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Endothelin receptors in the brain modulate autonomic responses and arrhythmogenesis during acute myocardial infarction in rats

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ABSTRACT

Aims: Endothelin has been implicated in various processes in the brain, including the modulation of sympathetic responses. The present study examined the pathophysiologic role of brain endothelin-receptors in the setting of acute myocardial infarction, characterized by high incidence of ventricular tachyarrhythmias.
Main methods: We investigated the effects of intracerebroventricular administration of antagonists of endothelin-receptors ET_A, ET_B, or both, during a 24 h-observation period post-coronary ligation in ($n = 70$) rats. Continuous recording was performed via implanted telemetry transmitters, followed by arrhythmia-analysis and calculation of autonomic indices derived from heart rate variability. The regional myocardial electrophysiologic properties were assessed by monophasic action potentials and multi-electrode recordings.
Key findings: Sympathetic-activity was decreased and vagal-activity was enhanced after intracerebroventricular ET_A-receptor blockade, thus attenuating regional myocardial repolarization inhomogeneity. As a result, the incidence of ventricular tachyarrhythmias was markedly lower in this group. Such effects were also observed after intracerebroventricular blockade of ET_B-, or both, ET_A- and ET_B-receptors, although to a lesser extent.
Significance: ET_A-receptors in the brain modulate sympathetic and vagal responses and alter arrhythmogenesis during evolving myocardial necrosis in rats. These findings provide insights into arrhythmogenic mechanisms during acute myocardial infarction and call for further investigation on the role of endothelin in the central autonomic network.

1. Introduction

Acute myocardial infarction (MI) is often complicated by ventricular tachyarrhythmias (VTs) that increase short-term morbidity and mortality [1]. In addition to early-stage arrhythmogenesis, sustained VTs during evolving MI (commonly referred to as phase-II) affect the outcome of patients, even those monitored in coronary care units [2]. Consequently, substantial research efforts are dedicated to deeper understanding of the mechanisms of VTs during acute MI.

Acute coronary occlusion invariably elicits progressive sympathetic activation that serves the maintenance of cardiac output, via its positive inotropic effects. However, sympathetic activation also alters ventricular electrophysiologic properties, and has been long recognized as an important arrhythmogenic mechanism [3]. Such activation consists of epinephrine-release in the ischemic myocardium and, subsequently, from chromaffin cells in the adrenal medulla. Additionally, central autonomic effects occur via afferent myocardial fibers, activated by hemodynamic changes and by the local production of various metabolites during ischemia [4]. Afferent stimuli reach the nucleus tractus solitarius, where sensory nuclei form circuits in the central autonomic network that elicit efferent discharges [5]; yet, current understanding on the factors regulating these processes remains incomplete, despite ample research efforts.

Endothelin-1 (ET-1) and its receptors, ET_A and ET_B, participate in the pathophysiology of acute MI [6], and modulate sympathetic activation at the myocardial and adrenal levels [7]. Moreover, ET-1 has been identified in a number of neural circuits in the brain of several species, including rats and humans, and has been implicated in

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autonomic responses [8]. Early reports demonstrated that intracerebroventricular (i.c.v.) administration of ET-1 stimulates sympathetic nervous activity and increases plasma epinephrine and norepinephrine [9,10]. ET_A-receptors appear to be the main mediator of these effects, as they were largely prevented in rats pre-treated with i.c.v. ET_A-blockade [11]. Recent evidence indicates that these processes are also operative during acute MI [12], but several aspects remain obscure. Furthermore, less information exists on the central actions of ET_B-receptors, which are also present in large numbers in various brain-regions [13,14].

Against this background, the present study investigated the pathophysiologic role of brain endothelin-receptors on autonomic responses and arrhythmogenesis during acute MI in rats. We examined the incidence of phase-II VTs and autonomic function after i.c.v. pharmacologic blockade of ET_A-, ET_B-receptors, or both. To shed further light into potential underlying mechanisms, we assessed regional electrophysiologic properties after each intervention.

2. Materials and methods

2.1. Animal study population and ethics

The experiments were conducted on 70 Wistar rats (16–18 weeks of age, weighing 272 ± 9 g); to overcome possible sex-related differences, all rats were male. The animals received humane care and every effort was made to minimize their suffering. They were housed individually, with free access to water and food, under optimal conditions, in terms of temperature (20–22 °C), humidity (~70%) and light-to-dark cycles (12:12 h). All procedures comply with the ARRIVE guidelines and were carried out in accordance with the EU Directive 2010/63/EU for animal experiments.

2.2. Anesthesia and intracerebroventricular injections

After induction of anesthesia with 5%-sevoflurane (*Abbott Laboratories*, Abbott Park, IL, USA), the rats were intubated and mechanically ventilated (respirator model-7025, *Ugo Basile*, Verona, Italy) at 85 breaths/min; anesthesia was maintained with a mixture of oxygen and 2.5% sevoflurane, a regimen known to exert only minimal effects on autonomic activation [15]. The animals were placed on a stereotaxic-apparatus (*David Kopf*, Tujunga, CA, USA) for intracerebroventricular (i.c.v.) injections at the following coordinates (relative to the bregma): 1.08 mm antero-posteriorly, ± 1.9 mm medio-laterally, and −3.7 mm dorso-ventrally. As in previous work [11,16], 10 μl-injections of 10 mM phosphate-buffered saline were performed manually (over a period of 10 min) via a Hamilton-needle, containing: (a) no active medication (control-group), (b) 10 nmol of BQ-123 (*Sigma*-Aldrich, St. Louis, MO, USA), a selective ET_A-receptor antagonist (ETA-group), (c) 10 nmol of BQ-788 (*Sigma*), a selective ET_B-receptor antagonist (ETB-group), (d) 10 nmol of BQ-123 *and* 10 nmol of BQ-788 (ETAB-group), and, (e) no active medication in rats without subsequent MI (sham-group). The study protocol is depicted in Fig. 1.

2.3. Induction of myocardial infarction

The left coronary artery was ligated midway between its origin and the apex in a consistent manner, ensuring comparable ischemic area in all experiments. In addition to visual confirmation of akinesis, MI was validated by prominent ST-segment elevation in a 6-lead ECG (QRS-Card, Pulse Biomedical Inc., *PBI*, Norristown, PA, USA) after amplification by software (Cardiology-Suite, *PBI*). At the end of the experiment, the rats were euthanized with KCl under deep anesthesia, and the hearts were quickly harvested. Infarct-size was measured by planimetry (Image*J*, NIH, USA) after triphenyltetrazolium-chloride staining, as described before [17]. In sham-operated animals, the left coronary artery was encircled, but not ligated. The opioid-analgesic buprenorfine (0.05 mg/kg) was injected intra-peritoneally, thus eliminating pain as a confounding factor. The protocol of the study consisted of two parts, namely (i) continuous electrocardiographic (ECG) recording ($n = 7$ in each of the five groups, i.e., ETA, ETAB, ETB, control and sham) and (ii) evaluation of arrhythmia mechanisms (in additional experiments, including also $n = 7$ rats in each group).

2.4. Continuous ECG-recording

ECG was continuously recorded for 24 h in the conscious, untethered state, with the use of miniature telemetry-transmitters (TCA−F40, Data Sciences International, *DSI*, Arden Hills, MN, USA); they were implanted in the abdominal cavity, with the leads secured under the right axilla and at the left hind-limb area, as previously [18]. The animals were placed on a receiver (RCA-1020, *DSI*), continuously capturing the signal during the 24 h-observational period.

2.4.1. Arrhythmia analysis

According to current guides [19], premature ventricular contractions (PVCs), couplets and triplets were counted, and episodes of four or more consecutive PVCs were reported collectively as VTs. The number and duration of bradyarrhythmic events were also recorded, including sinus pauses and atrio-ventricular block.

2.4.2. Autonomic responses

Heart rate (HR) was calculated from consecutive RR-intervals in sinus rhythm, averaged for the entire 24 h-period. Autonomic activation was evaluated by heart rate variability (HRV), aided by the Kubios-software, created and validated by the Department of Applied Physics, University of Eastern Finland, Kuopio, Finland [20]. In the *time domain*, we calculated the standard deviation (SDNN) of the RR-interval, and the coefficient of variance (C.V., defined as $100 \cdot SDNN \cdot RR^{-}$). In the *frequency-domain*, we calculated the low- (LF, 0.5–0.8 Hz) and high-frequency (HF, 0.8–2.4 Hz) band-power (as percent of the total power) after fast Fourier transformation. Sympathetic activity was further assessed with *detrended fluctuation analysis*, in which the early ($DF\alpha_1$) slope of double-log plots was calculated. Lastly, Poincaré plots were constructed in *nonlinear analysis*, with the width (SD1) of the distribution reflecting vagal activity.

2.4.3. Activity measurement

Voluntary activity was assessed by the number of counts produced by strength-variations in the telemetry-signal, in relation to the location of the animal.

2.5. Electrophysiologic milieu

We assessed regional electrophysiologic properties, by means of monophasic action potential (MAP) recordings and multi-electrode array (MEA) mapping, both performed 1 h post-ligation, as described previously [21]. Additional experiments were deemed necessary for this protocol, thereby maintaining uninterrupted ECG-recordings in the initial animal-groups.

2.5.1. MAP-recordings

A MAP-probe (*EPT*, Boston Scientific Corporation, Marlborough, MA, USA) was hand-held at the lateral left ventricular (LV) epicardium. Via a preamplifier (*EPT*), data were streamed into a personal computer, equipped with an analog-to-digital converter (BNC-2110, National Instruments, *NI*, Austin, Texas, USA) and a data-acquisition card (DAQ-6023E, *NI*). After filtering (digital notch-filter at 50 Hz and wavelet-filter), the signals were recorded with a software-program, custom-written in LabView (*NI*). Data analysis included maximum value of voltage rise (dV/dt_{max}) during phase 0, and action potential duration at 90% of repolarization (APD90).

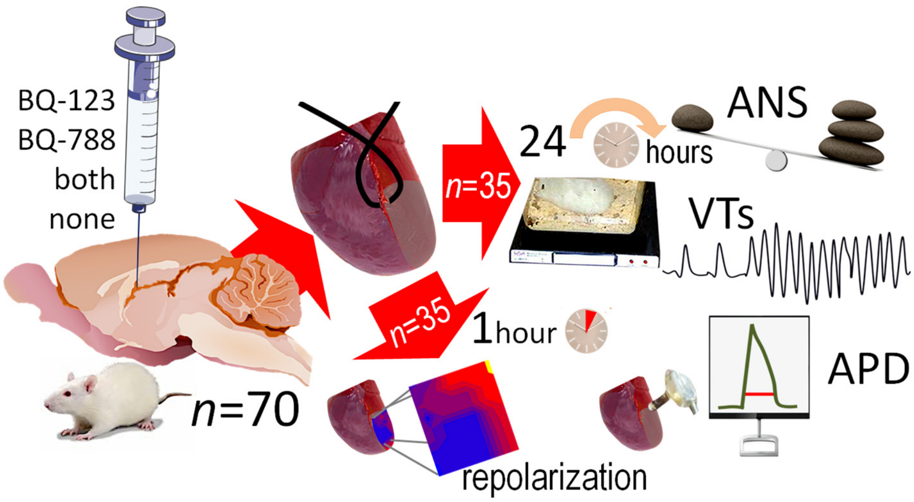

Fig. 1. Study protocol.
Myocardial infarction was induced after intracerebroventricular endothelin-receptor blockade. One hour thereafter, repolarization dispersion and action potential duration (APD) were measured from multi-electrode recordings and monophasic action potentials, respectively. Indices of autonomic nervous system (ANS) and the incidence of ventricular tachyarrhythmias (VTs) were assessed in 35 additional rats during a 24-hour observation period.

2.5.2. Multi-electrode array mapping

We used a 32-electrode MEA, selected from a commercially available device (FlexMEA72, Multi Channel Systems, *MCS*, Reutlingen, Germany), in which the 4 × 8 configuration (providing 1.25 × 1.50 mm inter-electrode distance) encompassed the ischemic and peri-ischemic myocardium. The array was connected via an adaptor (ADPT-FM-72, *MCS*) to two shielded connector-blocks (SCB-68A, 782536-01, *NI*), streaming the data into an acquisition system (PCI-6289, M-Series-DAQ, *NI*) via two shielded cables (SHC68-68-EPM, *NI*). Unipolar electrograms were recorded (at a sampling frequency of 5 kHz) from the antero-lateral LV epicardium, in reference to Wilson's central terminal. Electrical noise (50/60 Hz band-pass filter) and motion artifacts (Chebyshev, elliptic and wavelet filters) were removed by the software, also custom-written in LabView (*NI*). The repolarization heterogeneity index (μ) was calculated as the 95th minus the 5th percentile of the activation-recovery interval, divided by its median value, according to previous definitions [22]. Lastly, ventricular conduction was evaluated by the conduction-delay, i.e., the time-difference between the initial dV/dt_{min} of the first and last electrograms obtained in each set of 32 channels.

2.6. Statistical analysis

Values are reported as mean ± standard error of the mean. Normally distributed continuous variables (as per Kolmogolov-Smirnov test) were assessed with the analysis of variance, followed by Duncan's multi-stage test for post-hoc comparison between groups. After Box-Cox transformation to normality, these tests were also used for the comparison of the number and duration of tachyarrhythmic- and bradyarrhythmic-episodes, which displayed skewed distributions. Statistical significance was defined at $p < 0.05$.

3. Results

3.1. Infarct size

Infarct-size (as percent of the total LV area) was similar between the four MI-groups. Specifically, it was 27.6 ± 1.7% in the ETA-, 28.2 ± 2.2% in the ETAB-, 26.9 ± 1.4% in the ETB-, and 28.8 ± 1.5% in the control-group. Myocardial necrosis was absent in the sham-group.

3.2. Arrhythmias

3.2.1. Bradyarrhythmias

No bradyarrhythmias were present in sham-operated rats. By contrast, sinus pauses and episodes of atrio-ventricular block were occasionally observed in the four MI-groups, with a similar incidence.

3.2.2. PVCs, couplets and triplets

Only scarce PVCs were observed in sham-operated rats. Between the MI-groups, the total number of PVCs was lower in the ETA-group (152 ± 45), when compared to controls (475 ± 93), and comparable to the ETAB-group (290 ± 91); the difference in the number of PVCs between the ETA- and ETB-group (353 ± 133) was of marginal significance (p = 0.053). Likewise, the number of couplets was lower in the ETA- (8 ± 2) than in the control- (35 ± 7) or the ETB-group (36 ± 18), and similar to the ETAB-group (7 ± 1). The number of triplets was comparable in the four MI-groups.

3.2.3. VT/VF

No VTs were seen in sham-operated rats. The ETA-group had fewer episodes (10 ± 3) than the control-group (78 ± 18), although not different from those observed in the ETAB- (44 ± 23), or the ETB-group (47 ± 29). Moreover, the average duration of each VT-episode was longer in controls (10.2 ± 2.6 s), when compared to each of the treatment groups, i.e., the ETA- (3.3 ± 1.0 s), the ETAB- (1.6 ± 0.2 s), or the ETB-group (2.9 ± 1.2 s). As a result, the total duration of VTs during the 24 h-observational period was shorter in the three treatment groups (ETA, ETAB, ETB) than in controls, without differences between them (Fig. 2). Representative ECG-strips from each group are given in Fig. 3.

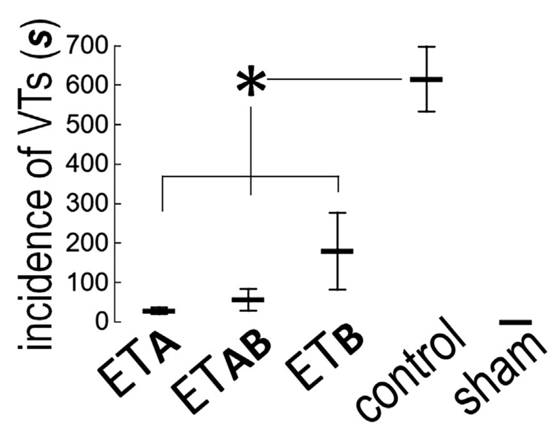

Fig. 2. Incidence of ventricular tachyarrhythmias.
The total duration of ventricular tachyarrhythmias (VTs) during a 24 h-observational period was shorter (asterisk) after intracerebroventricular blockade of endothelin-receptors ET_A, ET_B, or both.

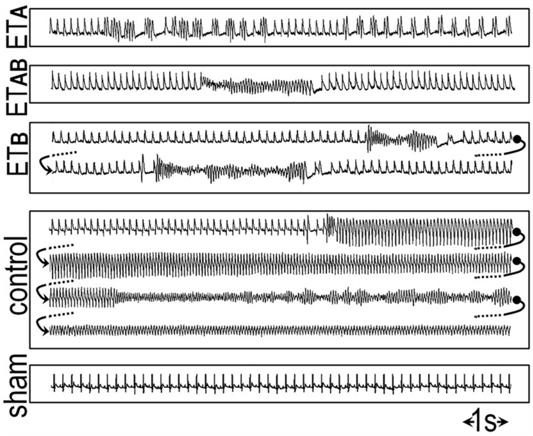

Fig. 3. Ventricular tachyarrhythmias.
Representative examples of ECG-strips in the five groups: the incidence of ventricular tachyarrhythmias was high in controls, low after intracerebroventricular blockade of endothelin ET_A-receptors, and absent in sham-operated rats.

3.3. Autonomic responses

Mean *HR* (in beats per minute, *bpm*) was lower in sham-operated animals (330 ± 5 *bpm*), but no differences were present between the four MI-groups; in these, HR was 411 ± 4 *bpm* in the ETA-, 406 ± 28 *bpm* in the ETAB-, 412 ± 13 *bpm* in the ETB-, and 433 ± 14 *bpm* in the control-group.

The results of the *time-domain analysis* of HRV are displayed in Fig. 4. SDNN was higher in the ETA-group than in controls, the ETAB- or the ETB-group. Moreover, the C.V. was higher in the ETA-group than in controls, the ETAB- or the ETB-group, and comparable to the sham-group.

The results of the *frequency domain analysis* of HRV are displayed in Fig. 5. The power of the LF-component (as percent of the total power) was lower (indicating lower sympathetic activation) in sham-operated rats (6.0 ± 0.1%), in comparison with the four MI-groups. However, no difference was found between the ETA- (9.6 ± 0.2%), the ETAB- (10.4 ± 1.1%), the ETB- (10.9 ± 1.0%) and the control-group (12.0 ± 1.5%). The power of the HF-component (as percent of the total power) was higher (indicating higher vagal activation) in sham-operated rats (31.4 ± 4.0%), in comparison with the four MI-groups; in these, values in the ETA-group (17.1 ± 3.4%) were higher than those in the control-group (8.5 ± 0.2%), although they did not differ from those in the ETAB- (13.2 ± 1.4%) or the ETB-group (13.8 ± 1.0%). As shown in the lower panel of Fig. 5, the sympatho-vagal balance, expressed as the LF·HF^{-1} ratio, was more favorable in the three treatment groups (ETA, ETAB, ETB) than in controls.

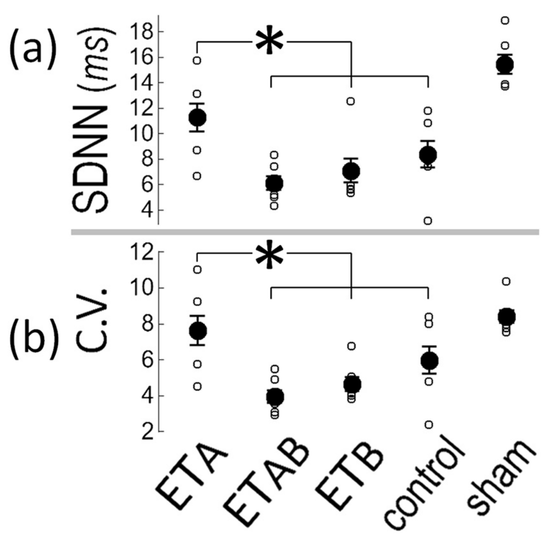

Fig. 4. Time-domain analysis of heart rate variability.
The time-domain analysis of heart rate variability indicated lower sympathetic activity after intracerebroventricular blockade of endothelin ET_A-receptors. The standard deviation (SDNN) of the RR-interval (a) and (b) the coefficient of variance (C.V., defined as 100·SDNN·RR^{-1}) were higher (asterisks) in the ETA-group.

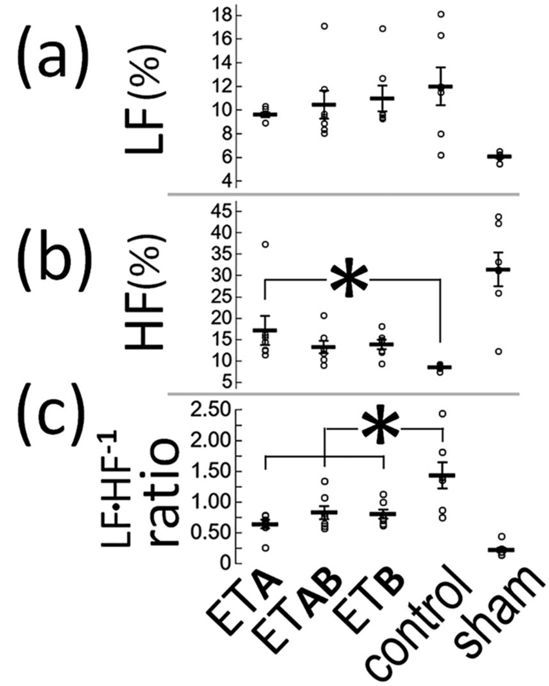

Fig. 5. Frequency-domain analysis of heart rate variability.
The power of the LF-component (a) was similar in the three treatment groups (ETA, ETAB, ETB) and controls, but the power of the HF-component (b) was higher (asterisk) after intracerebroventricular blockade of ET_A-receptors than in controls. The sympatho-vagal balance, expressed as the LF·HF^{-} ratio (c), was more favorable (asterisk) in the three treatment groups (ETA, ETAB, ETB) than in controls.

The DFAα_1, after *detrended fluctuation analysis*, was higher (indicating *lower* sympathetic activation) in sham-operated rats (1.21 ± 0.09), in comparison with the four MI-groups, in which values in the ETA- (1.00 ± 0.05), the ETAB- (0.93 ± 0.07), the ETB- (0.98 ± 0.04) and the control-group (0.87 ± 0.05) were similar. SD1, as an index of vagal activation after *non-linear analysis*, was higher (indicating higher vagal activation) in sham-operated rats (11.9 ± 2.2), in comparison with the four MI-groups, in which values in the ETA- (6.8 ± 1.4), the ETAB- (6.7 ± 1.8), the ETB- (4.1 ± 1.1) and the control-group (4.6 ± 0.2) were similar.

3.4. Voluntary activity

Total activity (in motion-counts) was higher in sham-operated rats (6042 ± 577), when compared to the MI-groups, i.e., to the ETA- (1621 ± 341), the ETAB- (1660 ± 228), the ETB- (1112 ± 272) or the control-group (877 ± 206). The observed differences between the MI-groups did not reach statistical significance, although a trend (p = 0.073) was noted towards higher activity in the ETA-group, when compared to controls.

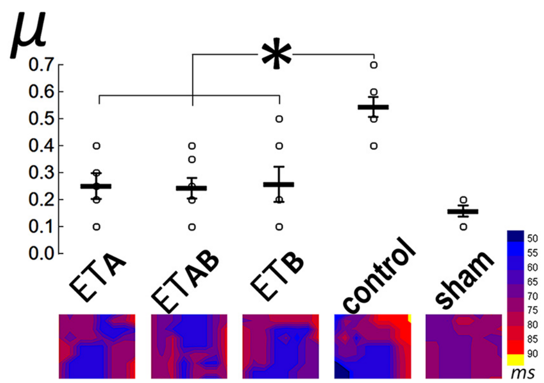

Fig. 6. Repolarization dispersion.
The repolarization heterogeneity index (μ) was lower (asterisk) in the three treatment groups (ETA, ETAB, ETB) than in controls, without differences between them. The lower panel shows representative isochronal repolarization maps in the five groups. Note the pronounced inhomogeneity in the control group, which is lower in the three treatment groups.

3.5. MAPs and MEA recordings

During phase 0, maximal voltage rise (dV/dt_{max}, in Volts·second^{-1}) in MAP-recordings was higher in sham-operated rats (28.0 ± 0.6V·s^{-1}) than in the four MI-groups, i.e., the ETA- (19.0 ± 1.3 V·s^{-1}), ETAB- (20.2 ± 4.5 V·s^{-1}), ETB- (15.7 ± 1.7 V·s^{-1}) and control-group (16.5 ± 0.8 V·s^{-1}), without differences between them. Likewise, the conduction-delay in MEA-recordings was shorter in sham-operated rats (9.3 ± 0.6 ms) than in the four MI-groups, i.e., the ETA- (17.6 ± 4.4 ms), ETAB- (16.62.6 ± ms), ETB- (17.0 ± 1.4 ms) and control-group (22.6 ± 1.7 ms), without differences between them.

No differences were detected in APD90 on MAP recordings, but significant variance was present in repolarization-dispersion in MEA recordings. As seen in Fig. 6, the repolarization heterogeneity index (μ) was lower in the three treatment groups (ETA, ETAB, ETB) than in controls, without differences between them. Representative isochronal repolarization maps are given at the bottom panel of Fig. 6.

4. Discussion

A growing body of evidence suggests that ET-1 participates in various brain-processes [8]. Based on this rationale, we explored the hypothesis that endothelin-receptors in the brain may alter arrhythmogenesis during acute MI, by modulating autonomic responses. To ensure effective ET_A- and ET_B-receptor blockade in the brain, the respective antagonists were administered by i.c.v. injections, as in previous work in rats [11]. This animal-model, used here, is particularly relevant, as repetitive self-terminating VTs are common in rats after coronary ligation, thereby maximizing the yield of each experiment. Accordingly, we observed high incidence of VTs in controls during evolving-MI, in sharp contrast to only occasional PVCs in sham-operated animals. Importantly, our four MI-groups were comparable in terms of infarct size after 24 h, evidenced by similar values of necrotic area.

We examined separately the sympathetic- and vagal-responses with HRV-analysis, which confers the advantage of continuous assessment in conscious, unrestraint animals. However, no single HRV-index can accurately depict the autonomic status; hence, we chose its versatile assessment after consideration of a cluster of indices, derived from the analysis in the time- and frequency-domain, as well as from detrended fluctuation and non-linear analyses.

4.1. Brain ET_A receptor blockade and sympathetic activation

SDNN and C.V. were higher in the ETA-group (compared to controls), although no significant differences were found in the power of the LF-component or in $DF\alpha_1$. Overall, our HRV-analysis indicates lower sympathetic activation in the ETA-group, a conclusion concurrent with the results of earlier work [11,23,24]; in these experiments, the modulatory actions of i.c.v. ET_A-receptor blockade on the sympathetic nervous system were demonstrated pharmacologically, as well as by direct recordings of sympathetic nerve activity. Of note, no differences in HR were found between our ETA-group and controls, a result also reported in anesthetized rats [11,23,24], as well as after chronic i.c.v. ET_A-receptor blockade in conscious, hypertensive rats [25]. Interestingly, voluntary activity tended to be higher after i.c.v. ET_A-receptor blockade, which may explain not only the relatively minor reduction in HR, but also the absence of bradyarrhythmic-episodes in this group. Taken together, our present findings, examined in light of previous reports, confirm the modulatory actions of i.c.v. ET_A-receptor blockade on sympathetic activation, and strongly suggest a pathophysiologic role during acute MI.

4.2. Brain ET_A-receptor blockade and vagal activation

The power of the HF-component, derived from the frequency-domain analysis, was higher in the ETA-group than in controls, indicating higher vagal activation in the former. This finding provides important input to current understanding of the effects of ET-1 on vagal-responses, which remains incomplete, despite their first observation as early as in 1991 [26]; in this early work, intracisternal ET-1 administration in conscious rats increased baroreflex sensitivity, which was attributed to the vagal component. In agreement with our results, it was subsequently demonstrated that the centrally-mediated vagal effects of ET-1 are exerted via the ET_A-receptors [27]. This relation was reiterated recently in conscious rabbits, although species-differences may be operative, which, in addition to dose-dependency, hamper the deduction of firm conclusions [28].

4.3. Brain ET_A-receptor blockade and VTs during MI

A prominent finding in our study was the lower incidence of phase-II VTs after i.c.v. ET_A-receptor blockade, in comparison with the control-group. This result cannot be explained by local myocardial differences in depolarization properties or in electrical conduction, based on the similar values of voltage rise (dV/dt_{max}) in MAPs and conduction-delay in MEA-recordings. On the other hand, the finding of *fewer* episodes of *shorter* average duration in treated animals points towards more favorable autonomic responses as an underlying mechanism. Indeed, focal automaticity and delayed afterdepolarizations are well described arrhythmogenic mechanisms, mediated by sympathetic activation during acute MI [29]. Moreover, enhanced sympathetic input to the heart exaggerates electrophysiologic inhomogeneity, by exerting diverse actions on the ischemic and non-ischemic ventricular myocardium [30]. Such effects have been thoroughly examined in the canine-model of acute MI, where it was demonstrated that sympathetic nerve activity shortens the effective refractory period in the non-ischemic zone, but simultaneously prolongs it in the ischemic area [31]. Lastly, homogeneous repolarization was recently reported after attenuated local myocardial sympathetic excitation on the ischemic porcine myocardium [32]. Thus, this antiarrhythmic mechanism may well explain the low incidence of VTs, observed here after i.c.v. ET_A-receptor blockade, based on the improved repolarization heterogeneity index (μ) in this group. This explanation retains its value, despite the absence of differences in APD90, as this variable cannot provide information on the spatial distribution of repolarization.

4.4. Vagal activation and VTs during MI

The effects of vagal stimulation on the ischemic ventricular myocardium have been increasingly recognized during the past years. For example, vagal stimulation, performed shortly after coronary occlusion in conscious dogs, ameliorated the electrophysiologic properties of the ischemic myocardium and prevented the onset of VTs [33]. This notion was recently expanded by the demonstration of vagal withdrawal contributing to arrhythmogenesis (in addition to sympathetic activation) in rats post-MI [34]. Taken together, our present findings, examined in light of previous work, indicate that i.c.v. ET_A-receptor blockade modulates sympathetic *and* vagal responses during acute MI, leading to improved local electrophysiologic milieu and lower incidence of phase-II VTs.

4.5. Potential sites of pharmacologic action of i.c.v. ET_A receptor blockade

Our study aimed to explore the pathophysiologic link between central ET-1 and arrhythmogenesis during acute MI, via modulation of autonomic responses. Based on such 'proof-of-concept', our study was not designed to identify potential sites of action; hence, ET_A-receptor blockade was attained by i.c.v. injections, a route providing pharmacologic effects throughout the brain. This (commonly used) strategy facilitates the deduction of conclusions on central pharmacologic actions, but has the disadvantage of necessitating additional investigation for identification of the responsible nuclei. Thus, further work is needed on the effects of central endothelin-ET_A receptor blockade during acute MI, focusing on sites of the central autonomic network, in which a critical role of ET_A-receptors has been demonstrated. In addition to brain-stem regions [14], such candidate sites include the paraventricular hypothalamic nucleus [35], which can directly modulate sympathetic *and* vagal outflow.

4.6. Blockade of ET_A, ET_B, or both receptors

An important part of our study was the examination of ET_B-receptors in the brain, the physiologic role of which remains uncertain. For this purpose, our protocol included two more groups, namely those derived after i.c.v. blockade of ET_B-, or both, ET_A- *and* ET_B-receptors. These groups displayed lower arrhythmogenesis than controls, with comparable incidence to that observed in the ETA-group. This effect was due to favorable electrophysiologic milieu, evidenced by similar values of the repolarization heterogeneity index (μ) in the three treatment groups (ETA, ETAB, ETB), which was, in turn, lower than controls. Nonetheless, sympathetic activation appeared more prominent in the ETAB- and ETB-groups (than in the ETA-group), based on the results of the time-domain analysis. These results are hard to explain, as the expression of ET_B-receptors has been demonstrated in glial cells, as opposed to ET_A-receptors located mainly on neurons [36]. Although vaso-active effects on the cerebral vasculature may be present after i.c.v. ET_B-receptor blockade, we favor the explanation of different compound selectivity, based on the previously described structural characteristics of brain endothelin-receptors [37]; in this regard, atypical ET_A-receptors have been postulated in the brain, having the capacity to bind with several antagonists, thus limiting their selectivity [38].

4.7. Strengths and limitations

The pathophysiology of arrhythmogenesis during acute MI, addressed here, is a challenging field, with its clinical significance underscored by the extensive prevalence of coronary artery disease. We investigated the modulatory effects of central ET-receptors on autonomic responses, a topic carrying potential ramifications for a wide variety of brain-processes. The comprehensive assessment of VTs, autonomic function and voluntary activity in conscious, freely-moving animals for an extended observation-window strengthens our study. Moreover, the in vivo evaluation of the electrophysiologic properties of the ischemic zone and the surrounding myocardium provides insights to the mechanisms of ischemia-induced VTs. Despite these merits, three limitations become apparent: *First*, the herein observed changes in autonomic variables have to be confirmed by additional methods, based on the limitations of HRV-analysis. *Second*, we did not attempt an hourly-analysis, as our study lacked adequate statistical power for meaningful conclusions. *Third*, we can only speculate on the pharmacologic targets responsible for the antiarrhythmic effects of i.c.v. endothelin-receptor blockade during acute MI, with further work required for this aim.

5. Conclusions

In the rat-model of acute MI, i.c.v. endothelin-receptor blockade ameliorates indices of autonomic activity and lowers the incidence of VTs during a 24 h-observation period, without inducing bradyarrhythmia. This action, mediated by the ET_A-receptors in the brain, consists of decreased sympathetic- *and* enhanced vagal-responses, leading to more homogenous electrophysiologic milieu. Further studies on the exciting field of brain-heart interactions are expected to extend current understanding, with a view to providing new antiarrhythmic approaches for acute MI.

Conflict of interest

The authors declare that there is no conflict of interest in the present study.

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