- *short communication* -

Intracerebroventricular endothelin receptor-A blockade in rats decreases phase-II ventricular tachyarrhythmias during acute myocardial infarction

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Short title: Central ETA-blockade in rats decreases ischemic arrhythmias

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Summary

Endothelin alters central sympathetic responses, but the resultant effects on arrhythmogenesis are unknown. We examined ventricular tachyarrhythmias after endothelin receptor-A blockade in the brain of Wistar rats with acute myocardial infarction. For this aim, BQ-123 (n=6) or phosphate-buffered saline (n=6) were injected intracerebroventricully. After 10min, the left coronary artery was ligated, followed by implantation of telemetry transmitters. Electrocardiography and voluntary activity (as a surrogate of acute left ventricular failure) were continuously monitored for 24h.

Infarct-size was similar in the two groups. There were fewer episodes of ventricular tachyarrhythmias of shorter average duration in treated rats, leading to markedly shorter total duration (12.3±8.9s), when compared to controls (546.2 ±130.3s). Voluntary activity increased in treated rats during the last hours of recording, but bradyarrhythmic episodes were comparable between the two groups.

Endothelin receptor-A blockade in the brain of rats decreases the incidence of ventricular tachyarrhythmias post-ligation, without affecting bradyarrhythmic episodes. These findings call for further research on the pathophysiologic role of endothelin during acute myocardial infarction.

Key words

Myocardial infarction; brain; endothelin; A-receptor; ventricular tachyarrhythmias

Acute myocardial infarction (MI) is often complicated by ventricular tachyarrhythmias (VTs) that occur during the early stage of ischemia, or subsequently, during evolving MI (Kolettis, 2013). The latter VTs, referred to as phase-II arrhythmogenesis, have an ominous prognosis not only in the out-of-hospital setting, but also in hospitalized patients (Piccini *et al*. 2008).

Central sympathetic activation, occurring frequently during acute MI, contributes to the genesis of phase-II VTs (Kolettis, 2018). Despite continuing research on this topic, the underlying pathophysiology remains incompletely understood. Endothelin-1 (ET-1) has attracted considerable interest in this regard, with its actions as a mediator of central sympathetic responses reported shortly after its discovery (Ouchi *et al*. 1989). For instance, intracerebroventricular (i.c.v.) injections of ET-1 in rats were shown to alter heart rate (HR) and plasma catecholamines (Kuwaki *et al*. 1997). These actions are likely exerted via ET$_A$-receptors, which are abundantly located in central neurons (Mosqueda-Garcia *et al*. 1993). The present study, performed in the rat-model of acute MI, explored the hypothesis that ET$_A$-receptor blockade in the brain decreases the incidence of phase-II VTs.

The experiments were conducted on 12 Wistar-rats (258±7g), housed under optimal conditions. The study was approved by the institutional ethics' committee and adheres to the European guidelines on laboratory animal care. The rats were mechanically ventilated at 85breaths/min and anesthetized with 2.5%-sevoflurane, a regimen not affecting autonomic responses (Kurosawa *et al*. 1989). They were placed on a stereotaxic frame (*David Kopf*, CA, USA), and 10μl of phosphate-buffered saline (PBS) was slowly (10min) injected i.c.v. via a Hamilton-needle, according to previous guides (DeVos and Miller, 2013). The following coordinates were used relative to the bregma: 1.08mm anteroposteriorly, ±1.9mm mediolaterally and -3.7mm dorsoventrally. Based on previous data (Mosqueda-Garcia *et al*. 1993), the injections contained 10nmol of (the selective ET$_A$-receptor antagonist) BQ-123 (directly dissolved in PBS) in the treatment group, with only PBS in controls; 10min thereafter, the left coronary artery was permanently ligated midway between its origin and the apex, with the induced MI validated by ST-segment elevation in a 6-lead ECG. After 30min, telemetry-transmitters (TCA-F40, *DSI*, MN, USA) were implanted quickly (<10min) in survivors. The opioid-analgesic buprenorfine (0.05mg/kg) was injected intraperitoneally prior to

extubation in both groups, thus eliminating pain as a confounding factor. The protocol, depicted in Fig.1, (i.c.v. administration, anesthesia, analgesia) ensures the comparability between the two groups. The rats regained consciousness within 3min after discontinuation of anesthesia, and were then placed on a receiver (RCA-1020, *DSI*), capturing the signal continuously for 24h; this setting enables the assessment in the conscious, unrestrained state.

Premature ventricular contractions, couplets and triplets were counted in the treatment and control groups, each of n=6 animals. The number and duration of VT-episodes, as well as bradyarrhythmic events were also recorded. Lastly, voluntary motor-activity was assessed by the number of counts, generated by strength-variations in telemetry-signal, relative to animal location; these counts correlate with the incidence and severity of acute left ventricular (LV) failure (Howarth *et al*. 2006). Based on previous work in rats, showing voluntary activity to occur mainly after the 10th hour post-MI (Kolettis *et al*. 2014), values are given separately for the early (initial 10h) and delayed periods (last 14h of recording). Mean sinus HR was calculated separately for these periods and for the entire experiment. At the end, infarct-size was measured by planimetry (ImageJ, NIH, USA) after triphenyltetrazolium-chloride staining, as described previously (Oikonomidis *et al*. 2010).

Values are reported as mean±standard error of the mean; after examination for normality with the Kolmogolov-Smirnov test, the variables displaying skewed distribution were transformed according to Box-Cox analysis. Subsequently, differences between the two groups were assessed with the Student's t-test, whereas changes over time were assessed with analysis of variance for repeated measures, followed by post-hoc Duncan's test. Statistical significance was set at $p<0.05$.

Infarct-size (as % of LV area) was similar between treated rats (28.7±1.9) and controls (27.3±1.6). HR remained stable in controls throughout the 24h-period, but it increased during the delayed stage in the treatment group (Fig.2A). The total number of bradyarrhythmic episodes was comparable, namely 52±23 in treated rats and 32±11 in controls; likewise, the total duration of these episodes did not differ, being 28.8±12.3s and 17.1±5.6s, respectively. The total number of premature ventricular contractions was also similar, at 125±63 in the treatment group and 207±52 in controls, but fewer couplets were observed in the treatment group (6±3) than in controls (13±2).

There were fewer VT-episodes in treated rats (8±5) than in controls (68±25, Fig.2B); moreover, the average duration of each episode was shorter in treated animals (0.7±0.4s) than in controls (11.3±3.6s). As a result, the total duration of VTs was markedly shorter in the treatment group (12.3±8.9s) than in controls (546.2±130.3s, Fig.2C). Compared to the early phase, voluntary activity increased in both groups during the delayed time-period, but such increase was more pronounced in treated rats (Fig.2D).

Acute MI is often complicated by VTs, with highest incidence during the initial 24h. Here, we investigated the antiarrhythmic potential of ETA-receptor blockade in the brain of rats; this model is considered particularly useful, as the rat displays multiple VT-episodes in response to coronary ligation (Opitz *et al*. 1995), thereby maximizing the yield of each experiment. Central ETA-receptor blockade was achieved by i.c.v. injections in the lateral ventricles, with this route favored over systemic administration, based on the absence of firm data on the blood-brain barrier permeability of ETA-receptor blockers. This setting is commonly used in the assessment of compounds with potential central cardiovascular activity, as they can act on many structures after spreading throughout the brain (Dashwood and Loesch, 2010). However, its major drawback lies within the inability to accurately identify the responsible nuclei, thus necessitating additional studies. The 24h-period in our protocol encompassed phase-II arrhythmogenesis, which remains an important therapeutic target, as such VTs coincide with evolving MI and terminate after the completion of the necrosis wavefront (Janse and Wit, 1989). As in previous work (Opitz *et al*. 1995), we observed multiple VT-episodes in the control group, ceasing after the 10thh post-MI.

The main finding of the present study was the markedly lower incidence of VTs in treated rats. Interestingly, this difference resulted from *fewer* episodes of *shorter duration*, supporting the hypothesis of ameliorated central sympathetic activation as the underlying mechanism. Indeed, central sympathetic responses, evident invariably after the 1sth post-MI (Jardine *et al*. 2005), are implicated in the genesis of phase-II VTs (Kolettis *et al*. 2018), mediated by enhanced focal automaticity and delayed afterdepolarizations (Di Diego and Antzelevitch, 2011). Additionally, sympathetic stimulation shortens the effective refractory period in the non-ischemic zone, which is

simultaneously prolonged in the ischemic area (Opthof *et al*. 1993); these opposite effects may enhance myocardial inhomogeneity, favoring reentry.

The observed antiarrhythmic effect of central ETA-receptor blockade during phase-II is in concert with previous findings, demonstrating ET-1 as an important modulator of sympathetic activity at the myocardial and adrenal levels (Kolettis *et al*. 2013). Our findings, in the absence of the confounding effects of anesthesia and pain, reinforce previous suggestions on ET-1 as a regulator of central sympathetic responses (Dashwood and Loesch, 2010). This intriguing hypothesis merits further investigation that could shed light to the function of ET-1 as a neurotransmitter and/or as a vasoconstrictor of cerebral arteries. Moreover, the pathophysiologic role of ETB-receptors during acute MI deserves particular attention, as these are widely distributed in high density across glial-cells of rats and humans (Morton and Davenport, 1992). Further to VTs, our analysis included bradyarrhythmic episodes, based on previously reported atrioventricular-block, counterbalancing the antiarrhythmic effect of decreased central sympathetic activity (Kolettis *et al*. 2015). Such episodes reflect not only conduction properties, but they are considered also indicative of acute LV failure in the rat-model (Opitz *et al*. 1995). In our experiments, bradyarrhythmic episodes did not differ between treated rats and controls, suggesting absence of treatment-effects on atrioventricular-conduction. This finding may reflect enhanced voluntary activity in the treated group, a statement supported by the higher activity counts and HR in this cohort during the delayed post-MI phase. Thus, the effects of central ETA-receptor blockade on the incidence of LV failure may constitute target for future research.

In summary, our study shows that ETA-receptor blockade in the brain decreases delayed VTs during acute MI in rats, without concurrent bradyarrhythmia. These preliminary findings justify further research on the effects of ET-1 on autonomic responses during acute MI.

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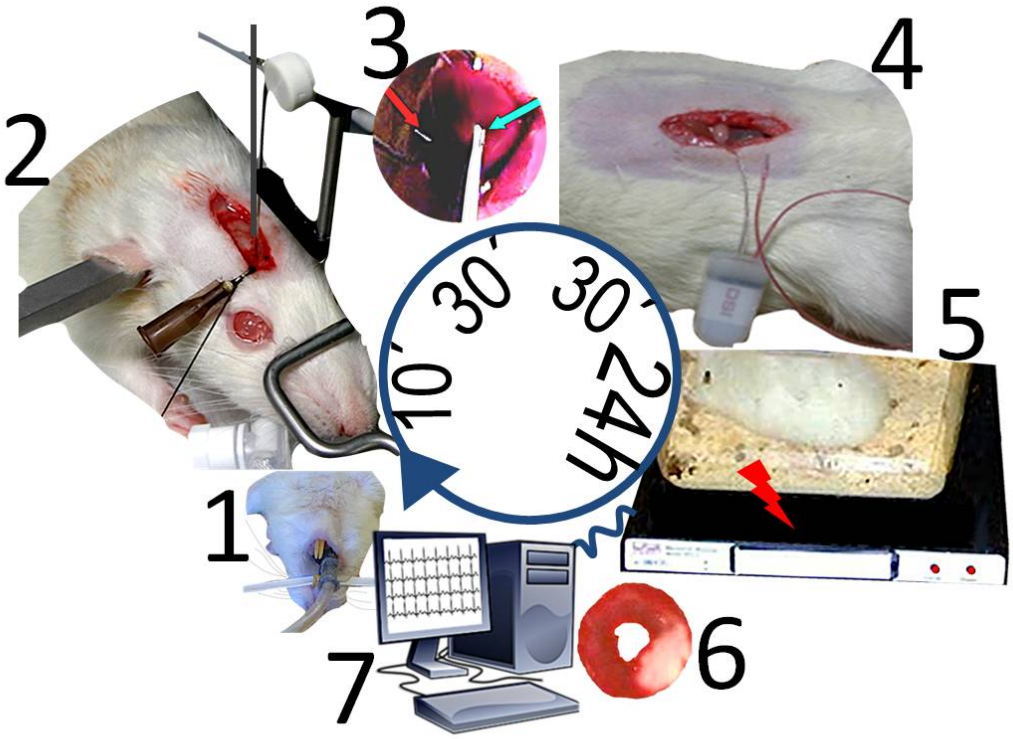
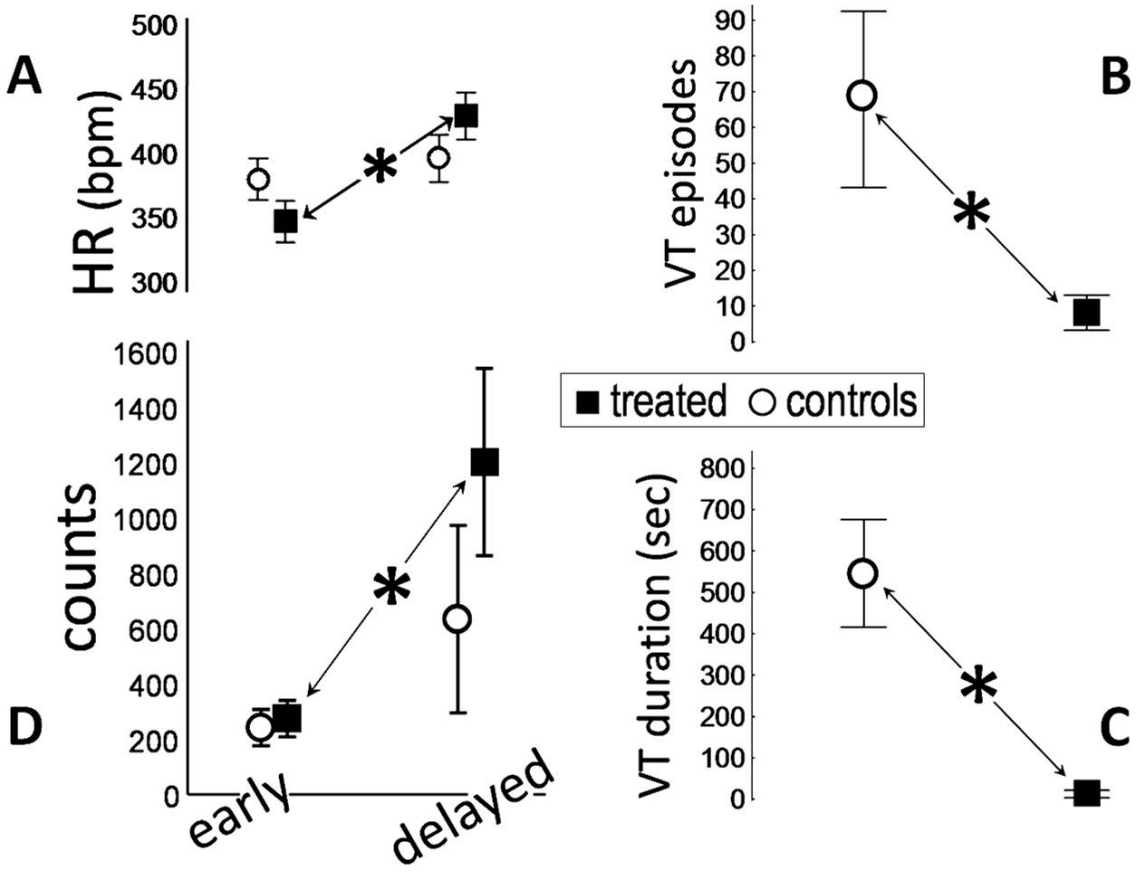
Figure 1



The study-protocol included, (1) intubation (2) intracerebroventricular injections, (3) induction of myocardial infarction, (4) implantation of telemetry-recorders, (5) 24h ECG-recording in conscious animals, (6) measurement of infarct-size, and (7) arrhythmia-analysis.

Figure 2



Heart rate (A) and voluntary activity (B) increased (asterisks, denoting p=0.0032 and p=0.025, respectively) in treated rats (solid squares) during the delayed period (last 14h of the recording), but remained stable in controls (open circles). In treated rats, there were fewer episodes (C, p=0.0058) of ventricular tachyarrhythmias (VTs) of shorter average duration, resulting in lower (p=0.00075) total duration (D).