Expressed monophasic action potential alternans before the onset of ventricular arrhythmias induced by intracoronary bolus administration of endothelin-1 in dogs

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ABSTRACT
We showed previously a direct arrhythmogenic effect of the intracoronary infusion of endothelin-1 (ET-1). We aimed to examine the electrophysiological effects of intracoronary bolus administration of ET-1 using monophasic action potential (MAP) recordings. Eight mongrel dogs received boli of ET-1 (1 and 2 nmol) into the left anterior descending coronary artery. These intracoronary ET-1 boli rapidly caused a marked decrease in coronary blood flow (1 nmol, $78 \pm 7\%$; 2 nmol, $89 \pm 7\%$). Ischaemic changes of MAP morphology, a decrease in upstroke velocity (baseline, $1.78 \pm 0.2$ V/s; 1 nmol, $0.95 \pm 0.18$ V/s; 2 nmol, $0.45 \pm 0.21$ V/s; $P < 0.01$) and a decrease in MAP duration at 90\% repolarization (MAPD$_{90}$) [1 nmol, from $191 \pm 3$ to $176 \pm 5$ ms ($P < 0.05$); 2 nmol, from $212 \pm 4$ to $180 \pm 8$ ms ($P < 0.05$)] occurred after ET-1 bolus administration. However, at 7–10 min after the 1 nmol bolus, a significant increase in MAPD$_{90}$ was observed (10 min, in the left ventricular anterior epicardial region: from $191 \pm 3$ to $206 \pm 6$ ms; $P < 0.05$). The incidence of ventricular arrhythmias was as follows: after the 1 nmol ET-1 bolus: ventricular tachycardia, 3/8 animals; ventricular fibrillation, 1/8; after the 2 nmol ET-1 bolus: ventricular tachycardia, 5/7; ventricular fibrillation, 5/7. MAP alternans was present in each animal (1 nmol, $18.2 \pm 5.8\%$; 2 nmol, $10.8 \pm 2.5\%$). Thus electrophysiological and coronary blood flow changes indicate the predominance of an ischaemic arrhythmogenic effect of the bolus administration of ET-1 (shortening of action potential duration; appearance of MAP alternans), whereas the observed delayed prolongation of MAPD$_{90}$ suggests a direct arrhythmogenic effect of ET-1. The expressed MAP alternans could have a pathogenic role in the onset of ventricular arrhythmias induced by an intracoronary bolus of ET-1.

INTRODUCTION
Endothelin-1 (ET-1), the best characterized isoform of a group of small endogenous peptides called the ETs, displays a potent and long-lasting coronary vasoconstrictory effect, and produces an increase in cardiac contractility via specific myocardial binding sites. The intracoronary bolus administration of ET-1 has been shown to induce severe ventricular tachyarrhythmias, associated with myocardial ischaemia [1–3]. We have demonstrated previously a direct arrhythmogenic effect of low-dose intracoronary infusion of ET-1 [4–6]. The

Key words: cardiac electrophysiology, monophasic action potential, ventricular arrhythmia.
Abbreviations: CBF, coronary blood flow; EAD, early afterdepolarization; ET-1, endothelin-1; LV$_{endo}$, left ventricular apical endocardial; LV$_{epi}$, left ventricular anterior epicardial; MAP, monophasic action potential; MAPD$_{90}$, monophasic action potential duration at 90\% repolarization; RV$_{endo}$, right ventricular apical endocardial.
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development of multifocal ventricular tachycardias was based on action potential prolongation and the occurrence of early after-depolarizations (EADs). However, the electrophysiological effects of the intracoronary bolus administration of ET-1 have not been studied.

In the present study we aimed to investigate the electrophysiological and arrhythmogenic effects of high-dose (1 and 2 nmol) intracoronary bolus administration of ET-1 using monophasic action potential (MAP) recordings.

MATERIALS AND METHODS

A total of eight mongrel dogs were anaesthetized with sodium pentobarbital (30 mg/kg, intravenous) and ventilated with humidified room air. Thoracotomy was carried out in the fifth intercostal space, and then the pericardial sac was opened and the left anterior descending artery was isolated. Mean arterial blood pressure was measured through a right femoral artery cannula, and coronary blood flow (CBF) was measured with an electromagnetic flowmeter placed on to the left anterior descending artery. Standard ECG leads were recorded throughout the study.

All animals received human care in compliance with the Principles of Laboratory Animal Care issued by the National Academy of Sciences and published by the National Society of Medical Research and the Guide for the Principles of Laboratory Animal Care issued by the National Institutes of Health (NIH Publication No. 86-23, revised 1996).

Left ventricular apical endocardial (LV endo) and right ventricular apical endocardial (RV endo) MAPs were recorded through a fractally coated electrode (Alcath 4F; Biotronik G.m.b.H.) [7]. A screw-in electrode (V-177; Biotronik) was used to record left ventricular anterior epicardial (LV epic) MAP signs. The analogue MAP signals were recorded on a 12-channel direct chart recorder (Madaus Schwarzer CU 12) and the digitized signals were stored on a PC. QT time and MAP duration at 90% repolarization (MAPD90) were determined at 300 ms cycle length, with stimulation applied for 10 ms (UHS 20 heart stimulator; Biotronik). Only data from recordings with a stable resting potential were included in the data analysis. Upstroke velocity was defined as the quotient of the depolarization amplitude and the depolarization duration of the MAP curve.

After the registration of baseline haemodynamic and electrophysiological values, an intracoronary bolus of 1 nmol of ET-1 was administered into the left anterior descending artery. In the seven dogs that survived the 1 nmol bolus, a 2 nmol ET-1 bolus was given 25 min after the first bolus, following stabilization and the disappearance of ischaemic ECG and MAP changes.

Data are expressed as means ± S.E.M. Comparisons within the groups were analysed by ANOVA for repeated measurements. Student’s paired t test was used to study changes in different variables following ET-1 administration. Significance was established at \( P < 0.05 \).

RESULTS

Both the 1 nmol and 2 nmol ET-1 boli caused a rapid decrease in CBF [max. decrease in CBF before the onset of tachyarrhythmias: 1 nmol, 78 ± 7% \( (P < 0.001) \); 2 nmol, 89 ± 7% \( (P < 0.005) \)] (Figure 1). In parallel, significant ST changes on ECG lead II were observed [max. change in ST: 1 nmol, 0.15 ± 0.04 mV \( (P < 0.005) \); 2 nmol, 0.18 ± 0.05 mV \( (P < 0.005) \)]. The maximal decrease in CBF and the maximal ST changes were seen 0.5–3 min after ET-1 bolus administration with both the 1 and 2 nmol doses.

The 1 nmol ET-1 bolus led to ischaemic changes in MAP morphology in all cases. For LV epic MAP signals, a decrease in upstroke velocity, with a maximum effect 9–10 min after the ET-1 bolus (baseline, 1.78 ± 0.2 V/s; 10 min, 0.95 ± 0.18 V/s; \( P < 0.01 \)) and a decrease in MAPD90 0.5–3 min after ET-1 bolus administration (baseline, 191 ± 3 ms; 2 min, 176 ± 5 ms; \( P < 0.05 \)) (Figure 1) were observed. This was followed by an increase in MAPD90 between 7 and 10 min (baseline, 191 ± 3 ms; 10 min, 206 ± 6 ms; \( P < 0.05 \)) (Figure 1). A continuous increase in MAPD90 was observed in LV endo, MAP, while MAPD90 in the RV endo region remained unchanged following ET-1 administration (Figure 1).

Administration of a 2 nmol ET-1 bolus led to a significant decrease in upstroke velocity in the LV epic MAP signals, with a maximum effect in the 1.5 min after bolus administration (baseline, 1.78 ± 0.2 ms; 1.5 min, 0.45 ± 0.21 V/s; \( P < 0.005 \)). MAPD90 was decreased sig-

Figure 1 Changes in MAPD90 and CBF following bolus administration of 1 nmol and 2 nmol of ET-1 ic., intracoronary.
DISCUSSION

Since its discovery [8], several reports have been published on the arrhythmogenic effects of ET-1 [1–3,9]. The first results referred to the secondary arrhythmogenic effect of ET-1 due to strong coronary vasoconstriction and ischaemia [1–3,9].

MAP recording is one of the most sensitive in vivo electrophysiological methods for the detection of local myocardial ischaemia [10,11]. Changes in MAP duration in the ischaemic area are thought to occur in two ways [12]: the more usual shortened action potential duration of ischaemic cells, and an alternans of action potential in ischaemic cells. Various clinical and experimental data suggest a relationship between alternation of action potential duration of the ischaemic myocardium and ventricular arrhythmias [13–15]. Induction of short-term ischaemia in dogs showed that alternans of action potential recorded from the ischaemic centre zone preceded ventricular fibrillation in 95% of cases [16]. The suggestion is that action potential duration alternans, causing inhomogeneities of ventricular repolarization in disparate myocardial regions, sets up the conditions for ventricular arrhythmias. Such differences in action potential duration could induce a regional conduction block due to differential refractoriness, or could result in a flow of injury current, both of which may facilitate ventricular arrhythmias. Ischaemic action potential duration alternans, reflecting cellular repolarization abnormalities, is probably the major cause of ischaemic T-wave alternans [17]. In one experimental study, severe conduction delay occurred concomitantly with episodes of alternans of action potential or ST segment elevation preceding ventricular arrhythmias, suggesting that re-entry is the mechanism of arrhythmia [18]. In another study that examined the role of ST alternans in the occurrence of ventricular fibrillation after reperfusion in dogs, the magnitude of ST alternans increased progressively until ventricular fibrillation occurred. Activation of ventricular premature complexes resulted in conduction block and formed re-entry, leading to the conclusion that ST alternans related to ventricular fibrillation by facilitating the formation of a re-entrant circuit [19].

In the present study, ET-1 caused a decrease in CBF immediately after bolus administration; this was followed by severe ischaemic ECG and MAP changes, shortening of the MAP duration and a decrease in the upstroke velocity in the LV_eqi region. The observed biphasic effect of the administration of a 1 nmol bolus of ET-1 on LV_eqi MAPD_90 could be the result of a marked epicardial ischaemic MAPD_90 decrease, followed by an increase in MAPD_90 due to a direct electrophysiological effect of ET-1. The continuous lengthening of MAP signals observed in the LV_endo region following the 1 nmol ET-1 bolus could be the result of dominant direct electrophysiological effects of ET-1. However, severe ventricular tachyarrhythmias, based mainly on an ischaemic arrhythmogenic effect, were observed after the 2 nmol ET-1 bolus.

In our previous study, the appearance of significant ventricular arrhythmias, induced by 30-min intracoronary infusion of ET-1 (60 pmol/min), was observed without ischaemic MAP and ECG changes [5]. ET-1 infusion for 30 min did not lead to ischaemic MAP or ECG changes. The lengthening of MAP duration and

**Figure 2** MAP and T-wave alternans
aVR, augmented unipolar right.

- **Figure 2** MAP and T-wave alternans
  aVR, augmented unipolar right.
expressed EAD formation were due to the direct arrhythmogenic effect of ET-1. The total dosage of the infusion was comparable with that applied as a bolus in the present study.

Expressed MAP alternans was present inside the ischaemic zone following the ET-1 bolus; however, MAP alternans did not occur during intracoronary ET-1 infusion. MAP alternans was observed after the appearance of ischaemic electrophysiological signs, just before the onset of serious ventricular tachyarrhythmias. The appearance of T-wave alternans was well correlated with the occurrence of MAP alternans, suggesting that T-wave alternans was due to changes in the MAP configuration.

In conclusion, electrophysiological (shortening of action potential duration; appearance of MAP alternans) and CBF changes suggest the predominance of an ischaemic (indirect) arrhythmogenic effect of a bolus administration of ET-1, whereas the observed delayed prolongation of MAPD_{50} suggests a direct arrhythmogenic effect of ET-1. MAP alternans caused by ET-1 has been demonstrated for the first time in this model. This well expressed MAP alternans could have a pathogenic role in the onset of ventricular arrhythmias induced by an intracoronary bolus of ET-1. Theoretically, in pathophysiological conditions, both mechanisms of arrhythmia may play a role in the development of severe life-threatening ventricular tachyarrhythmias.

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REFERENCES