Endothelin B-receptors and sympathetic activation: Impact on ventricular arrhythmogenesis during acute myocardial infarction

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ABSTRACT

Aims: We investigated the role of endothelin-B receptors on sympathetic activation originating from the adrenal gland or from the myocardium and its impact on arrhythmogenesis during acute myocardial infarction.

Main methods: We studied two groups of rats ($n = 120$, 284 ± 2 g), namely wild-type and ETB-deficient. Myocardial infarction was induced by permanent ligation of the left coronary artery and ventricular tachyarrhythmias were evaluated from continuous electrocardiographic recordings. Sympathetic activation, measured by indices of heart rate variability, was evaluated after adrenalectomy or catecholamine depletion induced by reserpine. Acute left ventricular failure was assessed by total animal activity.

Key findings: Adrenalectomy decreased the total duration of tachyarrhythmias in ETB-deficient rats, but their incidence remained higher, compared to wild-type rats. After reserpine, heart rate variability indices and tachyarrhythmias were similar in the two groups during the initial, ischaemic phase. During evolving infarction, tachyarrhythmia duration was longer in ETB-deficient rats, despite lower sympathetic activation. Heart rate was lower in ETB-deficient rats throughout the 24-hour observation period, whereas activity was comparable in the two groups.

Significance: Endothelin-B receptors modulate sympathetic activation during acute myocardial infarction not only in the ventricular myocardium, but also in the adrenal gland. Sympathetic activation markedly increases early-phase ventricular tachyarrhythmias, but other mechanisms involving the endothelin system underlie delayed arrhythmogenesis.

Introduction

Arrhythmogenesis during acute myocardial infarction (MI) accounts for most cases of sudden cardiac death (Rubart and Zipes, 2005), a major health-related problem worldwide. Two temporally distinct arrhythmia peaks have been described in acute MI-animal models that correspond to the pre- and in-hospital stages in the clinical setting (Di Diego and Antzelevitch, 2011). Polymorphic ventricular tachycardia (VT), degenerating into ventricular fibrillation (VF), during the early post-MI phase carries a particularly ominous prognosis, but VT and VF during the in-hospital phase are also associated with increased morbidity and mortality (Kolettis, 2013). Despite considerable research efforts during the past decades, several aspects of the pathophysiology of the MI-related VT/VF remain incompletely understood.

Acute coronary occlusion causes an immediate rise in plasma endothelin-1 (ET-1) (Miyauchi et al., 1989), aggravating myocardial ischaemia. Moreover, recent evidence indicates an emerging role of ET-1 in arrhythmogenesis during acute MI (Kolettis et al., 2013a). In addition to its direct arrhythmogenic actions mediated by enhanced spontaneous calcium transients (Proven et al., 2006), ET-1 modulates sympathetic activation, a well described participant in the genesis and maintenance of VT/VF (Schomig et al., 1991). This interaction is exerted

in the ventricular myocardium (Tawa et al., 2012), in which the presence of both (ETA and ETB) ET-receptors has been demonstrated in sympathetic nerve varicosities (Isaka et al., 2007). In the isolated beating heart model (Backs et al., 2005), ET-1 increased the net norepinephrine release by ETA receptor-mediated inhibition of re-uptake; this effect was partly counteracted by attenuated exocytotic norepinephrine release, mediated by ETB receptors (Backs et al., 2005). By means of pharmacological blockade (Isaka et al., 2007; Yamamoto et al., 2005) or with the use of an animal model with genetic deficiency of ETB receptors (Yamamoto et al., 2005), these actions of ET-receptors were demonstrated also during myocardial ischaemia in the same model; increased norepinephrine release was found after ETA receptor activation immediately after ischaemia, whereas ETB receptors exerted protecting effects (Isaka et al., 2007; Yamamoto et al., 2005).

The opposing actions of ET-receptors were recently confirmed in the in vivo rat model, in which genetic deficiency of ETB receptors was associated with enhanced sympathetic activation and arrhythmogenesis during the early post-MI phase (Oikonomidis et al., 2010). However, this pattern differed during the delayed phase, indicating varying effects of ETB receptors along the course of acute MI (Oikonomidis et al., 2010). We hypothesized that the markedly different contributions of ETB receptors during acute ischaemia and evolving MI can be attributed to catecholamine depletion, produced by excessive norepinephrine release during the early post-MI phase.

A pattern of opposing actions of ET-receptors, similar to that described in the myocardium, has been also demonstrated in the isolated, perfused rat adrenal gland (Nagayama et al., 2000). Based on these findings, the adrenal gland constitutes an additional site of interaction between ET-1 and the sympathetic system, but the potential impact of this effect on ventricular arrhythmogenesis during acute MI is unknown. Thus, the second aim of the present work was to further explore the pathophysiologic role of ETB receptors in the adrenal gland, focusing on the genesis of ischaemia-related VT/VF.

Materials and methods

Animal study population and ethics

The study was conducted on 120 male rats, 17–22 weeks of age, weighing 255–340 g. To maintain this animal study population, the animals were replaced, in case of death due to excessive bleeding resulting from surgical manipulations.

The present work adheres to the guiding principles of the Declaration of Helsinki regarding ethical conduct of animal research, held by the World Medical Association. In addition, all experiments conform to European legislation (*European Union directive for the protection of animals used for scientific purposes 609/1986*, revised in *2010/63/EU*). The experimental protocols were approved by the responsible regulatory state authorities. The animals were given humane care and were housed in Plexiglas-chambers in groups of two; ad libitum access to standard rodent pellet-diet and water was provided at all times. The laboratory conditions were kept optimal, in terms of temperature, humidity and light-to-dark cycles.

To examine the pathophysiologic role of ETB-receptors on ventricular arrhythmogenesis during acute MI, two animal strains were included, namely wild-type and a previously well characterized homozygous rat strain, deficient in ETB receptors (Gariepy et al., 1998, 2000). This strain carries a naturally occurring deletion of the ETB-receptor gene that abrogates the expression of functional ETB-receptors; these transgenic rats exhibit a partially rescued phenotype and live into adulthood, providing a valuable tool in the study of the pathophysiologic role of ETB-receptors (Gariepy et al., 1998, 2000). A colony of this strain has been bred in our animal facilities, kindly provided by Professor M. Yanagisawa (Southwestern Medical Center, Dallas, USA and University of Tsukuba, Tsukuba, Japan).

Study protocols

The present work consisted of two protocols:

(i) In the *first* protocol, the effects of ETB-receptors in the adrenal gland were examined by comparing arrhythmogenesis in four animal groups, namely wild-type or ETB-deficient rats, with or without prior adrenalectomy. This comparison aids in the characterization of the contribution of the adrenal gland to arrhythmogenesis and permits the identification of the role of ETB-receptors in the adrenal gland versus that in the ventricular myocardium. As the relative importance of sympathetic activation on arrhythmogenesis decreases markedly past the early MI-stage (Clements-Jewery et al., 2002), we opted to examine the incidence of VT/VF for the first hour post-MI (defined as phase I).

(ii) In the *second* protocol, we investigated the effects of pharmacological catecholamine depletion (from sympathetic nerve terminals and chromaffin cells) on the incidence of VT/VF during acute and evolving MI in wild-type and ETB-deficient rats. This protocol eliminates the effects of sympathetic activation, thereby facilitating the study on the role of ETB-receptors on arrhythmogenesis via alternative mechanisms.

Arrhythmia time-intervals

As previously (Kolettis et al., 2013b), the incidence of VT/VF is reported separately for phase I (corresponding to ischaemia and onset of necrosis), for the 2nd until the 11th hour post-ligation (phase IIA, corresponding to evolving MI) and for the 12th until the 24th hour post-ligation (phase IIB, corresponding to established myocardial necrosis). This distinction is useful for its translational value in the clinical setting, but also from a pathophysiologic point of view, as it may aid in the identification of the varying underlying mechanisms (Di Diego and Antzelevitch, 2011; Kolettis, 2013; Kolettis et al., 2013b).

Adrenalectomy

Bilateral adrenalectomy was performed 3 days prior to MI induction. After tracheal intubation with a 14G-catheter, the rats were mechanically ventilated with a rodent apparatus (model 7025, Ugo Basile, Comerio, Italy) and anaesthesia was maintained with a mixture of oxygen and 2% sevoflurane. The adrenal glands were removed through bilateral dorsal mid-flank incisions, followed by hydrocortisone treatment (5 mg/kg/day subcutaneously), as previously outlined (Rafiq et al., 2011).

ECG-telemetry transmitters

Continuous electrocardiographic (ECG) monitoring was performed with the use of miniature telemetry transmitters (TCA-F40, Dataquest, Data Sciences International, *DSI*, Transoma Medical, Arden Hills, MN, USA); implantation of these devices enables long-term recording in conscious, unrestricted animals (Agelaki et al., 2007; Baltogiannis et al., 2005).

The animals were mechanically ventilated and anaesthetized, as above. As in previous experiments (Kolettis et al., 2008), the transmitters were implanted in the abdominal cavity, with two leads secured under the right axilla and at the left hind-limb area, respectively. During recording, the rats were placed on a receiver (RCA-1020, *DSI*) that continuously captured the signal; ECG was displayed with the use of a software programme (A.R.T. 2.2, *DSI*) and saved for subsequent analysis.

Reserpine administration

Pharmacological catecholamine depletion was induced with reserpine, administered at a dosage of 0.15 mg/kg (as an intraperitoneal

injection 24 h prior to MI induction), as previously outlined (Banerjee et al., 1993). This regimen has been shown to result in effective exhaustion of catecholamine stores in several organs, including sympathetic nerve terminals in the heart and chromaffin cells in the adrenal glands (Martinez-Olivares et al., 2006).

Myocardial infarction induction

MI was generated as described (Elaiopoulos et al., 2007); briefly, the left coronary artery was ligated approximately 4 mm from its origin, extending from the left atrial appendage to the pulmonary cone. Experience from our laboratory (Agelaki et al., 2007; Baltogiannis et al., 2005; Kolettis et al., 2008) and from others (Opitz et al., 1995) demonstrates that following these anatomical landmarks ensures comparable size of the ischaemic area. In all experiments, MI induction was validated with a 6-lead ECG (QRS-Card digital PC-ECG, Pulse Biomedical Inc., *PBI*, Norristown PA, USA) after amplification by software (QRS Card Cardiology Suite version 4.05, *PBI*), in which prominent ST-segment elevation was observed in more than two leads. A single intraperitoneal injection of buprenorfine, (0.05 mg/kg) was given for analgesia.

Heart rate

Sinus heart rate (HR) was calculated as an average of 10 consecutive RR-intervals, after exclusion of non-sinus beats. HR is reported for the following time-points: baseline, 5th, 30th and 60th minutes post-ligation and hourly thereafter.

Arrhythmia analysis

Episodes of monomorphic VT, polymorphic VT and VF were identified and the duration of each episode was measured using the time-scale provided by the analysis-software. Nonetheless, their distinction can be occasionally difficult, according to our experience (Agelaki et al., 2007; Baltogiannis et al., 2005; Kolettis et al., 2008; Elaiopoulos et al., 2007) and that of others (Opitz et al., 1995); hence, we report the sum of these arrhythmias collectively (termed VT/VF). The total duration of VT/VF episodes is reported for each hourly interval post-ligation.

ECG-indices of sympathetic activation

Sympathetic activation was assessed using ECG-indices of heart rate variability, as previously described (Kruger et al., 1997). These indices were calculated separately for phases I, IIA and IIB. We used the Kubios HRV-software (version 2.1, Biosignal Analysis and Medical Imaging Group, Department of Applied Physics, University of Eastern Finland, Kuopio, Finland) (Niskanen et al., 2004). As an index of sympathetic activity, we report the ratio of low-frequency (LF, 0.195–0.605Hz) to high-frequency (HF, 0.605–2.5Hz) bands (Kolettis et al., 2013b), which displays good correlation with catecholamine measurements (Oikonomidis et al., 2010).

Monophasic action potential recordings

Monophasic action potentials (MAPs) are extracellularly recorded waveforms that reproduce the depolarization and repolarization sequence of transmembrane action potentials with high fidelity. MAP recordings in our laboratory were performed as in earlier experiments (Elaiopoulos et al., 2007), following established guides (Franz, 1999). Briefly, a probe (model 200, EP Technologies, *EPT*, San Jose, CA, USA) was placed on the lateral left ventricular (LV) wall and the signal was amplified with a pre-amplifier (model 300, *EPT*). A digital notch filter was applied at 50 Hz and a band pass filter for ranges 0.05 Hz–500 Hz. Two-minute recordings were stored into a personal computer, equipped with an analogue-to-digital converter (model BNC-2110, National

Instruments Corporation, Dallas, TX, USA). Fifty consecutive sinus beats per recording were analysed and the LV action potential duration at 90% (APD90) and 75% (APD75) of repolarization was measured at baseline and 5 min post-ligation. The standard deviation of these measurements was used as a measure of electrical alternans (Franz, 1999), whereas right ventricular (RV) recordings were used as reference.

Activity measurement

As before (Kolettis et al., 2013b), the incidence and severity of acute LV failure were assessed by voluntary motor activity, recorded by the Dataquest A.R.T. analysis software (*DSI*). This programme records strength-variations in the telemetry-signal, in relation to the location of the animal; changes in these signals are depicted as counts, the number of which depends on total animal activity.

Statistical analysis

All values are given as mean $\pm$ standard error of the mean. Kaplan–Meier survival curves were constructed and heterogeneity was assessed by chi-square; differences between two groups were examined with the Peto-and-Peto Wilcoxon test. Differences in continuous variables between two groups were compared with Student's t-test, whereas differences between three or more groups were compared using the analysis of variance, followed by the post-hoc Duncan's multi-stage test. Changes in continuous variables over time were assessed with analysis of variance for repeated measures. Statistical significance was defined at an alpha value of 0.05.

Results

Adrenal gland protocol

In this part of the study, 60 animals (all male, 17–22 weeks of age) were included. Four groups were formed, namely wild-type rats with adrenalectomy ($n = 15$, 288 ± 6 g), wild-type rats without adrenalectomy ($n = 15$, 286 ± 4 g), ETB-deficient rats with adrenalectomy ($n = 15$, 287 ± 6 g) and ETB-deficient rats without adrenalectomy ($n = 15$, 282 ± 4 g).

Mortality

Mortality one hour after ligation was 46% in ETB-deficient rats without adrenalectomy, 26% in ETB-deficient rats with adrenalectomy, 20% in wild-type rats without adrenalectomy and 26% in wild-type rats with adrenalectomy. These differences between groups did not reach statistical significance (χ^2 for heterogeneity = 3.96, p = 0.26), although a trend (p = 0.092) was present towards
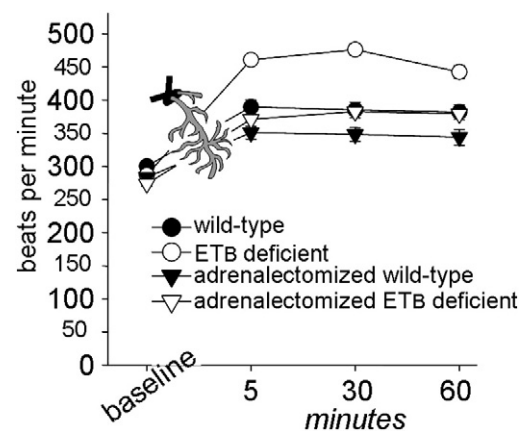

Fig. 1. Heart rate after adrenalectomy. Heart rate increased in all groups after ligation, but adrenalectomy attenuated this increase, mainly in ETB-deficient rats.

higher mortality in non-adrenalectomized ETB-deficient rats, compared to non-adrenalectomized wild-type rats.

Heart rate

No differences were found in HR at baseline. As shown in Fig. 1, HR increased in all groups after ligation. Adrenalectomy attenuated the HR increase after ligation in both groups, but this effect was more pronounced (all $p < 0.05$) in ETB-deficient rats at all time-points.

VT/VF episodes and duration

The number of VT/VF episodes and their total duration during phase I are shown in Fig. 2. Adrenalectomy did not affect ($p = 0.49$) the number of VT/VF episodes in wild-type rats, nor their total duration ($p = 0.11$). Likewise, adrenalectomy did not affect ($p = 0.37$) the number of VT/VF episodes in ETB-deficient rats, but tended ($p = 0.067$) to decrease their mean duration; as a result, total duration of VT/VF episodes was shorter ($p < 0.001$) in adrenalectomized ETB-deficient rats. When adrenalectomized wild-type and adrenalectomized ETB-deficient rats were directly compared, there was a trend ($p = 0.091$)
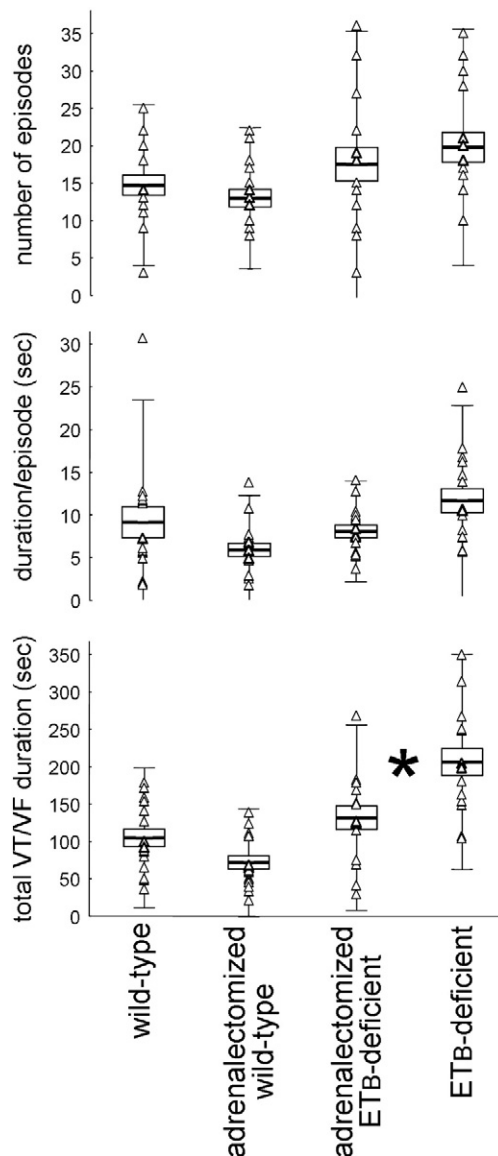

Fig. 2. Effects of adrenalectomy on ventricular arrhythmias. Scatter diagrams with box (±standard error) and whisker plots (±2 standard deviations) of the number of ventricular tachycardia (VT) and fibrillation (VF) episodes, their mean and total duration. Total duration of VT/VF was shorter (asterisk) in adrenalectomized ETB-deficient rats.

towards less VT/VF episodes in the former group (13.0 ± 1.2 versus 17.5 ± 2.2), with a shorter ($p = 0.064$) mean duration (5.91 ± 0.82 s versus 8.07 ± 0.76 s); these differences resulted in shorter ($p = 0.0031$) total duration of VT/VF episodes in adrenalectomized wild-type rats (71.8 ± 9.3 s) than in adrenalectomized ETB-deficient rats (131.6 ± 16.0 s). Representative examples from the 4 groups are shown in Fig. 3.

Reserpine protocol

The reserpine protocol included 60 rats (all male, 20–22 weeks of age), of which 30 were wild-type (280 ± 3 g) and 30 were ETB-deficient (283 ± 22 g).

Mortality

No differences ($p = 0.88$) in Kaplan–Meier survival curves were present between ETB-deficient and wild-type rats after reserpine administration.

Heart rate

Compared to wild-type animals, baseline heart rate was lower ($p < 0.001$) in ETB-deficient rats and remained so after MI induction, throughout the 24-hour observation period (Fig. 4).

VT/VF duration

The duration of VT/VF episodes for each hourly interval is shown in Fig. 5. During the first hour (phase I), VT/VF duration was comparable ($p = 0.72$) in the two animal groups. By contrast, total VT/VF duration was longer ($p = 0.049$) in ETB-deficient (104.0 ± 43.7 s) than in wild-type rats (23.2 ± 10.5 s) during phase IIA. Likewise, VT/VF duration was longer ($p = 0.025$) in ETB-deficient (87.2 ± 42.8 s) than in wild-type rats (0.9 ± 0.5 s) during phase IIB.

Sympathetic activation

Sympathetic activation was comparable ($p = 0.40$) in the two animal groups (LF/HF: 0.95 ± 0.21 in ETB-deficient versus 1.22 ± 0.22 in wild-type rats) during phase I. By contrast, sympathetic activation was lower ($p = 0.014$) in ETB-deficient rats (0.45 ± 0.09) than in wild-type rats (0.85 ± 0.10) during phases IIA and IIB (0.53 ± 0.07 versus 0.83 ± 0.09, respectively, $p = 0.022$).

Monophasic action potential recordings

At baseline, no differences were observed in APD90 or APD75 in the LV or in the RV. During ischaemia, the duration at both measurements was more prolonged in the LV ($p = 0.022$ and $p = 0.012$, respectively) and in the RV ($p = 0.018$ and $p = 0.031$, respectively) in ETB-deficient rats, compared to wild-type rats (Fig. 6).

Beat-to-beat variability of APD90 5 min after ligation was higher ($p = 0.0028$) in ETB-deficient rats (3.22 ± 0.22 ms), as compared to wild-type rats (2.30 ± 0.15 ms), this difference being indicative of enhanced electrical alternans in the former group.

Activity measurement

Activity counts were comparable in the two groups during phase I (456 ± 28 in ETB-deficient versus 471 ± 24 in wild-type rats, $p = 0.66$), as well as during phase IIA (469 ± 27 in ETB-deficient versus 437 ± 36 in wild-type rats, $p = 0.89$) and during phase IIB (466 ± 26 in ETB-deficient versus 436 ± 36 in wild-type rats, $p = 0.92$).

Discussion

After its first description in 1989 (Wennmalm et al., 1989), the interaction between ET-1 and the sympathetic nervous system and its impact during MI has attracted considerable research interest (Tawa et al., 2012). In-depth understanding of the underlying mechanisms may have significant implications in the pathophysiology of ischaemia-related VT/VF, which often lead to sudden cardiac death.
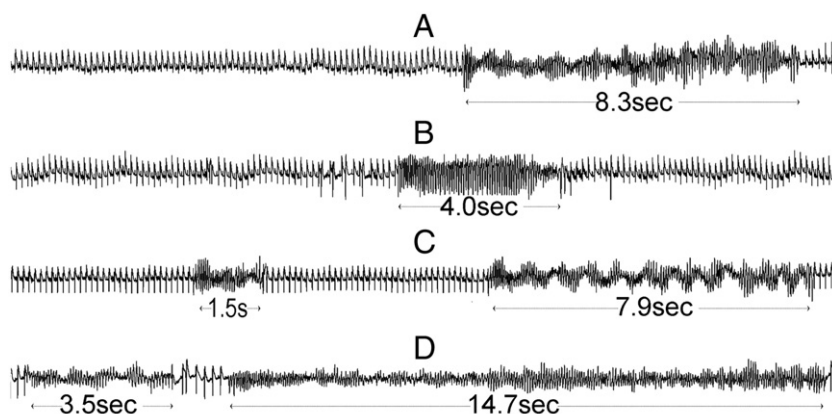
Fig. 3. Arrhythmias after adrenalectomy. Examples of ventricular tachyarrhythmias from (A) wild-type, (B) adrenalectomized wild-type, (C) adrenalectomized ETB-deficient and (D) ETB-deficient rats. Note the higher duration in the latter group.

In the present work, we examined the role of ETB receptors on sympatho-adrenal activation during acute MI and its implications on ventricular arrhythmogenesis. Our study indicates that ETB receptors modulate ventricular arrhythmogenesis during acute MI, by controlling sympathetic activation in the ventricular myocardium, as well as in the adrenal gland.

ETB receptors in the adrenal gland

Acute MI produces an immediate sympatho-adrenal response that increases arrhythmogenesis (Schomig et al., 1991), but the relative contribution of myocardial versus adrenal origin is unclear. In our experiments, arrhythmogenesis after reserpine (which induces catecholamine-depletion in myocardial sympathetic nerve terminals and in chromaffin cells) was lower compared to that observed after adrenalectomy, which, in turn, was lower than in controls; these findings point towards contribution of both, myocardium and adrenal glands, in phase I arrhythmogenesis. Moreover, our findings on HR and VT/VF in the two animal-groups suggest that the participation of the adrenal medulla in the sympathetic response and arrhythmogenesis after acute MI varies, based on its regulation by the ETB receptor. This inference is in keeping with our previous study (Kolettis et al., 2008), reporting increased plasma epinephrine levels after dual (ETA and ETB) receptor blockade.

Our conclusions are also in agreement with that derived by Nagayama et al. (2000) after pharmacological blockade in the perfused rat adrenal gland model; in this study, ETA receptor blockade inhibited the epinephrine and norepinephrine output, but this inhibition was abolished by pre-treatment with ETB receptor blockade (Nagayama et al., 2000). The present findings expand these observations, indicating that activation of ETB receptors mitigates the contribution of the adrenal gland to ventricular arrhythmogenesis during acute MI; in the absence of functional ETB receptors, the adrenal gland increases ventricular arrhythmogenesis during acute MI, mainly by prolonging the duration of VT/VF episodes. This pattern is consistent with enhanced sympathetic activation, a well established parameter of VT/VF maintenance, via its facilitation of re-entrant circuits at the border zone between the ischaemic and normal myocardium (Opthof et al., 1993).

Our experiments shed further light to the (surprisingly limited) available information on the role of adrenal activation during myocardial ischaemia and acute MI. A relatively minor impact was previously suggested in anaesthetized cats (Kuo et al., 1993), an inference supported by the findings of a clinical report, examining the effects of ischaemia induced by atrial pacing (Remme et al., 1994); this study found much higher rises in plasma norepinephrine than in epinephrine during acute ischaemia, indicating primarily cardiac origin of sympathetic stimulation (Remme et al., 1994). By contrast, significant changes in both catecholamines were demonstrated in patients with acute anterior MI (as well as in a subset of patients presenting with rhythm disturbances), whereas lower norepinephrine values were observed in patients with posterior MI (Slavikova et al., 2007); these findings imply a dual origin, with norepinephrine values possibly correlating with the size of myocardial ischemic area (Slavikova et al., 2007).
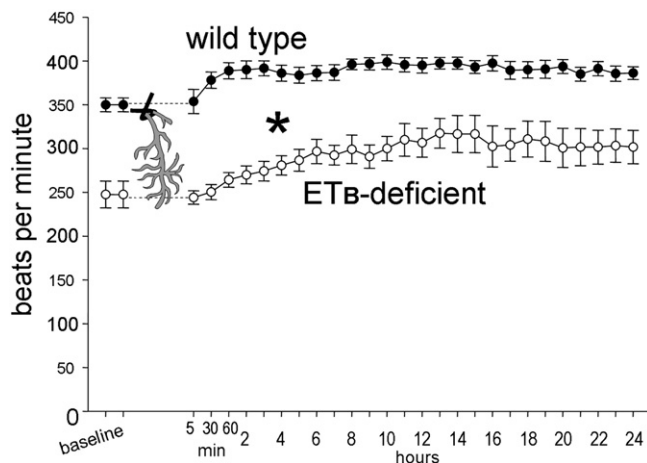

Fig. 4. Heart rate after reserpine. Heart rate was lower (asterisk) after reserpine in ETB-deficient rats pre- and post-ligation, throughout the 24-hour observation period.
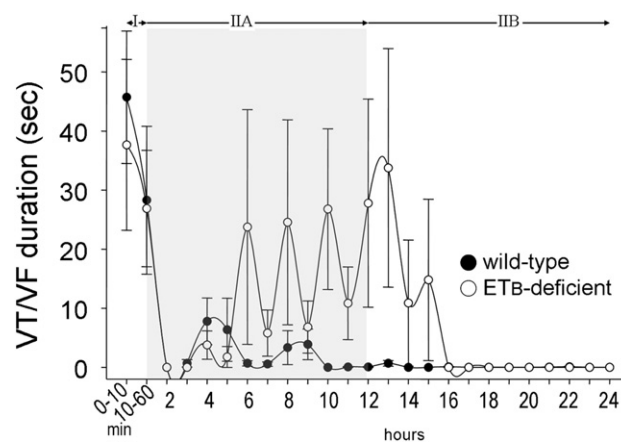

Fig. 5. Ventricular arrhythmias after reserpine. Total duration of ventricular tachycardia (VT) and fibrillation (VF) was longer in ETB-deficient rats during the delayed phases (IIA and IIB) post-ligation.
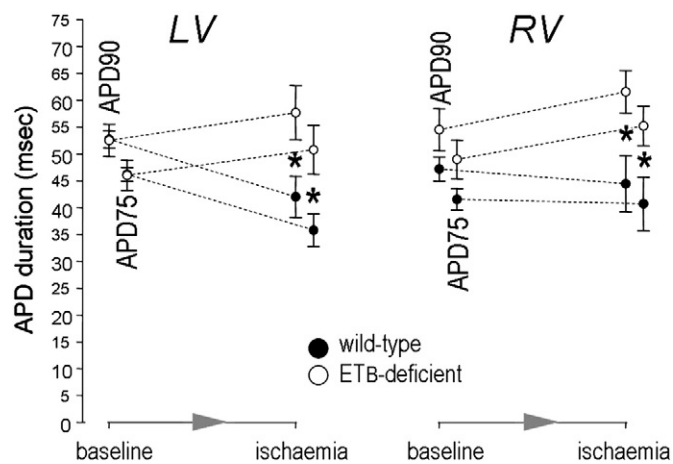
Fig. 6. Action potential duration. Left (LV) and right ventricular (RV) action potential duration (APD) was prolonged (asterisks) in ETB-deficient rats 5 min postligation.

Our conclusions on the role of the adrenal glands in the genesis of ischaemia-related VT/VF may aid in the ongoing investigation to identify the factors regulating catecholamine release (Douglas et al., 2010). Amidst the plethora of substances acting as endocrine or paracrine/ autocrine modulators of adrenomedullary activity, we believe that the role of ET-1 and its receptors is critical and merits further research.

ETB receptors and phase I arrhythmogenesis

In healthy and failing rat hearts, opposing actions of ET-receptors were previously shown on myocardial sympathetic nerve endings, consisting of ETA receptor-mediated inhibition of norepinephrine re-uptake, versus ETB receptor-mediated attenuation of exocytotic norepinephrine release (Backs et al., 2005). These actions were confirmed in the early post-MI setting, with ETB receptors exerting protective effects in terms of sympathetic activation (Isaka et al., 2007; Yamamoto et al., 2005) and resultant arrhythmogenesis (Oikonomidis et al., 2010). Our present results reiterate the importance of ETB receptors in the myocardium, based on the nearly two-fold higher incidence of VT/VF in adrenalectomized ETB-deficient rats (compared to adrenalectomized wild-type animals); furthermore, depletion of catecholamines, induced by reserpine pre-treatment, resulted in comparable sympathetic activation and arrhythmogenesis in the two groups.

An interesting finding in our experiments was the different responses in the duration of the action potential (at both measurements, APD90 and APD75) between ETB-deficient and wild-type rats, evident 5 min after the onset of ischaemia. Of note, these differences in APD were observed not only in the ischaemic antero-lateral LV myocardium, but also in reference recordings obtained from the RV. Prolongation of the APD was previously reported in isolated rat ventricular cardiomyocytes after catecholamine depletion induced by reserpine, mediated by decreased transient outward potassium current (Bru-Mercier et al., 2002). Indeed, this current plays an important role in the action potential morphology and duration, but also in the genesis of electrical alternans (Zhao et al., 2012). However, it should be noted that changes in the action potential duration reflect a complex interplay between numerous factors, including sympathetic activation, rate dependence, and alterations in ion channel function; thus, no conclusions can be rendered from the present study and more research is required towards this direction.

Based on the present findings, we conjecture that catecholamine depletion in ETB-deficient rats in our experiments might have been more pronounced after reserpine pre-treatment, despite the absence of significant differences in non-invasive indices of sympathetic

activation during phase I. This notion is strengthened (a) by the increased variation of APD90 in ETB-deficient rats; (b) by the lower heart rate observed in this animal group prior to MI induction and throughout the 24-hour observation period; and, (c) by the lower sympathetic activation in ETB-deficient rats during phase II after reserpine pre-treatment. Further investigation on the mechanisms underlying this putative effect is warranted.

ETB receptors and phase II arrhythmogenesis

After the acute ischaemic phase, evolving myocardial infarction is associated with a second wave of arrhythmogenesis, which terminates after completion of the necrotic waveform (Kolettis et al., 2013b). An important (and perhaps unexpected) finding in our experiments was the increased incidence of VT/VF during phase II, in the absence of ETB receptors. This finding cannot be explained by a higher incidence of acute LV failure in the ETB-deficient rat group, as activity was similar between groups. Moreover, increased arrhythmogenesis was observed despite lower sympathetic activation in ETB-deficient rats. This observation is in line with previous experiments in isolated, perfused rat hearts, indicating that mechanisms other than sympathetic activation underlie phase II arrhythmogenesis (Clements-Jewery et al., 2002).

The mechanisms responsible for the genesis of delayed VT/VF (in the course of acute MI) are incompletely understood, but are likely different from those during phase I (Di Diego and Antzelevitch, 2011; Kolettis, 2013; Clements-Jewery et al., 2002; Kolettis et al., 2013b). Phase II arrhythmogenesis is clinically important due to its associated increases in short-term morbidity and mortality, despite its occurrence invariably in the hospital setting (Kolettis, 2013), and necessitates continuing investigation (Clements-Jewery et al., 2002, 2005). The present study indicates that ET-1 and its receptors are involved in phase II VT/VF, the role of which is revealed in the setting of enhanced catecholamine depletion. Future studies should address potential candidate mechanisms, such as the effects of ET-1 on repolarizing potassium channels (Kiesecker et al., 2006) and on gap junctional remodelling that affects the electrical impulse conduction velocity (Reisner et al., 2009).

Strengths and limitations

We feel that our study adds to the current understanding on the pathophysiology of VT/VF during acute and evolving MI. The in vivo rat-model used in the present study presents distinct advantages, due to the similarities of the post-ligation arrhythmia pattern to that observed in patients with acute MI (Di Diego and Antzelevitch, 2011; Opitz et al., 1995). We used only male rats to eliminate possible gender-related differences in outcome. The evaluation of arrhythmogenesis for an extended time-period without the confounding effects of anaesthesia represents an additional strength. Lastly, the use of the previously characterized (Gariepy et al., 1998, 2000) ETB-deficient rat model overcomes certain limitations associated with pharmacological blockade, such as pharmacokinetic or selectivity issues.

Three limitations should be acknowledged: *first*, our study was under-powered to adequately evaluate survival data; thus, the observed differences in mortality between groups did not reach statistical significance, despite the variation in the incidence of VT/VF. *Second*, infarct size measurements were not included in the present work; nonetheless, this issue has been extensively addressed in our previous studies (Oikonomidis et al., 2010; Baltogiannis et al., 2005; Kolettis et al., 2008), in which no effect on infarct size was detected after pharmacological blockade of ET-receptors or with the use of ETB-deficient animals. Moreover, the use of activity counts as indices of acute LV failure partly counterbalances this limitation, although the use of additional indices would have been advantageous. *Third*, we used only non-invasive indices of sympathetic stimulation, without measurements of plasma catecholamines. It should be noted, however, that heart rate variability in our previous study (Oikonomidis et al., 2010)

displayed very good correlation with plasma measurements. This finding is in accordance with earlier (Kruger et al., 1997) and more recent (Kruger et al., 2000; Lee et al., 2013) studies, signifying heart rate variability as a valuable tool for the evaluation of autonomic function in the MI rat-model. Additionally, the use of non-invasive indices of sympathetic activation permits the assessment at several time-points in the course of acute MI.

Conclusions

The ETB receptor modulates sympathetic activation during acute MI in the ventricular myocardium, but also in the adrenal gland. This regulation has significant implications on ventricular tachyarrhythmias during the early post-MI phase. During evolving MI, other mechanisms entailing the endothelin system underlie arrhythmogenesis; these may have potential clinical significance and merit further research.

Conflict of interest statement

No competing interests.

References

Agelaki MG, Pantos C, Korantzopoulos P, Tsalikakis DG, Baltogiannis GG, Fotopoulos A, et al. Comparative antiarrhythmic efficacy of amiodarone and dronedarone during acute myocardial infarction in rats. Eur J Pharmacol 2007;564(1–3):150–7.

Backs J, Bresch E, Lutz M, Kristen AV, Haass M. Endothelin-1 inhibits the neuronal norepinephrine transporter in hearts of male rats. Cardiovasc Res 2005;67(2):283–90.

Baltogiannis GG, Tsalikakis DG, Mitsi AC, Hatzistergos KE, Elaiopoulos D, Fotiadis DI, et al. Endothelin receptor-A blockade decreases ventricular arrhythmias after myocardial infarction in rats. Cardiovasc Res 2005;67(4):647–54.

Banerjee A, Locke-Winter C, Rogers KB, Mitchell MB, Brew EC, Cairns CB, et al. Preconditioning against myocardial dysfunction after ischemia and reperfusion by an alpha 1-adrenergic mechanism. Circ Res 1993;73(4):656–70.

Bru-Mercier G, Deroubaix E, Rousseau D, Coulombe A, Renaud JF. Depressed transient outward potassium current density in catecholamine-depleted rat ventricular myocytes. Am J Physiol Heart Circ Physiol 2002;282(4):H1237–47.

Clements-Jewery H, Hearse DJ, Curtis MJ. Independent contribution of catecholamines to arrhythmogenesis during evolving infarction in the isolated rat heart. Br J Pharmacol 2002;135(3):807–15.

Clements-Jewery H, Hearse DJ, Curtis MJ. Phase 2 ventricular arrhythmias in acute myocardial infarction: a neglected target for therapeutic antiarrhythmic drug development and for safety pharmacology evaluation. Br J Pharmacol 2005;145(5):551–64.

Di Diego JM, Antzelevitch C. Ischemic ventricular arrhythmias: experimental models and their clinical relevance. Heart Rhythm 2011;8(12):1963–8.

Douglas SA, Sreenivasan D, Carman FH, Bunn SJ. Cytokine interactions with adrenal medullary chromaffin cells. Cell Mol Neurobiol 2010;30(8):1467–75.

Elaiopoulos DA, Tsalikakis DG, Agelaki MG, Baltogiannis GG, Mitsi AC, Fotiadis DI, et al. Growth hormone decreases phase II ventricular tachyarrhythmias during acute myocardial infarction in rats. Clin Sci (Lond) 2007;112(7):385–91.

Franz MR. Current status of monophasic action potential recording: theories, measurements and interpretations. Cardiovasc Res 1999;41(1):25–40.

Gariepy CE, Williams SC, Richardson JA, Hammer RE, Yanagisawa M. Transgenic expression of the endothelin-B receptor prevents congenital intestinal aganglionosis in a rat model of Hirschsprung disease. J Clin Invest 1998;102(6):1092–101.

Gariepy CE, Ohuchi T, Williams SC, Richardson JA, Yanagisawa M. Salt-sensitive hypertension in endothelin-B receptor-deficient rats. J Clin Invest 2000;105(7):925–33.

Isaka M, Kudo A, Imamura M, Kawakami H, Yasuda K. Endothelin receptors, localized in sympathetic nerve terminals of the heart, modulate norepinephrine release and reperfusion arrhythmias. Basic Res Cardiol 2007;102(2):154–62.

Kiesecker C, Zitron E, Scherer D, Lueck S, Bloehs R, Scholz EP, et al. Regulation of cardiac inwardly rectifying potassium current IK1 and Kir2.x channels by endothelin-1. J Mol Med (Berl) 2006;84(1):46–56.

Kolettis TM. Coronary artery disease and ventricular tachyarrhythmia: pathophysiology and treatment. Curr Opin Pharmacol 2013;13(2):210–7.

Kolettis TM, Baltogiannis GG, Tsalikakis DG, Tzallas AT, Agelaki MG, Fotopoulos A, et al. Effects of dual endothelin receptor blockade on sympathetic activation and arrhythmogenesis during acute myocardial infarction in rats. Eur J Pharmacol 2008;580(1–2):241–9.

Kolettis TM, Barton M, Langleben D, Matsumura Y. Endothelin in coronary artery disease and myocardial infarction. Cardiol Rev 2013a;21(5):249–56.

Kolettis TM, Kontonika M, Valenti MC, Vilaeti AD, Baltogiannis GG, Papalois A, et al. Arrhythmogenesis after acute myocardial necrosis with and without preceding ischemia in rats. J Basic Clin Physiol Pharmacol 2013b:1–11.

Kruger C, Kalenka A, Haunstetter A, Schweizer M, Maier C, Ruhle U, et al. Baroreflex sensitivity and heart rate variability in conscious rats with myocardial infarction. Am J Physiol 1997;273(5 Pt 2):H2240–7.

Kruger C, Landerer V, Zugck C, Ehmke H, Kubler W, Haass M. The bradycardic agent zatebradine enhances baroreflex sensitivity and heart rate variability in rats early after myocardial infarction. Cardiovasc Res 2000;45(4):900–12.

Kuo JS, Lin NN, Cheng WY, Cheng FC, Chai CY. Effects of reducing sympathetic activities on acute myocardial ischemia in cats. Chin J Physiol 1993;36(2):101–7.

Lee HW, Han TH, Yi KJ, Choi MC, Lee SY, Ryu PD. Time course of diurnal rhythm disturbances in autonomic function of rats with myocardial infarction. Auton Neurosci 2013;179(1–2):28–36.

Martinez-Olivares R, Villanueva I, Racotta R, Pinon M. Depletion and recovery of catecholamines in several organs of rats treated with reserpine. Auton Neurosci 2006;128(1–2):64–9.

Miyauchi T, Yanagisawa M, Tomizawa T, Sugishita Y, Suzuki N, Fujino M, et al. Increased plasma concentrations of endothelin-1 and big endothelin-1 in acute myocardial infarction. Lancet 1989;2(8653):53–4.

Nagayama T, Kuwakubo F, Matsumoto T, Fukushima Y, Yoshida M, Suzuki-Kusaba M, et al. Role of endogenous endothelins in catecholamine secretion in the rat adrenal gland. Eur J Pharmacol 2000;406(1):69–74.

Niskanen JP, Tarvainen MP, Ranta-Aho PO, Karjalainen PA. Software for advanced HRV analysis. Comput Methods Programs Biomed 2004;76(1):73–81.

Oikonomidis DL, Tsalikakis DG, Baltogiannis GG, Tzallas AT, Xourgia X, Agelaki MG, et al. Endothelin-B receptors and ventricular arrhythmogenesis in the rat model of acute myocardial infarction. Basic Res Cardiol 2010;105(2):235–45.

Opitz CF, Mitchell GF, Pfeffer MA, Pfeffer JM. Arrhythmias and death after coronary artery occlusion in the rat. Continuous telemetric ECG monitoring in conscious, untethered rats. Circulation 1995;92(2):253–61.

Opthof T, Coronel R, Vermeulen JT, Verberne HJ, van Capelle FJ, Janse MJ. Dispersion of refractoriness in normal and ischaemic canine ventricle: effects of sympathetic stimulation. Cardiovasc Res 1993;27(11):1954–60.

Proven A, Roderick HL, Conway SJ, Berridge MJ, Horton JK, Capper SJ, et al. Inositol 1,4,5-trisphosphate supports the arrhythmogenic action of endothelin-1 on ventricular cardiac myocytes. J Cell Sci 2006;119(Pt 16):3363–75.

Rafiq K, Nakano D, Ihara G, Hitomi H, Fujisawa Y, Ohashi N, et al. Effects of mineralocorticoid receptor blockade on glucocorticoid-induced renal injury in adrenalectomized rats. J Hypertens 2011;29(2):290–8.

Reisner Y, Meiry G, Zeevi-Levin N, Barac DY, Reiter I, Abassi Z, et al. Impulse conduction and gap junctional remodeling by endothelin-1 in cultured neonatal rat ventricular myocytes. J Cell Mol Med 2009;13(3):562–73.

Remme WJ, Kruyssen DA, Look MP, Bootsma M, de Leeuw PW. Systemic and cardiac neuroendocrine activation and severity of myocardial ischemia in humans. J Am Coll Cardiol 1994;23(1):82–91.

Rubart M, Zipes DP. Mechanisms of sudden cardiac death. J Clin Invest 2005;115(9):2305–15.

Schomig A, Haass M, Richardt G. Catecholamine release and arrhythmias in acute myocardial ischaemia. Eur Heart J 1991;12(Suppl. F):38–47.

Slavikova J, Kuncova J, Topolcan O. Plasma catecholamines and ischemic heart disease. Clin Cardiol 2007;30(7):326–30.

Tawa M, Yamamoto S, Ohkita M, Matsumura Y. Endothelin-1 and norepinephrine overflow from cardiac sympathetic nerve endings in myocardial ischemia. Cardiol Res Pract 2012;2012:789071.

Wennmalm A, Karwatowska-Prokopczuk E, Wennmalm M. Role of the coronary endothelium in the regulation of sympathetic transmitter release in isolated rabbit hearts. Acta Physiol Scand 1989;136(1):81–7.

Yamamoto S, Matsumoto N, Kanazawa M, Fujita M, Takaoka M, Gariepy CE, et al. Different contributions of endothelin-A and endothelin-B receptors in postischemic cardiac dysfunction and norepinephrine overflow in rat hearts. Circulation 2005;111(3):302–9.

Zhao Z, Xie Y, Wen H, Xiao D, Allen C, Fefelova N, et al. Role of the transient outward potassium current in the genesis of early afterdepolarizations in cardiac cells. Cardiovasc Res 2012;95(3):308–16.