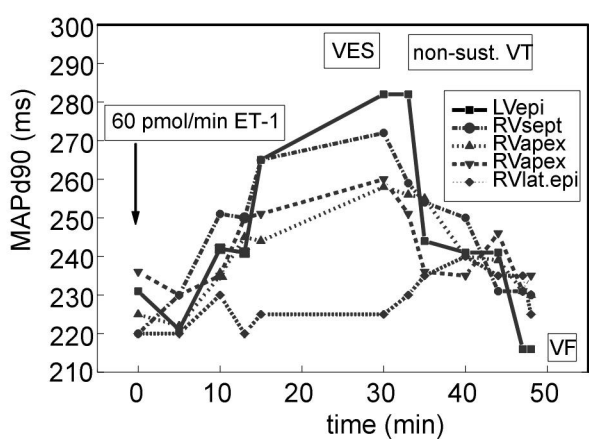


Figure 5. During the i.c. infusion of ET-1 the MAP duration of the LV epicardium and the endocardial RV apical and setal recording showed the most pronounced increase of 35-60 ms as seen in the left slide while in the lateral recordings of the MAP from the right ventricle a slight increase of 15 ms was observed. Onset of VES were observed 20 min after start of the infusion. After 30 min runs of non-sustained and sustained VT were recorded followed by VF after 45 min.

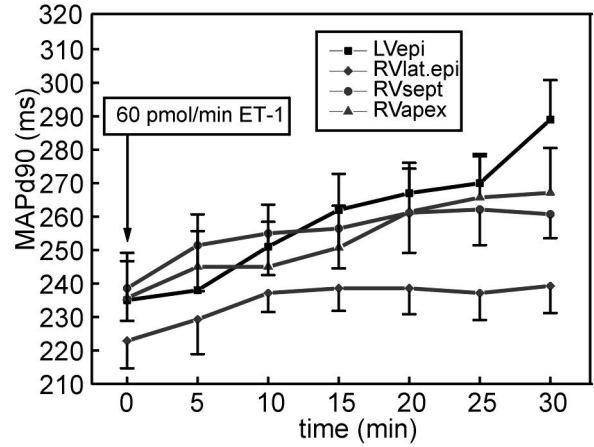


Figure 7. All dogs of group C show this spatial dispersion of MAP duration during infusion of ET-1. After 20 min the dispersion increased with time. 30 min after start of the infusion the dispersion attained its maximum. Afterwards the occurrence of frequent premature contractions and non-sustained VTs did not allow to measure MAP duration at 70 ppm due to the appearance of disturbing frequency-related artifacts.

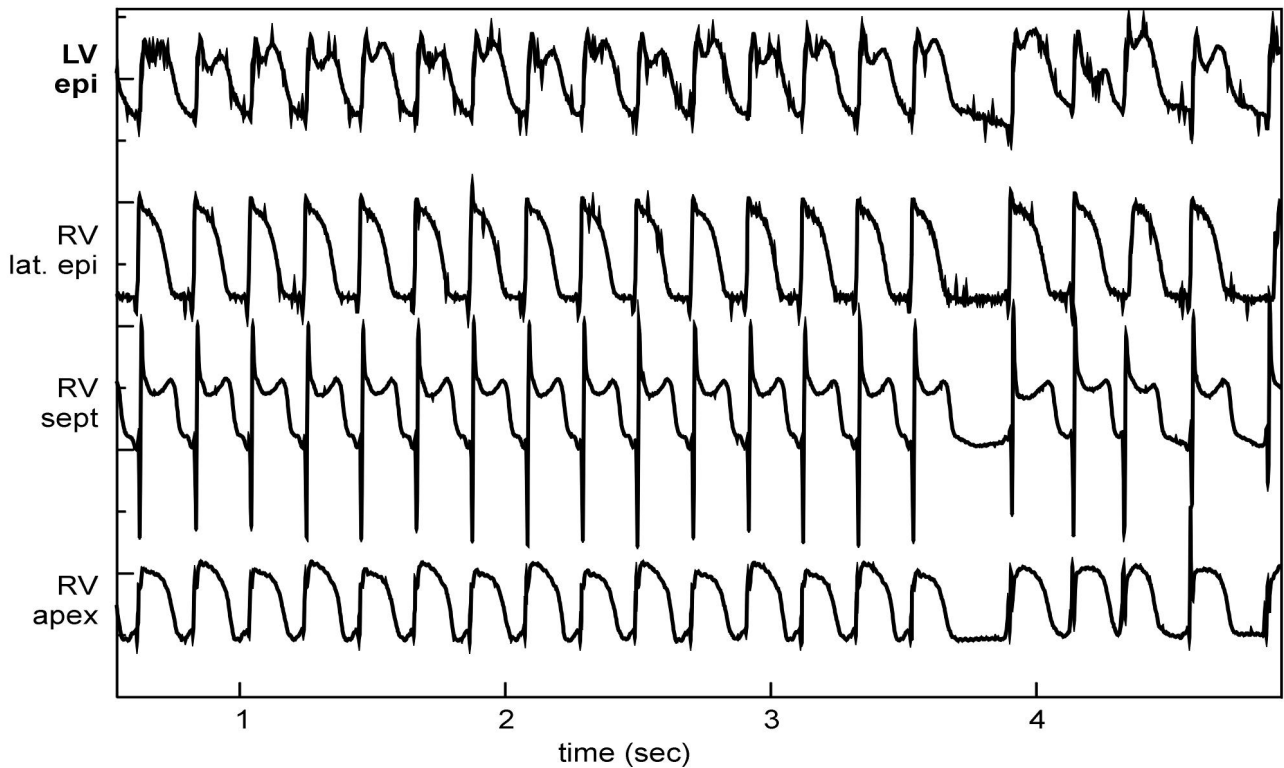


Figure 6. The digital four-channel MAP recording during an incessant sustained ventricular tachycardia.

taneously exist and either of them can play an important role in arrhythmia induction due to i.c. ET-1 administration [13-15].

In conclusion, the data of this study prove that low-dose i.c. ET-1 administration precipitates severe ventricular arrhythmias before signs of myocardial ischemia appear. ET-1 has a direct arrhythmogenic effect, too. Myocardial ischemia probably increases the arrhythmogenic properties of ET-1. The prolongation of the MAP duration and the development of afterdepolarization represent the most characteristic electrophysiologic effects of ET-1. Ventricular arrhythmias caused by ET-1 could be the prototype of rhythm disturbances leading to sudden cardiac death [11][12].

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