



## Rapid communication

## Local conduction during acute myocardial infarction in rats: Interplay between central sympathetic activation and endothelin

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## ABSTRACT

We investigated the effects of autonomic dysfunction and endothelin on local conduction and arrhythmogenesis during myocardial infarction. We recorded ventricular tachyarrhythmias, monophasic action potentials, and activation sequences in wild-type and  $ET_B$ -deficient rats displaying high endothelin levels. Central sympathetic inputs were examined after clonidine administration. Clonidine mitigated early and delayed arrhythmogenesis in  $ET_B$ -deficient and wild-type rats, respectively. The right ventricular activation delay increased in clonidine-treated  $ET_B$ -deficient rats and slightly decreased in wild-type rats. The left ventricular voltage rise decreased in all groups, whereas the activation delay increased mainly in clonidine-treated  $ET_B$ -deficient rats. Central sympathetic activation and endothelin modulate ischemia-induced arrhythmogenesis. Ischemia alters excitability, whereas endothelin impairs local conduction, an action partly counterbalanced by central sympathetic activity.

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## 1. Introduction

Abnormal local conduction is a key mechanism of arrhythmogenesis, often complicating acute myocardial infarction (MI) [1]. The accompanying autonomic dysfunction, modulated by the release of endothelin-1 (ET-1) [2], exerts a prominent role [3], but their relative effects on the electrophysiological milieu remain unclear [4].

Here, we recorded ventricular tachyarrhythmias (VTs), monophasic action potentials (MAPs) and local activation sequences in rats. To identify the effects of central sympathetic activity, we used the “subtraction-model” offered by clonidine, whereas the effects of ET-1 were examined in  $ET_B$ -deficient rats.

## 2. Material and methods

Sustained inhibition of sympathetic preganglionic neurons was induced by clonidine, administered at 0.5 mg/kg in wild-type and  $ET_B$ -deficient rats 1 h prior to MI; the latter well-characterized rat-strain, kindly provided by Professor M. Yanagisawa (University of Tsukuba, Japan), displays 10-fold higher ET-1 levels [5]. The study protocol conformed to EU Directive 2010/63/EU, and was approved by the regulatory authorities (6003, 19/04/2013).

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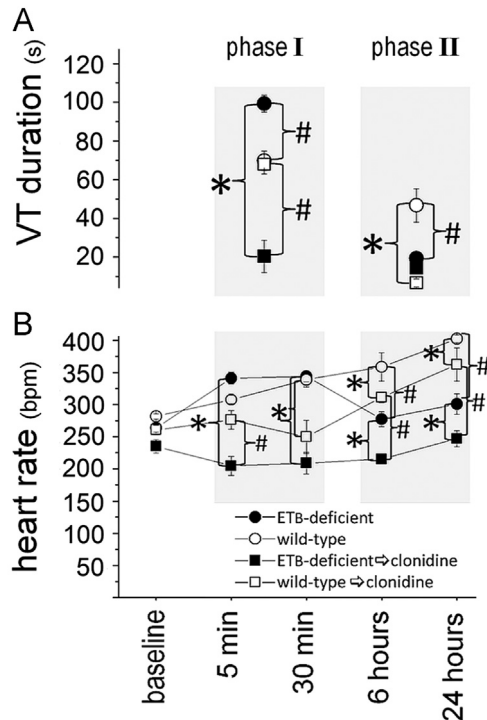
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Under 2.5% sevoflurane anesthesia, exerting minimal effects on sympathetic activation [6], acute MI was induced by ligation of the mid-left coronary artery. VTs were recorded by implanted telemetry transmitters in conscious rats ( $n=24$ ) for 24 h, and infarct size was measured at the end of the experiment, as previously [7]. No major bleeding occurred during surgery. In another similar animal cohort ( $n=32$ ), MAPs were recorded from the anterolateral left ventricular (LV) and right (RV) ventricular epicardium; voltage rise ( $dV/dt_{max}$ ) was measured, providing information on sodium-channels. Measurements were carried out in anesthetized animals (as above) at baseline and at 5 and 30 min (phase I) and at 6 and 24 h (phase II) post-ligation. Unipolar electrograms were recorded from the LV and RV with a 32-electrode array, in reference to Wilson's central terminal. We measured RR intervals and activation delay, defined as the time difference between the onset of the first and last electrograms in each recording.

Variables are reported as mean  $\pm$  standard error of the mean. Differences were assessed with one-way analysis of variance (ANOVA) or with ANOVA for repeated measures, as appropriate, followed by Duncan's test. Significance was set at  $p < 0.05$ .

## 3. Results

Compared to the respective untreated groups, the VT duration was shorter in clonidine-treated  $ET_B$ -deficient rats during phase I and in wild-type rats during phase II (Fig. 1A). Heart rate (HR)



**Fig. 1.** Ventricular tachyarrhythmias and heart rate. Ventricular tachyarrhythmias post-ligation (A): note the effects of clonidine in ET<sub>B</sub>-deficient rats during phase I, and in wild-type rats during phase II. Also note the blunted heart rate response after clonidine, particularly in ET<sub>B</sub>-deficient rats (B). Asterisks denote significant differences in comparisons between treated and untreated rats of the same strain, illustrating the effects of central sympathetic activation. Hash tags denote significant differences in comparisons between similarly treated wild-type and ET<sub>B</sub>-deficient rats, illustrating the effects of endothelin.

increased in both untreated groups, but this response was blunted after clonidine, mainly in ET<sub>B</sub>-deficient rats (Fig. 1B). Infarct size (as percent of the LV) was almost identical in the four groups ( $30.8 \pm 1.7\%$  and  $31.2 \pm 1.4\%$  in untreated ET<sub>B</sub>-deficient and wild-type rats, and  $30.3 \pm 1.9\%$  and  $29.7 \pm 1.1\%$  in clonidine-treated groups, respectively).

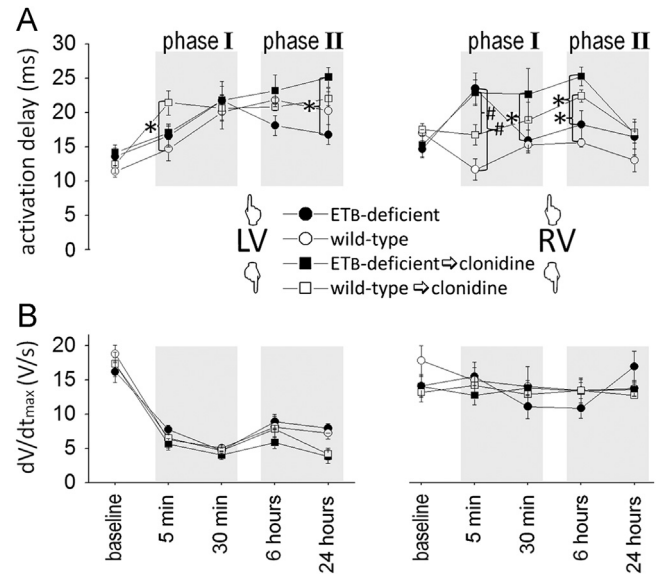
RV activation delay decreased marginally ( $p=0.080$ ) from baseline in untreated wild-type rats at 5 min post-ligation, but remained unchanged in clonidine-treated wild-type rats throughout the observation period. In contrast, it increased in untreated ET<sub>B</sub>-deficient rats at 5 min, and in clonidine-treated ET<sub>B</sub>-deficient rats at 5 min, 30 min, and 6 h. The LV  $dV/dt_{max}$  decreased equally in both groups, with or without clonidine treatment, whereas the RV  $dV/dt_{max}$  remained stable (Fig. 2).

The LV activation delay increased from baseline in clonidine-treated and untreated wild-type rats, although this effect was delayed in the latter group. Similar values were found in clonidine-treated and untreated ET<sub>B</sub>-deficient rats at 5 min and 30 min, but there was a trend ( $p=0.091$ ) toward higher values in clonidine-treated ET<sub>B</sub>-deficient rats at 6 h, reaching significance at 24 h (Fig. 3).

#### 4. Discussion

Despite the importance of local conduction properties in arrhythmogenesis, the factors regulating its course during acute MI remain poorly defined. We demonstrate intricate effects of central sympathetic inputs and ET-1 on activation delay and VTs during a 24-h observation period.

Early-phase VTs decreased after clonidine in ET<sub>B</sub>-deficient rats, with a delayed effect observed in wild-type animals. The corresponding HR



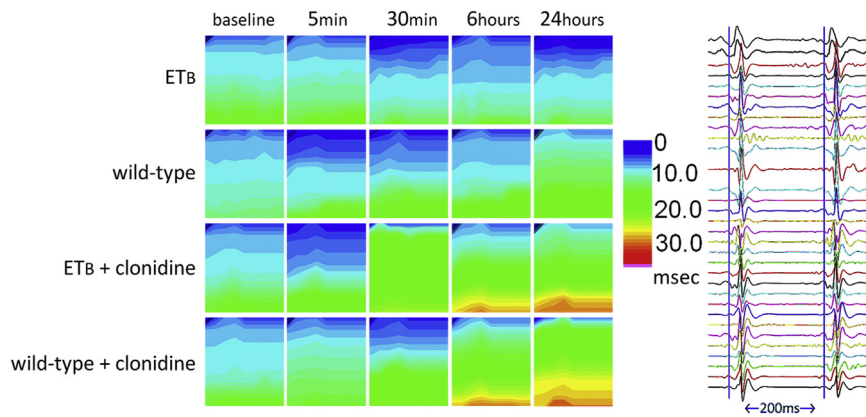
**Fig. 2.** Conduction delay and voltage rise. Conduction delay (A) in the left ventricle (left panel): note the effects of clonidine in ET<sub>B</sub>-deficient rats during the delayed phase. In right ventricular recordings (right panel), note the early difference between untreated ET<sub>B</sub>-deficient and wild-type rats, as well as the effects of clonidine in the two groups at 30 min and 6 h. See text for discussion. No differences were seen in  $dV/dt_{max}$  (B) in the left (right panel) or the right (right panel) ventricle. Asterisks denote significant differences in comparisons between treated and untreated rats of the same strain. Hash tags denote significant differences in comparisons between similarly treated wild-type and ET<sub>B</sub>-deficient rats. LV, left ventricle; RV right ventricle.

changes reiterate the proposed interaction between central inputs and ET-1 [2], affecting ischemia-induced arrhythmogenesis [4]. Using activation mapping, we found subtle decreases of conduction delay in the RV myocardium of untreated wild-type rats during phase I, as opposed to stable values in their clonidine-treated counterparts. This finding indicates minor effects of central sympathetic inputs on conduction velocity, and compares favorably with an 8% increase after left stellate ganglion stimulation, previously reported in the non-ischemic canine myocardium [8]. Although such action was also evident in ET<sub>B</sub>-deficient rats, it was counteracted by conduction delays, indicating the modulating effects of ET-1. The absence of differences in  $dV/dt_{max}$  argues against noticeable effects of ET-1 on sodium current, implicating gap junctions, another major determinant of conduction. This view is consistent with the impaired conduction caused by gap junction remodeling observed in rat ventricular cardiomyocytes after incubation with ET-1 [9].

In the LV myocardium, the activation delay was prolonged in wild-type rats post-MI, accompanied by a marked decrease in  $dV/dt_{max}$ . This time course, similar to that reported in dogs [10], is secondary to the well-described effect of ischemia on excitability, whereas small differences after clonidine suggest only a minimal influence by autonomic inputs.

The mechanisms underlying arrhythmogenesis during evolving MI are more complex. We have presented evidence supporting a role of central sympathetic activation [11], but the similar conduction delay seen in our untreated and clonidine-treated wild-type rats refutes major effects on conduction; alternative mechanisms such as repolarization dispersion appear to be more likely [12].

Distinct responses to clonidine were observed in the LV conduction delay in ET<sub>B</sub>-deficient rats, with higher values in treated animals during the delayed phase, further indicating modulation of sympathetic inputs by ET-1. Nonetheless, this pattern raises the possibility of additional mechanisms, with the transient outward potassium current appearing as a potential candidate; this current,



**Fig. 3.** Conduction maps. Examples of recordings (right panel) and representative isochronal conduction maps from the left ventricular myocardium in the four groups (left panel).

implicated in conduction, is regulated by ET-1 and catecholamines and warrants further study. Future work should also assess intramural conduction and repolarization abnormalities, as well as activation patterns during eccentric depolarization, as in that arising from premature beats.

In summary, our experiments demonstrate altered excitability and local conduction during ischemia. ET-1 modulates arrhythmogenesis and impairs local conduction, an action partly counterbalanced by concurrent central sympathetic activation.

#### Conflicts of interest

All authors declare no conflict of interest related to this study.

#### Acknowledgment

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