Multiple Monophasic Action Potential Recording during Ischemia and Intracoronary Endothelin-1 Infusion - Similarities and Differences

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Summary

Endothelin-1 (ET-1) a strong coronary constrictor peptide is supposed to have a direct arrhythmogenic property, however the latter effect has not been clearly proven yet. Our study was aimed to characterize the electrophysiologic changes during left anterior descending artery (LAD) occlusion and intracoronary (ic.) ET-1 infusion and to differentiate between the supposed direct and indirect (ischemic) arrhythmogenic actions of ET-1. Changes of monophasic action potential duration (e.g. $MAPD_{90}$) and upstroke velocity (UV) are capable of detecting both local ischemic changes and triggered activity. LV apical endocardial and anterior epicardial (LV_{ENDO}, LV_{EPI}) and RV lateral epicardial and anteroseptal endocardial (RV_{EPI}, RV_{ENDO}) MAPs were recorded by fractally coated BIOTRONIK MAP catheters. $MAPD_{90}$, MAP dispersion (MAP_{DISP}) and UV from different sites of the ventricles were determined at stimulation with 300 ms cycle length. In group A (n = 8) 30 min LAD occlusion was followed by a 60 min reperfusion period. In groups B and C ET-1 was administered into LAD at rates of 30 (n = 8) and 60 pmol/min (n = 10). Standard ECG, BP and CBF were monitored continuously. In group A after the LAD occlusion both $MAPD_{90}$ and UV decreased significantly in the LAD supplied region (LV_{EPI} and LV_{ENDO} 186 ± 7 and 196 ± 6 vs 152 ± 8 and 180 ± 5 ms, $p < 0.05$, and 2.9 ± 0.4 and 2.7 ± 0.3 vs 1.0 ± 0.2 and 1.3 ± 0.2 V/s, $p < 0.05$, - results are presented as control and 30 min values in all groups) whereas the increase of MAP_{DISP} remained unsignificant (18 ± 6 vs 28 ± 7 ms). No severe arrhythmias were noticed in this group [non-sustained VT (nsVT): 3 cases]. CBF decreased significantly in groups B and C ($DCBF_{30MIN}$ B: 21 ± 2%, $p < 0.05$; C: 35 ± 2%, $p < 0.05$). In group B both $MAPD_{90}$ and MAP_{DISP} increased significantly (LV_{EPI} and LV_{ENDO} 181 ± 2 and 184 ± 3 vs 201 ± 3 and 217 ± 8ms $p < 0.05$, MAP_{DISP}: 12 ± 4 vs 36 ± 6 ms, $p < 0.05$) whereas UV remained unchanged at the end of the infusion. Early afterdepolarizations (EADs) were present in 3 instances. In group C both $MAPD_{90}$ and MAP_{DISP} increased significantly (LV_{EPI} and LV_{ENDO} 188 ± 5 and 203 ± 3 vs 210 ± 13 and 255 ± 11 ms, $p < 0.05$, MAP_{DISP}: 15 ± 3 vs 48 ± 10 ms, $p < 0.05$) and UV decreased slightly, but not significantly in the LAD region. EADs were observed in 5 instances. Severe arrhythmias were observed in both groups [group B-C: nsVT 8 - 10, sustained VTs (sVT) 3 - 7, VF 2 - 8 cases]. The increases of $MAPD_{90}$ and MAP_{DISP} lasted until the appearance of polymorphic nsVTs and sVTs, thereafter both $MAPD_{90}$ and MAP_{DISP} decreased. MAP prolongation, increased MAP dispersion and development of EADs all contribute to the arrhythmogenic action of ET-1. The lack of the almost prompt decrease of UV and $MAPD_{90}$ in groups B and C which was observed in group A support the probability of the direct arrhythmogenic effect of ET-1.

Key Words

Cardiac electrophysiology, monophasic action potential, ventricular arrhythmias

Introduction

Endothelin-1 (ET-1) is an endothelium-derived potent vasoconstrictor, consisting of 21 amino acids. ET-1 secretion is stimulated by vascular stretch, shear stress, endothelial injury, sympathetic activation, impaired release of endothelium derived relaxing factor (EDRF), endotoxin and hypoxia [1]. Raised levels of ET-1 have been detected in myocardial infarction, chronic heart failure and cardiogenic shock. Besides

being a vasoconstrictor, ET-1 may also exhibit a primary arrhythmogenic property that is not solely attributable to myocardial ischemia. Earlier studies have shown the direct arrhythmogenic effect of ET-1 on isolated cardiac tissues [1,8]. Development of severe ventricular tachyarrhythmias were noticed on low dose intracoronary (ic.) and intrapericardial infusions of ET-1 [5-8]. The data of these investigations suggest that the arrhythmogenic effect of ET-1 may differ from the ischemic arrhythmogenesis, however direct evidence is still lacking. Therefore, we employed monophasic action potential (MAP) recording which is suitable for studying the characteristics of local myocardial repolarization and identifying the bases for triggered ventricular arrhythmias, moreover this method is one of the most informative and sensitive in vivo electrophysiological method to detect possible myocardial ischemia [1,2]. The aims of our study were

1) to compare a 30 min total LAD occlusion to two different doses of ic. administered ET-1 infusion and to determine the hemodynamic and electrophysiologic changes during the observation period and
2) to investigate the ischemic or direct pathomechanism of ET-1 arrhythmias.

Materials and Methods

General Preparation

Acute experiments were conducted on three groups of mongrel dogs (weight: 22.8 ± 0.5 kg). Each animal was initially anesthetized with sodium pentobarbital (30 mg/kg iv.), and additional anesthesia was given as needed to maintain a constant level. After endotracheal intubation the dogs were ventilated with room air. The right femoral artery was cannulated for monitoring arterial blood pressure and the left great saphenous vein was cannulated for infusion of intravenous fluids. Standard ECG leads were recorded throughout the experiment. Thoracotomy was performed in the 5th intercostal space and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery (LAD) was isolated and an electromagnetic flow probe was placed around the vessel. The coronary sinus (CS) was cannulated and 200 U/kg heparin sulfate was administered. In Group A a snare occluder was placed around the LAD under its first diagonal branch. In Groups B and C distal to the flow probe a 24G cannula was positioned in LAD to allow ic. infusion of ET-1 and saline. Continuous recordings of coronary blood flow (CBF), blood pressure (BP), heart rate (HR) and ECG were performed on a PC using Dasylab 4.0 software.

Experimental Protocol

After surgical instrumentation each animal underwent a 20 min equilibration period to ensure stability of the preparation, which was followed by a 20 min baseline period. At the end of the baseline period coronary blood flow, blood pressure, heart rate, ECG, endo- and epicardial MAPs were recorded, and PES was carried out.

Group A:

Effects of 30 min LAD occlusion and 60 min reperfusion were studied in 10 dogs. After the beginning of LAD occlusion, MAP parameters were determined in 5 min intervals by PES. The 30 min occlusion was followed by a 60 min reperfusion period, determination of MAP was again done in 5 minute intervals.

Groups B and C:

Effects of intracoronary ET-1 infusion were examined in 18 dogs. ET-1 was administered into LAD at 30 pmol/min (Group B, n = 8) and 60 pmol/min (Group C, n = 10) doses, respectively.

Intracoronary ET-1 infusion (30 pmol/min in Group B, 60 pmol/min in Group C) was started, MAP recording and PES followed the same protocol as in Group A. In the 30th min ET-1 infusion was stopped.

In the post ET-1 phase (60 min) MAP determination and PES were performed as in Group A.

Monophasic Action Potential Registration

Fractally coated monophasic action potential (MAP) catheters (YP-60-BP, Biotronik, Germany) were inserted through the right internal jugular vein into the apex of the right and left ventricle. Fractally coated epicardial MAP electrodes (MAPOX, Biotronik, Germany) were placed onto the left and right anterolateral epicardium. The duration of MAP was determined during programmed electrical stimulation (PES) (UHS 20 heart stimulator Biotronik, Germany) at 90% repolarization ($MAPD_{90}$) applying 30 stimuli at 300 ms cycle length and 0.5 ms pulse duration at a voltage of twice the ventricular threshold. Upstroke velocity (UV) was defined as the quotient of the depolarization amplitude and the depolarization duration of the MAP curve. MAP parameters were digitized by a twelve bit analogue to digital converter after being amplified and filtered and were stored on a PC.

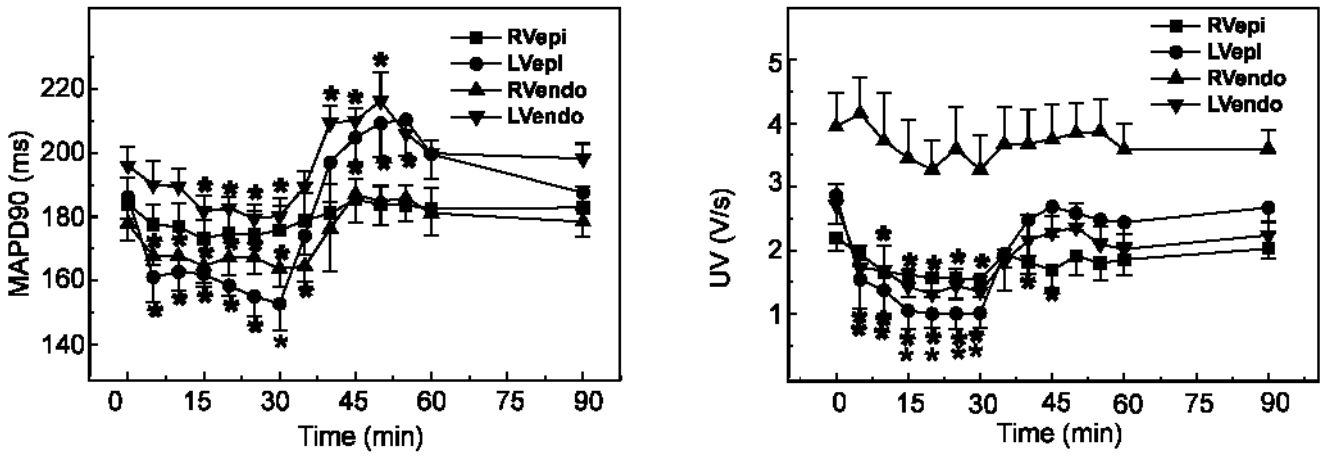

Figure 1. Changes of $MAPD_{90}$ (left) and UV (right) during 30 min LAD occlusion and 60 min reperfusion (= $p < 0.05$).*

Statistical analysis

All results are expressed as mean ± SEM. Differences between means in two groups at chosen time points were evaluated using analysis of variance for repeated measures. $p < 0.05$ was considered to be statistically significant.

Results

Effects of 30 min LAD ischemia and 60 min reperfusion

Coronary blood flow (CBF) ceased during the 30 min occlusion, a reactive hyperemic response (control vs. maximum: 34 ± 3 vs. 85 ± 12 ml/min, $p < 0.01$) was observed on release of the occlusion. CBF returned to normal value at 20 min of reperfusion. BP and HR did not change significantly. In the occlusion period some ventricular premature contractions (VPC) were observed in all dogs. Upon reperfusion VPC-s were noticed in all of the ten dogs and nsVT-s occurred in two instances. Significant ST-segment elevation (greater than 0.2 mV) was observed in all but one cases.

Left ventricular epicardial and endocardial (LV_{EPI} and LV_{ENDO}) and right ventricular endocardial (RV_{ENDO}) $MAPD_{90}$-s and LV_{EPI}, LV_{ENDO} and RV_{ENDO} upstroke velocities (UV) decreased significantly during the occlusion. RV_{EPI} upstroke velocity and MAP dispersion did not change significantly during the occlusion (Figure 1 and Table 1). Even 5 min after the release of the occlusion significant increase was observed in the $MAPD_{90}$-s of the LAD supplied area (Figure 1). This increase was significant compared to the baseline values, as well. By the end of the reperfusion all observed MAP parameters returned to the baseline values.

Effects of 30 pmol/min intracoronary ET-1 infusion

Coronary blood flow gradually decreased during the ic. ET-1 infusion (control vs. 30 min: 38 ± 4 vs. 30 ± 3 ml/min, $p < 0.05$), but returned to 90% of the control value at 60 min after terminating the infusion. BP and HR remained unaltered. Significant ST-segment elevation was observed only once. All dogs exhibited VPC-s in increasing numbers during the ET-1 infusion, two dogs had multiple nsVT-s. No VF was observed in the first 30 min. In the reperfusion period multiple nsVT-s occurred in two dogs, while in one animal multiple nsVT-s and sVT-s propagated into VF in the 9th min of reperfusion. In that dog CBF was virtually unaffected (control vs. 39 min: 55 vs. 53 ml/min) at the onset of VF.

LV_{EPI}, LV_{ENDO}, and RV_{ENDO} $MAPD_{90}$ values and MAP dispersion increased significantly, whereas RV_{EPI} $MAPD_{90}$ values and upstroke velocities did not change significantly during ET-1 infusion even in the LAD supplied area. After terminating the infusion $MAPD_{90}$ values returned to initial value by the 30th min (Figure 2 and Table 1). Third phase early afterdepolarizations (EAD) were observed in two cases (Figure 4).

Effects of 60 pmol/min intracoronary ET-1 infusion

Coronary blood flow gradually decreased during the ic. ET-1 infusion (control vs. 30 min: 40 ± 3 vs. 27 ± 4 ml/min, $p < 0.05$) and increased only slightly after ter-

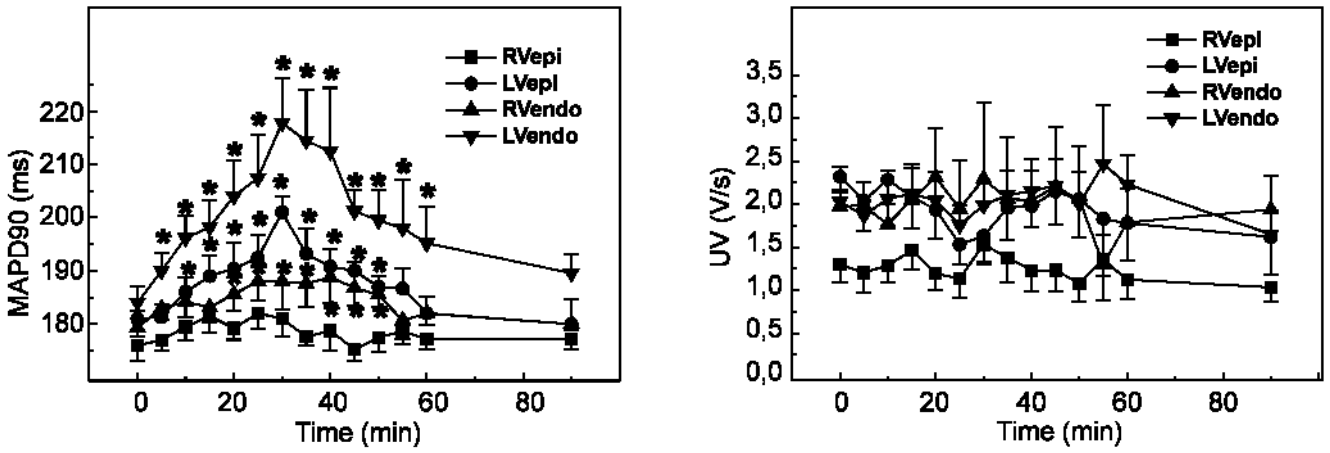

Figure 2. Changes of $MAPD_{90}$ (left) and UV (right) during and after 30 pmol/min ET-1 infusion (= $p < 0.05$).*

minating the infusion. In two instances CBF did not change throughout the ET-infusion despite the observed severe ventricular arrhythmias. BP and HR were unaltered.

Significant ST-segment elevations were observed in three instances. Multiple VPC-s and multiple nsVT-s were observed in all dogs. In the post ET-1 phase the arrhythmias worsened in all of the remaining seven animals. Multiple nsVT-s occurred in all, sVT-s in seven dogs. Five dogs fibrillated in the first 10 min after discontinuing the infusion, whereas other three dogs died of VF in the next 10 min.

LV_{EPI}, LV_{ENDO}, RV_{ENDO} $MAPD_{90}$ and MAP dispersion increased significantly during the ET-1 infusion. Upstroke velocities tended to decrease but failed to reach significance (Figure 3 and Table 1) Presence of EAD-s were noticed in 5 instances (Figure 4).

Discussion

In this study we aimed to compare the effects of a total LAD occlusion to low dose ic. ET administration and we wanted to determine the efficacy of monophasic action potential recording in differentiating between the ischemic and proposed direct arrhythmogenic effect of ET-1.

Monophasic action potential recording provides a highly sensitive measure of localized myocardial ischemia. The MAP registers electrical events in the immediate proximity of the exploring electrode and is

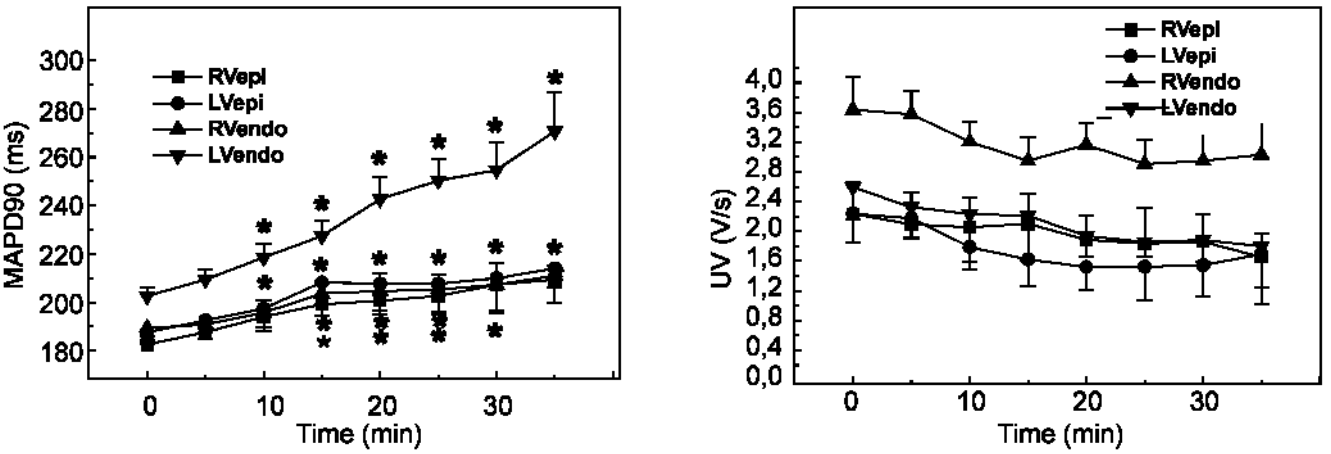

Figure 3. Changes of $MAPD_{90}$ (left) and UV (right) during and after 60 pmol/min ET-1 infusion (= $p < 0.05$).*

	MAPD$_{90}$ (ms)			UV (V/s)		
Group A: 30 min LAD occlusion						
Time	**0 min**	**30 min**	**90 min**	**0 min**	**30 min**	**90 min**
RV epicardial	184±8	176±8	182±7	2.2±0.2	1.5±0.3**	2.0±0.2
LV epicardial	186±7	151±8**	187±9	2.9±0.5	1.0±0.2**	2.7±0.2
RV endocardial	177±5	163±5*	178±4	3.9±0.5	3.3±0.5	3.6±0.3
LV endocardial	196±6	180±5**	198±5	2.70±.3	1.3±0.2**	2.2±0.2
MAP dispersion	18±6	28±7	19±5			
Group B: ET-1 30 pmol/min						
Time	**0 min**	**30 min**	**90 min**	**0 min**	**30 min**	**90 min**
RV epicardial	176±3	181±3	177±2	1.3±0.2	1.5±0.3	1.1±0.2
LV epicardial	181±2	206±3**	180±5	2.8±0.1	2.6±0.3	2.6±0.4
RV endocardial	176±3	181±3	177±2	3.0±0.5	3.2±0.5	2.9±0.4
LV endocardial	184±3	217±8**	189±4	2.0±0.1	2.0±0.3	1.7±0.3
MAP dispersion	12±4	36±6**	12±5			
Group C: ET-1 60 pmol/min						
Time	**0 min**	**30 min**	**35 min**	**0 min**	**30 min**	**35 min**
RV epicardial	182±4	207±9*	209±6*	2.2±0.4	1.9±0.4	1.7±0.3
LV epicardial	188±5	210±13**	214±8**	2.2±0.4	1.5±0.4	1.6±0.6
RV endocardial	189±5	207±10*	211±11*	3.6±0.4	3.0±0.3	3.0±0.4
LV endocardial	203±3	255±11**	270±16**	2.6±0.4	1.9±0.3	1.8±0.5
MAP dispersion	15±3	48±10**	61±15**			

* p<0.05; ** p<0.01

Table 1. Changes of electrophysiological parameters during LAD occlusion (group A) and ET-1 infusion (groups B and C).

thereby a measure of very early localized changes. Fractally coated electrodes were proven to be suitable tools for recording monophasic action potentials not only in experimental settings but in clinical circumstances, as well. Ischemia has been shown to have several effects on the action potential, namely shortening of the duration, loss of amplitude and diastolic potential and reduction in maximum UV [7-10].

In our study clear ischemic circumstances were created by total LAD occlusion. Both UV and MAPD$_{90}$ decreased significantly in the LAD area indicating ischemia almost immediately after the occlusion. After the release of LAD occlusion the MAPD$_{90}$ values increased significantly in the LAD supplied area, which may be due to the release of endogenous repolarization prolonging factors. In earlier studies the role of endothelin release in reperfusion was proven. Endogenous ET-1 release during reperfusion presumably contributes to the observed MAP-prolongation, but this phenomenon needs to be further investigated. In this group the significant UV and MAPD$_{90}$ decrease preceded the onset of VPC-s and nsVT-s in all instances. Contrary to these findings in the endothelin groups (group B and C) MAPD$_{90}$-s increased significantly in the LAD supplied area, but UV-s did not change significantly. In these groups the significant MAPD$_{90}$ increase and the unaltered UV preceded the development of nsVTs and sVTs in all cases. The significant increase of MAP dispersion which was observed in both endothelin groups suggests different electrophysiological circumstances in both chambers and even in endo- and epicardium. The alteration of MAP dispersion possibly contribute to the arrhythmogenic action of ET-1.

Ventricular premature contractions occurred in the first 10 min after starting the ET-infusion followed by nsVT-s between 20 and 30 min. Severe sVT-s and VF appeared after discontinuing the infusion. Ischemic hemodynamic- and electrophysiologic-alterations were not present in the endothelin group at the onset of the arrhythmias. The different features of MAP changes during LAD occlusion and endothelin infusion in our study strongly support the postulated direct arrhythmogenic effect of ET-1, as well.

In summary, MAP recording was found to be suitable method in monitoring the precise location and extent

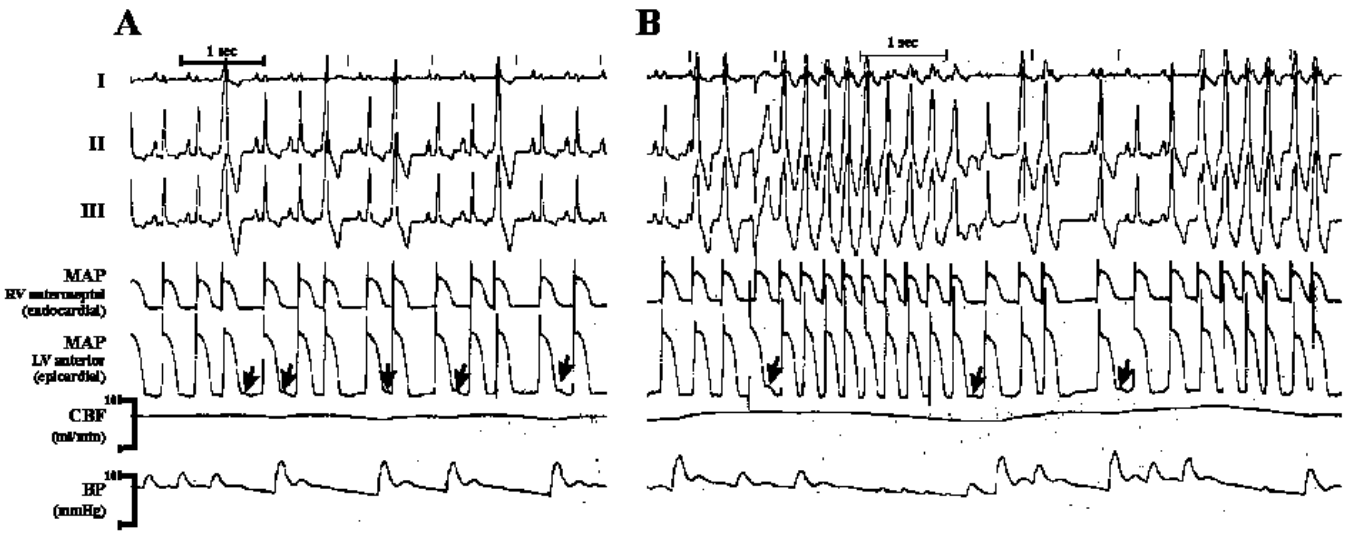

Figure 4. Part A shows VES-s based on triggered activity. Arrows show EADs. Part B depicts a non sustained VT which is presumably based on triggered activity, as well.

of myocardial ischemia, moreover enabled us to determine the patho-mechanism of the arrhythmias. The most characteristic features at the onset of ET-arrhythmias were: slightly decreased CBF, unchanged UV-s and significantly increased $MAPD_{90}$ in the infused region. Low dose ET-1 administration precipitated severe ventricular arrhythmias before signs of myocardial ischemia appeared. At the onset of arrhythmias neither ischemic ECG signs with MAP changes nor significantly reduced CBF were present. The ET-1 induced major ventricular arrhythmias were not proportional to the observed - comparatively moderate - CBF changes. The increased MAP dispersion may have important role in the patho-mechanism of ET-1 induced ventricular arrhythmias, since it may contribute to the development and maintenance of both triggered and reentry based arrhythmias. The patho-mechanism of ET-induced and ischemic arrhythmias presumably differ, but they may complement each other under certain pathologic conditions.

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