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COMMENTARY

Endothelin-1 during myocardial ischaemia: a double-edged sword?

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INTRODUCTION

Ischaemic heart disease remains a major health-related problem worldwide. Despite the widespread use of reperfusion strategies, acute myocardial ischaemia and infarction are associated with considerable morbidity and mortality. In addition to ventricular arrhythmias, acute cardiac failure and cardiogenic shock constitute the most common clinical sequelae. Therefore, the pathophysiology of ischaemia-induced left ventricular dysfunction has been at the centre of cardiovascular research for decades.

ET-1 DURING MYOCARDIAL ISCHAEMIA

Endothelin-1 (ET-1), a 21 amino acid peptide identified by Yanagisawa *et al.* in 1988,[1] is produced by vascular endothelial and smooth muscle cells, as well as by cardiomyocytes. Transcription of the prepro-ET-1 gene leads to the production of prepro-ET-1, a 203 amino acid peptide, which is cleaved to the inactive 38 amino acid precursor big-ET-1.[1] ET-1 is generated from big-ET-1 by endothelin-converting enzyme (ECE), a protease widely expressed in various tissues, including the myocardium. The production of ET-1 increases markedly within minutes after the onset of ischaemia, peaks at 6 h and returns to normal values within 24 h, but remains elevated in patients with complicated infarction.[2] The source of increased ET-1 levels is likely multiple, involving intra- and extracardiac production sites, as well as reduced pulmonary clearance.

The effects of ET-1 are mediated by two (ETA and ETB) G-protein-coupled receptors,[3] which are widely distributed in mammalian organs, including the heart, the adrenal gland and peripheral neurons. Most of the effects of ET-1 are exerted through activation of ETA receptors, which account for more than 90% of endothelin receptors in the ventricular myocardium and the vasculature.[3] In contrast, ETB receptors are not only involved in the clearance of ET-1 and inhibition of ECE, but also in the release of vasodilatory mediators, such as nitric oxide. However, the precise effects of the ETB receptor are yet to be defined, as they may differ in various pathological conditions.

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PATHOPHYSIOLOGICAL ROLE OF ET-1

A growing body of evidence indicates an important role of ET-1 during acute myocardial ischaemia/infarction, which is mainly threefold: (1) ET-1 causes coronary vasoconstriction,[4] thereby ET-1 reduces myocardial perfusion and increases infarct size. (2) ET-1 exerts direct and indirect electrophysiological effects and participates in arrhythmogenesis. In this respect, experimental studies have shown that endothelin receptor blockade decreases ischaemic ventricular arrhythmias.[5,6] (3) The effects of ET-1 on left ventricular function during myocardial ischaemia (with or without reperfusion) are complex, frequently disparate and perhaps the least comprehended. ET-1 increases intracellular calcium concentration (secondary to the stimulation of sarcoplasmic reticulum calcium release and to enhanced L-type calcium current) and can thereby provide short-term inotropic support to the ischaemic myocardium. Conversely, ET-1 decreases cardiac output by increasing left ventricular afterload, secondary to peripheral vasoconstriction induced by the ETA receptor. Finally, an important aspect of the pathophysiology of acute myocardial ischaemia is the interrelation between ET-1 and the autonomic nervous system, as sympathetic stimulation may increase myocardial oxygen demand, deteriorate ischaemia and promote cell death. It has been known for years that ET-1 regulates sympathetic stimulation, but the precise mechanisms and the relative importance of ETA and ETB receptors during myocardial ischaemia are still debated.

Against this background, Tawa *et al.* in this issue of *Hypertension Research* advance current understanding on the effects of ET-1 on post-ischaemic left ventricular function.[7] In Langendorff-perfused rat hearts, subjected to global ischaemia (40-min) followed by (30-min) reperfusion, big-ET-1 was administered in the perfusate, beginning 15 min before ischaemia until 5 min after the onset of reperfusion. Three concentrations (0.1, 0.3 and 1 nM) were tested, and indices of left ventricular function after ischemia/reperfusion were measured. Big-ET-1 increased left ventricular developed pressure and the maximum value of the first derivative of left ventricular pressure and decreased end-diastolic pressure.

These findings were probably unexpected and contradict, in part, those reported[8] previously by the same group under identical experimental conditions; in this study,[8] endogenously generated and exogenously administered ET-1 evoked noradrenaline overflow and produced left ventricular dysfunction. The elegant study by Tawa *et al.* raises several questions that were thoroughly addressed in their experiments. The main ensuing issues are briefly discussed below:

(1) *Were the observed effects due to conversion of big-ET-1 to ET-1*? In search of an explanation for the findings of the present study, one could speculate that big-ET-1 is not really an inactive molecule and may actually exert independent

effects. Despite the fact that only the concentration of 0.3 nM was examined, the experiments in the study by Tawa *et al.* proved that this is not the case, as the effects of big-ET-1 were markedly attenuated, when administered in the presence of an ECE inhibitor. Moreover, ET-1 content in the coronary effluent (measured 5 min after reperfusion) increased, corresponding to big-ET-1 concentrations. These findings indicate that exogenously applied big-ET-1 converted to ET-1 (at a ratio of approximately 25%) and the observed effects were due to this conversion.

(2) *Were the observed effects dose dependent*? The cardioprotective effects of big-ET-1 were clear-cut only in the middle (0.3 nM) concentration, whereas they were less pronounced with the low (0.1 nM) and high (1 nM) concentrations. These results indicate a bell-shaped dose–response curve.

(3) *What is the likely explanation for the beneficial effects of big-ET-1 on left ventricular function*? Based on the study by Tawa *et al.*, the explanation for the effects of big-ET-1 after ischaemia/reperfusion may be twofold: first, it may be attributed to an increase in coronary blood flow (after reperfusion), as was observed after big-ET-1 administration (only at the concentration of 0.3 nM); second and foremost, the improved left ventricular function appeared to be mainly secondary to decreased sympathetic stimulation, evidenced by a decrease in noradrenaline overflow in the coronary effluent. This conclusion was based on the similar dose–response pattern for the three tested concentrations of big-ET-1 for both effects, namely (1) for the decrease in noradrenaline overflow and (2) for the improvement in left ventricular function. Again, it should be noted that the decrease in noradrenaline overflow reached statistical significance only after perfusion of 0.3 nM big-ET-1 and not with the other two tested concentrations. Co-administration of big-ET-1 (0.3 nM) with an ECE inhibitor prevented its effects. Correlation analysis revealed significant (albeit moderate) linear correlation between the decrease in noradrenaline overflow and the improvement in left ventricular function.

(4) *What was the relative contribution of ETA and ETB receptors*? ETB receptor blockade reversed both effects of big-ET-1, strongly suggesting that they were mediated by ETB receptor stimulation. This observation reinforces previous conclusions by the same group[8] on the protective role of the ETB receptor during acute myocardial ischaemia. Moreover, it is in line with recent findings in our laboratory;[9] using the *in vivo* rat model of coronary ligation, we compared two animal cohorts, namely wild-type rats and homozygous ETB-receptor-deficient rats (developed and characterized by Professor M Yanagisawa and colleagues) during a 24-h observation period. During the first hour after ligation, indices of sympathetic activation (such as heart rate and the ratio of low- to high-frequency spectra after fast Fourier analysis of heart rate variability), as well as serum adrenaline and noradrenaline were higher in ETB-deficient rats. As a result, ventricular arrhythmogenesis and total (arrhythmic and non-arrhythmic) mortality rates were markedly increased in ETB-deficient rats. However, these effects diminished during subsequent phases (2–24 h) of evolving myocardial infarction. The data reported by Tawa *et al.*, in context with previous work,[8,9] shed further light to a long-standing caveat on the role of the ETB receptor. Nevertheless, it should be emphasized that this information applies only to acute myocardial ischaemia and should not be extrapolated to other chronic disease states.

(5) *How can the diverse actions of endogenous ET-1 and exogenous big-ET-1 be explained*? The findings of this study are intriguing, but difficult to explain. The explanation provided by the authors, based on previous work,[10] is currently the only plausible one. A graphic illustration is shown in Figure 1. According to this explanation, endogenous and exogenous big-ET-1 may be cleaved by two ECE-1 isoforms: endogenous big-ET-1 may be cleaved by ECE-1a, which is located intracellularly, whereas exogenous big-ET-1 is cleaved by ECE-1b, which is located on the cell surface, probably in proximity to the ETB receptor. Therefore, in contrast to endogenous ET-1, ET-1 produced by exogenous big-ET-1 preferentially functions on the ETB receptor. Obviously, this interesting hypothesis merits further investigation.

FUTURE DIRECTIONS

The study by Tawa *et al.* provides valuable information on the effects of exogenous big-ET-1 administration on left ventricular function and sympathetic activation, as well as on the role of ETA and ETB receptors. The Langendorff-perfused isolated rat heart model provides high degree of accuracy in the evaluation of left ventricular function, but it is associated with all the limitations of an *ex vivo* model. As such, Langendorff preparations are not only devoid of sympathetic innervation, but also they do not examine adrenal catecholamine secretion. The latter is important, as the regulatory effects of ET-1 on sympathetic stimulation are exerted not

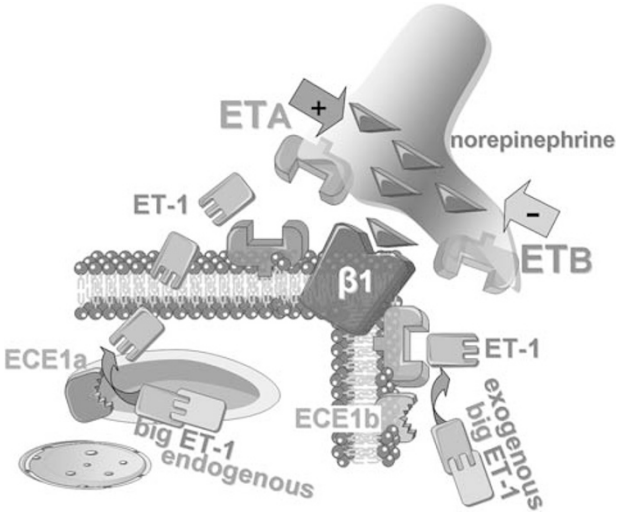

Figure 1 Proposed explanation for the diverse effects of endogenous and exogenous big-ET-1. Note the two isoforms of endothelin-converting enzyme (ECE) and the proximity of ECE1b to the endothelin-B-receptor (ET_B). As a result, ET-1 from exogenous big-ET-1 activates preferentially the ET_B over the ETA receptor.

only in the myocardium, but also in the adrenal gland.[11] Therefore, the results of Tawa *et al.* should be confirmed in *in vivo* experiments that will facilitate more comprehensive evaluation and will permit the extension of the observation period after ischaemia/infarction.

1 Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988; **332**: 411–415.
2 Stewart D, Kubac KB, Costello KB, Cernacek P. Increased plasma endothelin-1 in the early hours of acute myocardial infarction. *J Am Coll Cardiol* 1991; **18**: 38–43.
3 Molenaar P, O'Reilly G, Sharkey A, Kuc RE, Harding DP, Plumpton C, Gresham GA, Davenport AP. Characterization and localization of endothelin receptor subtypes in the human atrioventricular conducting system and myocardium. *Circ Res* 1993; **72**: 526–538.
4 Kyriakides ZS, Kremastinos DT, Psychari SN, Kolettis TM, Sbarouni E, Webb DJ. Coronary vasoconstriction after coronary angioplasty is attenuated by endothelin receptor-A antagonism. *Am J Cardiol* 2001; **87**: 1011–1013.
5 Baltogiannis GG, Tsalikakis DG, Mitsi AC, Hatzistergos KE, Elaiopoulos D, Fotiadis DI, Kyriakides ZS, Kolettis TM. Endothelin receptor-A blockade decreases ventricular arrhythmias after myocardial infarction in rats. *Cardiovasc Res* 2005; **67**: 647–654.
6 Kolettis TM, Baltogiannis GG, Tsalikakis DG, Tzallas AT, Agelaki MG, Fotopoulos A, Fotiadis DI, Kyriakides ZS. Effects of dual endothelin receptor blockade on sympathetic activation and arrhythmogenesis during acute myocardial infarction in rats. *Eur J Pharmacol* 2008; **580**: 241–249.
7 Tawa M, Fukumoto T, Ohkita M, Yamashita N, Geddawy A, Imamura T, Ayajiki K, Okamura T, Matsumura Y. Effects of exogenous big endothelin-1 on postischemic cardiac dysfunction and norepinephrine overflow in rat hearts. *Hypertens Res* 2011; **34**: 218–224.
8 Yamamoto S, Matsumoto N, Kanazawa M, Fujita M, Takaoka M, Gariepy CE, Yanagisawa M, Matsumura Y. Different contributions of endothelin-A and endothelin-B receptors in postischemic cardiac dysfunction and norepinephrine overflow in rat hearts. *Circulation* 2005; **111**: 302–309.
9 Oikonomidis DL, Tsalikakis DG, Baltogiannis GG, Tzallas AT, Xourgia X, Agelaki MG, Megalou AJ, Fotopoulos A, Papalois A, Kyriakides ZS, Kolettis TM. Endothelin-B receptors and ventricular arrhythmogenesis in the rat model of acute myocardial infarction. *Basic Res Cardiol* 2010; **105**: 235–245.
10 Emoto N, Nurhantari Y, Alimsardjono H, Xie J, Yamada T, Yanagisawa M, Matsuo M. Constitutive lysosomal targeting and degradation of bovine endothelin-converting enzyme-1a mediated by novel signals in its alternatively spliced cytoplasmic tail. *J Biol Chem* 1999; **274**: 1509–1518.
11 Yamaguchi N. Role of ETA and ETB receptors in endothelin-1-induced adrenal catecholamine secretion *in vivo*. *Am J Physiol* 1997; **272**: R1290–R1297.