Endothelin and the ischaemic heart

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Introduction

It has been widely documented that plasma ET-1 and big ET-1 levels are increased in patients within a day of onset of acute myocardial infarction [1,2,3,4]. Similar changes in systemic plasma ET-1 are seen in animal models of myocardial ischaemia [5,6]. The source of the released ET-1 appears to be the area subjected to ischaemic damage, since in a study in anaesthetised pigs both short and long periods of left anterior descending (LAD) coronary artery occlusion were found to enhance overflow of ET-1 from the area of myocardium subjected to the ischaemia/reperfusion insult [7]. The myocardial tissue level of ET-1 has been found to be increased by 3 to 7 fold in the ischaemic area compared to the non-ischaemic myocardium, suggesting an increased biosynthesis of ET-1 during ischaemia/reperfusion [5,7]. Indeed, the demonstration of increased ET-1 mRNA levels in cardiomyocytes subjected to ischaemia [8], supports this notion that that the raised circulating levels of endothelin are not simply due to release of stored peptide. The observation that ischaemia and reperfusion increases ^{125}I-labelled ET-1 binding sites in rat cardiac membranes [9] further supports the view that the endothelin system is up-regulated during these events, although the observation that there is no difference in ET-1 mRNA levels between animals subjected to ischaemia only and hearts subjected to ischaemia followed by reperfusion, implies that it is ischaemia that stimulates enhanced ET-1 production. Taken together, these observations have led to the concept that endothelin may play an important role in determining the outcome of myocardial ischaemia/reperfusion.

Based on our knowledge that ET-1 is a potent vasoconstrictor peptide, early hypotheses focused on the role of ET-1 as a detrimental factor in the

ischaemic/reperfused heart. However, over the last decade or so, as our understanding of the cellular effects of endothelin has extended beyond that of vasoconstriction, evidence is emerging that endothelin may play a very complex role in the setting of the ischaemic heart, with the potential to both contribute to cellular injury and cellular repair. The aim of this review therefore is to provide an overview of the effects of endothelin on the cardiomyocyte, in the setting of myocardial ischaemia, and within this to consider not only its actions mediated through vasoconstriction, but also through its effects on ion channels, cellular integrity and inflammatory cells.

Contribution of endothelin to ischaemia/reperfusion-induced myocardial injury.

The possibility that the coronary vasoconstrictor effects of endothelin could play a role in the development of injury resulting from myocardial ischaemia arose following a number of demonstrations that intracoronary infusion of exogenous ET-1 reduces coronary blood flow by approximately 90% [10-12], resulting in marked myocardial ischaemia. Substantive evidence for a contributory role of *endogenous* ET-1 in infarct extension subsequently emanated from studies that demonstrated that a monoclonal antibody to ET-1 can reduce infarct size in rats following ischaemia/reperfusion [5]. The ensuing development of both selective ET_A receptor and mixed ET_A/ET_B receptor antagonists led to the opportunity to demonstrate that blocking the actions of endogenous ET-1 prior to the onset of ischaemia resulted in preservation of the myocardium. Subsequently, there is now a very large literature to demonstrate that both ET_A-selective (e.g. BQ123; [13]) and mixed ET_A/ET_B receptor antagonists (e.g. LU135252 and bosentan; [12, 14]) can reduce myocardial infarct size following ischaemia and reperfusion. Since ET_B receptor blockade does not appear to be a

prerequisite to observe this effect, this led to the conclusion that ET_A receptors mediate the actions of endothelin that contribute to tissue injury.

Blockade of the intense coronary vasoconstriction induced by endothelin, which would contribute to the "no-reflow" phenomenon, can be considered a prime candidate for the mechanism underlying the protective effects of endothelin antagonists on cardiomyocyte integrity. However, there are other actions of endothelin that may also contribute to tissue injury. For example, ET-1 is known to activate polymorphonuclear leukocytes to generate reactive oxygen species [15,16], which have been identified as mediators of reperfusion injury, and studies with the ET_A receptor antagonist LU135252 have shown that this agent reduces both myocardial necrosis and tissue myeloperoxidase activity in the ischaemia/reperfused heart [17]. Furthermore, the protective effects of ET-1 antagonists have been proposed to involve inhibition of ET-1-induced activation of phospholipase C and subsequent release of Ca^{2+} [18], superoxide production [19] and suppression of NO production [20,21].

Although the majority of studies assessing the effects of ET-1 antagonists on infarct size demonstrate a cardioprotection, there are also numerous studies that report no beneficial effects in the setting of myocardial ischaemia. Although both bosentan [12] and BQ123 [13,22,23] have shown cardioprotective effects in various animal models, there are several studies using these agents that have failed to demonstrate protection against myocardial infarction [24,25]. Studies with other ET_A selective (FR-139317 and PD-156707) antagonists have also shown either a reduction in [26,27] or no effect on [28] infarct size. There are several explanations for these conflicting findings.

First, the outcome may be dependent upon the animal model used, although this can not always explain the discrepancy since studies with the same antagonist in the same species do not always agree [29,30]. A second explanation could be the dose employed since there is evidence that high doses of ET_A receptor antagonists may block the low affinity ET_B binding site linked to nitric oxide release [31], which would abrogate any potentially protective contribution of nitric oxide production through activation of these receptors by endogenous endothelin, but not the high affinity ET_B binding site linked to vasoconstriction. A similar argument may apply to mixed ET_A/ET_B receptor antagonists, since they too appear to possess different affinities for two different ET_B receptor subtypes [32]. Neither of these explanations can account for all of the incongruities between the large number of studies using ET receptor antagonists against myocardial infarction, giving us an inkling that perhaps, in the setting of the ischaemic heart, the actions of ET-1 in myocardial survival cannot all be labelled as "bad".

Cardioprotective effects of ET-1 mediated by the ET_A receptor

Following on from the endothelin antagonist revolution, a small number of studies have emerged to demonstrate that, in the *in vivo* and *in vitro* rabbit heart, exogenous administration of ET-1 as a preconditioning stimulus can reduce myocardial infarct size [33,34], with the subsequent confirmation of this effect in the isolated rat heart [35]. These studies also provided evidence that this effect appeared to be achieved through ET_A receptor-mediated activation of protein kinase C and K_{ATP} channels, as has been suggested for ischaemic preconditioning [36]. This has recently been corroborated in an *in vivo* rat study [37] which provides evidence to support the hypothesis that it is activation of the mitochondrial K_{ATP} channel (through the ET_A

receptor; [38]) that is responsible for ET-1 induced cardioprotection. However, as we begin to understand ET-1 physiology a little more, there are several alternative paradigms that might explain ET_A receptor mediated cardioprotection.

Inhibition of cardiac myocyte apoptosis.

There is growing evidence that apoptosis is associated with acute myocardial infarction, with apoptotic cardiomyocytes being found predominantly in the hypo-perfused border area lying between the "normal" myocardium and the core of the infarct (reviewed in [39]). Moreover, hypoxia and re-introduction of oxygen in isolated cardiomyocytes has been shown to induce apoptosis [40,41]. Evidence for a pathophysiological significance of apoptosis emanates from studies that have demonstrated the ability of caspase inhibitors to reduce infarct size [42]. There is now increasing evidence to show that ET-1 can inhibit apoptosis in cardiomyocytes [43], as well as other cells, through a signalling mechanism that involves induction of GATA-4 [44], which is one of a number of DNA binding proteins responsible for regulating cell lineage differentiation, progenitor cell proliferation and organ morphogenesis [39]. ET-1 has been shown to induce phosphorylation of GATA-4 [44] in an atrial cell line, to increase the interaction between GATA-4 and NFATc in cardiomyocytes [45] and increase GATA-4 DNA-binding activity [44], all of which may contribute to its anti-apoptotic effect. Furthermore, PPARγ has been implicated in the perturbation of the anti-apoptotic signalling pathway of ET-1, since activators of PPARγ inhibit the cardiomyocyte protection afforded by ET-1 [46]. Moreover, ET-1 has recently been shown to activate a protective pathway that is unique to cardiomyocytes, in addition to common pathways among other cell types [47], which provides an exciting avenue to explore for selective inhibition of apoptosis within the

heart. Interestingly, ET-1 has been shown to act through stabilisation of the mitochondrial membrane to protect the cardiomyocyte from apoptosis, rather than through preservation of the mitochondrial respiratory chain [48], thus implying that its action is required prior to the induction of injury, rather than once injury has begun, Taking this evidence together, it would therefore be of great interest to elucidate whether or not inhibition of apoptosis represents a mechanism by which exogenous ET-1 improves cardiomyocyte survival during ischaemia/reperfusion.

Mast cell activation

While the traditional view regarding ET-1 release in the ischaemic heart is that it largely emanates from the vascular endothelium, we now know that it is generated by a range of cells, including granulation tissue and fibroblasts [49,50], at the site of infarction. One very early observation of an alternative source of ET-1 comes from studies by Ehrenreich et al [51], who demonstrated that mast cells synthesize and secrete ET-1. Furthermore, these cells were also seen to express ET_A receptors. Since then, ET-1 has been shown to induce mast cell degranulation in the gut [52] and, more recently, in the rat heart [53]. Recent work from our own group has similarly demonstrated mast cell degranulation in response to ET-1 in the murine heart, with an associated increase in tryptase activity in the myocardium [54]. Mast cells are recognised as important sentinels of the innate immune response [55] and have long been implicated in the inflammatory events that occur after a myocardial infarction. The effects of ET-1 on mast cell degranulation appear to be dependent upon both the type of mast cell (e.g. bone-marrow-derived versus fetal skin-derived mast cells) [56] and on the microenvironment (reviewed in [57]) and, until very recently, a phenomenon that could not be easily explained. However, the recent study

by Maurer et al [58] has provided unique insight into what now appears to be an ingenious homeostatic mechanism by which ET-1 induced stimulation of mast cells results in protection against ET-1 mediated toxicity. In these studies, mast cell deficient mice administered ET-1 intraperitoneally fared much worse with respect to toxicity to ET-1 than wild type mice. Through an elegant series of experiments, the authors demonstrated that one of the mechanisms by which mast cell-mediated protection against ET-1 associated pathology is achieved is through a reduction in the concentration of ET-1 by release of proteolytic enzymes. This observation implies a "double-edged sword" role for endothelin, whereby not only is it responsible for inducing damage, but also for limiting its own effects. In the setting of myocardial ischaemia/reperfusion, therefore, this may offer a possible explanation for the cardioprotective effects of an agent that, upon its original discovery, was automatically labelled as a "bad guy".

Cardioprotective effects mediated through the ET_B receptor.

The above evidence implying a cardioprotective role for ET-1 via an ET_A receptor mechanism is all well and good, but we should not overlook the potential role of the action of exogenous ET-1 at the ET_B receptor. Based upon observations that short term ischaemia/reperfusion in the isolated rat heart results in a trend to an increase in ET_B, but not ET_A receptor, binding sites [59]) and an increase in mRNA expression for both ET_A and ET_B receptors [60], we recently undertook a series of studies to determine the effects of ET_B receptor activation with the ET_B receptor agonist sarafotoxin 6c (S6c) on myocardial infarct size and on ischaemia/reperfusion induced changes in ET_A and ET_B receptor mRNA levels in the *in vivo* rat heart. We demonstrated that while ischaemia/reperfusion resulted in a marked reduction in

mRNA for both receptor subtypes in the ischaemic (but not the normal) zone of the heart, pre-treatment with S6c significantly reduced myocardial infarct size concomitant with a preservation of ET_B receptor mRNA [61]. Preservation of ET_B receptors within the ischaemic zone could conceivably benefit the ischaemic heart in two ways. First, by preferentially preserving the ET_B receptor; this would allow endogenously generated ET-1 to act at this receptor, which mediates physiological responses (such as vasodilatation) that could be categorised as protective rather than destructive, rather than inducing damaging effects through the ET_A receptor. The second benefit could be achieved through increased clearance of ET-1 from the local environment, since this is largely achieved through the ET_B receptor [62]. In addition to preserving ET_B receptor mRNA, activation of ET_B receptors prior to ischaemia could similarly activate a number of mechanisms that could afford protection to the heart, such as direct release of prostacyclin and/or nitric oxide, both of which are cardioprotective [63,64] or induction of a leukocyte-mediated oxidative stress response, which has similarly been linked to cardioprotection [65]. In support of the latter, we have previously demonstrated that S6c can increase the *ex vivo* generation of reactive oxygen species from leukocytes following *in vivo* administration [66].

Thus, the role of endothelin in the cellular outcome following myocardial ischaemia is far from straightforward and involves both cytotoxic and cytoprotective effects, providing various paradigms for therapeutic targets (summarised in Figure 1).

Contribution of endothelin to ventricular arrhythmias.

Cellular injury is not the only adverse outcome in the ischaemic heart to which ET-1 is thought to contribute. The earliest studies aimed at determining the effects of intra-

coronary ET-1 in the normal heart all demonstrated in pigs [67], rats [68] and dogs [69] that there was marked arrhythmic activity associated with a powerful vasoconstriction that induced ischaemia. However, a subsequent study by Yorikane et al [70] provided the first implication that exogenous ET-1 may exert direct electrophysiological effects (prolongation of the action potential and induction of early after-depolarisations) that underlie its ability to induce arrhythmias. However, the effect of exogenous ET-1 on arrhythmias in the setting of myocardial ischaemia was not addressed until, in an early study from our group [71], we demonstrated that exogenous administration of ET-1 during *in vivo* myocardial ischaemia in rats substantially increased arrhythmia severity and that this could be abrogated by concomitant administration of the ET_A antagonist BQ123, implying an action of ET-1 through the ET_A receptor. The importance of the ET_A receptor in arrhythmogenesis was subsequently corroborated in an *in vitro* study in rat hearts, whereby BQ-485 prevented ET-1-induced arrhythmias in normal, non-ischaemic hearts [72]. However, our study also demonstrated that BQ123 alone could also reduce ischaemia-induced arrhythmias, providing the first demonstration of a role for *endogenous* ET-1 in arrhythmogenesis in the ischaemic heart [71]. The potential for ET_A antagonists to act as anti-arrhythmic agents as well as to reduce myocardial injury has subsequently been well examined and, overall, the evidence points to a potentially beneficial effect of these agents against ischaemic arrhythmias (for a thorough review of this topic see [73]), although this is by no means universal [74]. As with cellular injury, however, there remains some debate as to whether selective ET_A receptor blockade is preferable to mixed ET_A/ET_B receptor inhibition, since some studies show that ET_B receptor blockade with either of the ET_B selective antagonists IRL-1038 and IRL-1025 has no effect on arrhythmias [75], thus precluding the need for mixed antagonism in this

setting, whereas others demonstrate an antiarrhythmic effect of ET_B receptor blockade [76]. The antiarrhythmic effect of ET-1 antagonism has also been observed against high-glucose induced electrical instability (QT prolongation) through an action predominantly at the ET_A receptor, since both an ET_A selective (FR139317) and a mixed ET_A/ET_B (SB209670) antagonist, but not a selective ET_B antagonist (BQ788), was able to block its effects [77]. As with all studies on arrhythmias, consideration should always be made of the possible direct effects of agents on cardiac electrophysiology independent of their action at endothelin receptors. Indeed, there is some evidence that, at least in the case of BQ123, such a direct effect on cardiac electrophysiology may, in part, contribute to its antiarrhythmic effects [78]. Notwithstanding this, while there is clear evidence for an antiarrhythmic effect of ET-1 antagonism, what remains highly controversial is whether the pro-arrhythmic effect of ET-1 itself is mediated through a direct electrophysiological effect or occurs as a consequence of its other actions, such as vasoconstriction, and warrants detailed review.

Mechanisms underlying the pro-arrhythmic effect of ET-1

Several *in vivo* studies have attempted to identify the mechanisms by which exogenous ET-1 induces a pro-arrhythmic effect. In view of ET-1's known coronary vasoconstrictor action leading to myocardial ischaemia, the *in vivo* electrophysiological actions of ET-1 have been compared to those of myocardial ischaemia. Such studies, all carried out in anaesthetised dogs, have concluded that ET-1 has a direct arrhythmogenic effect independent of its ability to cause myocardial ischaemia. The evidence from a study by Szabo *et al*. [79] was that ET-1, given directly into the coronary artery, induced arrhythmias without any electrocardiographic or metabolic signs of ischaemia. Indeed, ET-1 significantly

increased left ventricular epicardial (LV_{epi}) and right ventricular endocardial (RV_{endo}) monophasic action potential duration (MAPD) with no change in the upstroke velocity. This was in direct contrast to coronary artery occlusion, which decreased MAPD and upstroke velocity in the ischaemic area [79]. In another study from the same group by Becker *et al.* [80], intra-coronary administration of ET-1 induced ventricular arrhythmias that were focal in nature, without changing local refractory periods, left ventricular conduction time or the overall activation pattern. This was also quite distinct from the changes induced by coronary artery occlusion, which resulted in local conduction delay, prolonged refractoriness in all layers and arrhythmias that were maintained by re-entrant mechanisms [80]. Low dose intra-coronary ET-1 (at a concentration that did not cause a significant reduction in coronary flow) was also shown to prolong epicardial and endocardial MAPD [81]. An increase in the spatial dispersion of MAPD was demonstrated with the MAP close to the infusion site showing the most pronounced prolongation. Significant differences were observed between the lengthening of the right and left ventricular MAPD times and the lengthening of the epicardial and endocardial MAPD times. This increase in dispersion of MAPD is thought to play an important role in the pathogenesis of ventricular arrhythmias, albeit that this is in the formation of re-entry circuits. Support for ET_A receptors mediating these proposed electrophysiological effects of ET-1 comes from studies with the ET_A antagonist LU 135.252. In these experiments, LU 135.252, was found to be anti-arrhythmic and was able to inhibit the main electrophysiological actions of ET-1 in the anaesthetised dog, namely MAPD prolongation and early after depolarisation (EAD) formation, while being unable to prevent the vasoconstriction caused by ET-1 suggesting that these direct electrophysiological effects of ET-1 are mediated through the ET_A receptor [82].

In contrast to these *in vivo* studies, our group have examined the electrophysiological effects of ET-1 and S6c, the ET_B receptor specific agonist, in an isolated paced working rabbit heart model which is devoid of neural and humoral influences. Over a wide concentration range, ET-1 reduced $MAPD_{90}$ but did not increase endocardial or epicardial $MAPD_{90}$ or refractoriness as has been reported *in vivo*. Furthermore, the abbreviation of $MAPD_{90}$ was, at all concentrations, associated with a concomitant reduction in coronary flow, providing no evidence of any direct electrophysiological effect of ET-1. S6c lacked any marked electrophysiological effect in either the endo- or the epicardium suggesting that the ET_B receptor does not play a major role in any observed electrophysiological action in the ventricles [83; In Press]. Our study further investigated whether ET-1 or S6c could modify the electrophysiological changes induced during myocardial ischeamia/reperfusion. As shown in Fig 2, ET-1 but not S6c, shortened $MAPD_{90}$ before coronary artery occlusion but did not cause further shortening than that induced during myocardial ischaemia. This data is not, therefore, in agreement with the *in vivo* studies in the dog and may suggest that an intact nervous system or the presence of blood is necessary to observe electrophysiological effects that are independent of myocardial ischaemia.

There are, however, also *in vitro* studies in isolated cardiac tissue or cells which support the view that ET-1 has direct electrophysiological effects on the ventricles. In isolated canine cardiac tissue ET-1 causes a prolongation of the action potential duration, measured at the plateau phase, i.e. APD_{50} in the right bundle branch, Purkinje cells and ventricle with the prolongation being most marked in the right bundle branch [70]. This study also demonstrated the development of EADs during

the prolonged plateau phase after ET-1 administration. Nicardipine was shown to abolish these EADs, suggesting that the voltage sensitive calcium current is involved in their genesis [70]. It should be noted, however, that the concentrations of ET-1 used in this study were more than 10 fold higher than the concentration of ET-1 (10^{-10}M) required to cause a significant decrease in coronary flow in our study in the isolated rabbit heart. Thus, in the intact heart it may not have been possible to observe this ET-1 induced prolongation in action potential because of the counteracting effect of myocardial ischaemia. In neonatal rat ventricular myocytes, ET-1 was found to increase Ca^{2+} entry through the sarcolemmal T-type Ca^{2+} channel, possibly through a pathway involving PKC [84]. An increase in L-type Ca^{2+} current (I_{CAL}), which would be expected to prolong the plateau phase of the action potential and its duration has also been reported in adult rat ventricular myocytes. This effect was found to be ET receptor mediated and involved a PKC mediated pathway [85].

In contrast to the studies that have shown that ET-1 can stimulate Ca^{2+} current there have been several studies reporting an inhibition of Ca^{2+} current or lack of effect on basal Ca^{2+} current. Thus, ET-1 administration decreased the L-type Ca^{2+} current in isolated canine ventricular myocytes [86,87], in guinea pig [88], rabbit [89] and human myocytes [90]. In guinea pig ventricular myocytes, ET-1 had little effect on basal L-type Ca^{2+} current but it did reduce this current enhanced by β_1 adrenoceptor stimulation [91]. This anti-adrenergic effect of ET-1 on I_{CAL} has been reported also in canine ventricular myocytes [86]. Thus, there is controversy in the literature about the role of I_{CAL} in the observed prolongation of action potential duration by ET-1 with the majority of studies supporting the view that an increase in this current is unlikely to be the main underlying mechanism.

The delayed rectifier K^+ current (I_K), is an important determinant of action potential duration, which has been demonstrated to be regulated by ET-1. ET-1 has been shown either to enhance or decrease I_K in guinea pig ventricular myocytes [92,93], to decrease it in human myocytes [90] and to have no effect on this current in canine myocytes [86]. ET-1 has a more clear-out effect to inhibit an enhanced I_K, following β_1-adrenoceptor stimulation in a range of species [86,93]. This effect was mediated by the ET_A receptor and through a PTX sensitive G-protein/Protein Kinase A (PKA) pathway [93]. ET-1 has also been shown to modulate the ATP sensitive K^+ channel (K_{ATP}). In guinea pig ventricular cells ET-1 caused a partial inhibition of the K_{ATP} current, which was abolished by BQ-485, an ET_A receptor antagonist [94], suggesting involvement of the ET_A receptor. In the same study, ET-1 had no effect on normal action potential duration (measured in papillary muscle), but, it partially reversed the cromakalim, a K_{ATP} channel opener, induced shortening of duration. Overall, ET-1 effects on potassium currents are also controversial with no clear explanation of an ionic basis for a prolongation of normal action potential duration. Given the pronounced anti-adrenergic effect of ET-1 on both calcium and potassium current augmented by β_1-adrenoceptor stimulation it is possible that ET-1 will exhibit electrophysiological effects that are seen *in vivo* but not *in vitro*. That these anti-adrenergic effects would, in turn, be pro-arrhythmic is hard to reconcile with the known pro-arrhythmic actions of sympathetic stimulation.

There are other possible mechanisms by which ET-1 might act to alter cardiac electrophysiology and arrhythmogenicity. In both rat and cat atrial myocytes ET-1 was found to exhibit an arrhythmogenic effect, which was suppressed by the inositol

1,4,5 trisphosphate (IP3) receptor antagonist, aminoethoxydiphenyl borate (2-APB), suggesting that activation of IP3 may be involved through an increase in Ca^{2+} release from the sarcoplasmic reticulum [95,96]. Activation of the cardiac Na^{+}-Ca^{2+} exchanger (reverse mode activation) has been shown in guinea pig ventricular myocytes following ET-1 administration, which was found to be through a PKC dependent mechanism [97]. Reverse mode activation of the Na^{+}-Ca^{2+} exchanger results in Ca^{2+} influx and may underlie arrhythmias involving after-depolarisations. The arrhythmogenic effects of ET-1 may also involve activation of the Na^{+}-H^{+} exchanger leading to acidosis since activation of the Na^{+}-H^{+} exchanger has been observed in rabbit ventricular myocytes following ET-1 administration [98]. Acidosis, in turn, results in Ca^{2+} overload through the activation of the Na^{+}-Ca^{2+} exchanger. Moreover, Na^{+}-H^{+} exchanger activation has been linked with a shortening of the monophasic action potential duration in pigs following coronary artery occlusion [99]. It is noteworthy, however, that these direct electrophysiological effects of ET-1, with the exception of the studies on Na^{+}-H^{+} exchanger, have been observed with concentrations that are greater than those which cause coronary vasoconstriction raising the question of their relevance to ET's pro-arrhythmic action *in vivo*.

Evidence for an antiarrhythmic effect of endothelin

Since ET-1 can mimic ischaemic preconditioning against myocardial injury (as described above), and given that ischaemic preconditioning can also protect against ventricular arrhythmias [100], it would seem feasible that ET-1 may also induce an anti-arrhythmic effect when given exogenously. In the aforementioned studies demonstrating an infarct-reducing effect of ET-1 when given prior to the onset of a

period of ischaemia the effect of this exogenous ET-1 administration on consequent arrhythmias was not reported. Our group undertook a study to specifically address this question and observed that ET-1 can indeed reduce ischaemic arrhythmias in an *in vivo* rat model of ischaemia and reperfusion [76]. In those studies we were unable to elucidate the receptor responsible for mediating the antiarrhythmic effect of ET-1, due to inherent antiarrhythmic effects of antagonists masking any blockade of the antiarrhythmic action of ET-1. However, in a subsequent study we demonstrated that the ET_B agonist S6c was also able to reduce the incidence of ischaemic arrhythmias [101], implying that the antiarrhythmic action of ET-1 is mediated through the ET_B receptor, rather than through the ET_A receptor. Although the mechanisms underlying the antiarrhythmic effects of ET-1 remain undetermined, we have observed that its effects are much less evident in the isolated heart compared to the *in vivo* heart, signifying that the mechanisms are likely to be more complex than a direct effect on the myocardium, the coronary blood vessels, or both [102]. In contrast, in the studies with S6c we found that this was able to protect equally well in both the *in vivo* and *in vitro* heart. Taken together this suggests that the antiarrhythmic effect of ET-1 is no less complex than the cytoprotective effects, probably involving a number of mechanisms. However, more studies are required to fully elucidate this protective effect of endothelin.

Concluding Comments

As implied in the introduction to this review, the role of ET-1 in the setting of the ischaemic heart is much more complex than originally perceived. Clearly there are benefits of blocking the actions of ET-1, yet the strong evidence now pointing to protective effects of this peptide tells us that we need to resolve a number of issues

before the most appropriate form of anti-endothelin therapy can be developed. For example, can we block the injurious facet of ET-1's action on cardiomyocyte integrity, while at the same time harness its ability to contribute to tissue repair or change the homeostatic balance of the levels of the peptide in favour of tissue survival? Furthermore, with respect to the pro-arrhythmic effects of endothelin, will we ever be able to resolve the argument surrounding the direct versus indirect action of ET-1, or is the combined physiology of the ischaemic heart and of ET-1 too intricate to dissect out completely? If we can answer these questions then we should have a very powerful tool available to us to modulate ET-1 in the ischaemic heart for the best possible outcome.

Acknowledgement

The authors are grateful to the British Heart Foundation for their support of studies into the effects of endothelin in the ischaemic heart.

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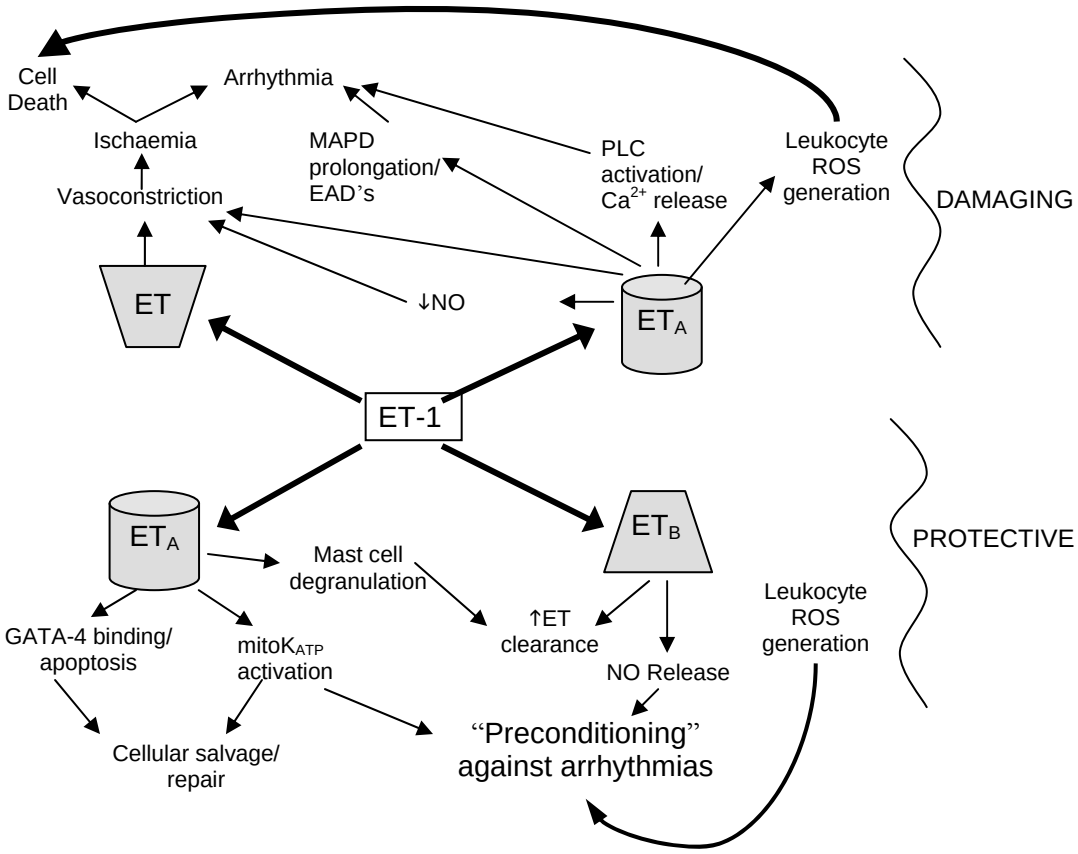

Figure 1: Paradigm for the complex actions of endothelin in the ischaemic heart that contribute to both cellular damage and to cardioprotection (see text for full details). MAPD – monophasic action potential duration; EAD's – early after depolarisations; mitoKATP – mitochondrial ATP-dependent potassium channel; ROS – reactive oxygen species.

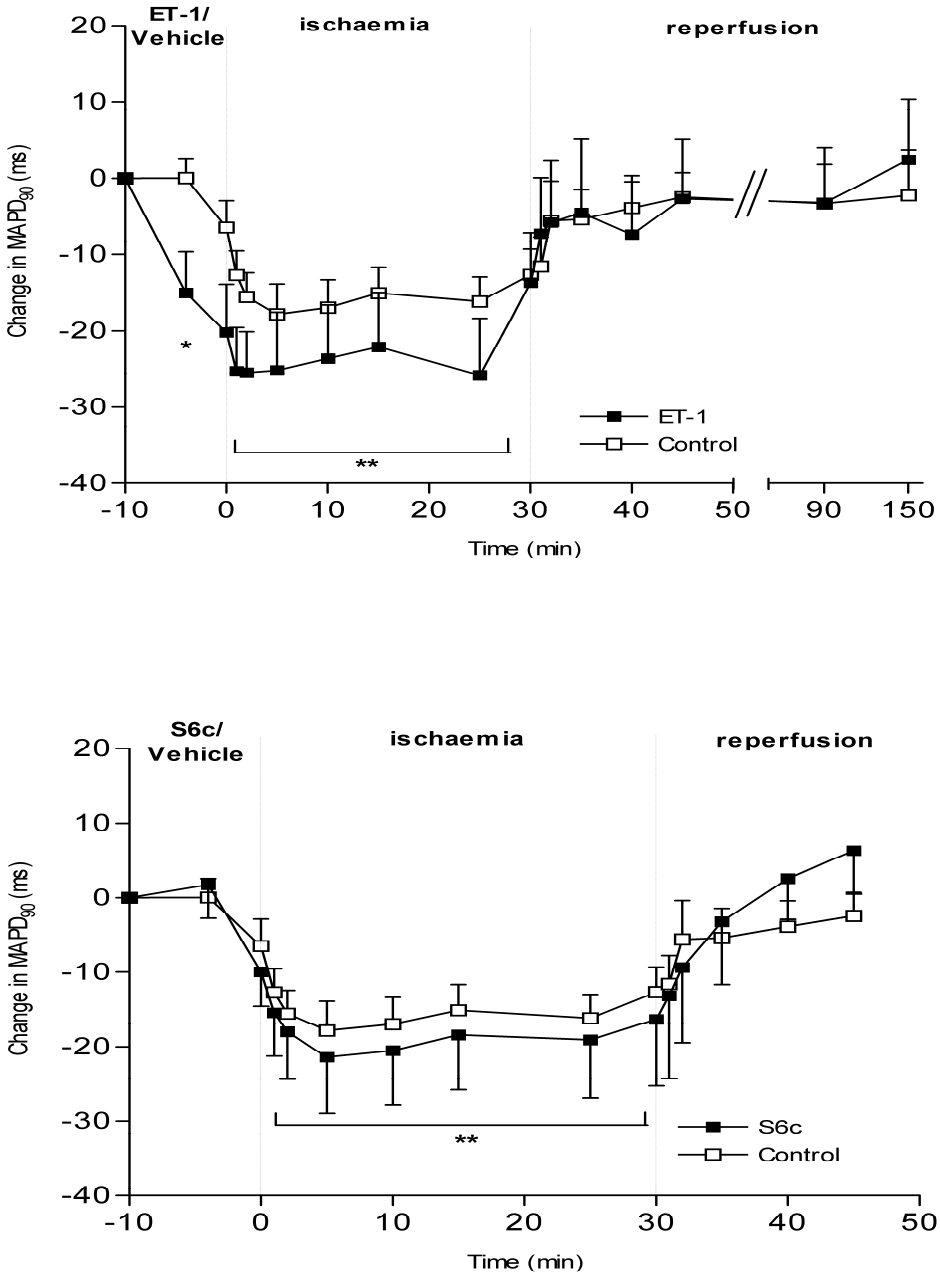

Figure 2. Change in $MAPD_{90}$ in the endocardium of working rabbit hearts treated with vehicle, 10^{-10}M ET-1 (top panel) or 10^{-8}M S6c (bottom panel) before and during acute regional ischaemia and reperfusion. '*' indicates significantly different from pre-drug value. '**' indicates statistically significant differences from respective pre-ischaemic values, ($P<0.05$ in each case). (n=6-12).