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Cardiac Effects of Endothelin-1 (ET-1) and Related C-Terminal Peptide Fragment: Increased Inotropy or Contribution to Heart Failure?

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Summary

The contrasting pattern of cardiac inotropy induced by human peptide endothelin-1 (ET-1) has not been satisfactorily explained. It is not clear whether ET-1 is primarily responsible for increased myocardial ET-1 expression and release with resultant inotropic effects, or for the induction of myocardial hypertrophy and heart failure. There are at least two subtypes of endothelin receptors (ET_A and ET_B) and the inotropic effects of ET-1 differ depending on the receptor involved. Along with some other groups, we reported significant subtype-ET_B endothelin receptor down-regulation in human cardiac cells preincubated with endothelin agonists (Dřímal *et al.* 1999, 2000). The present study was therefore designed to clarify the subtype-selective mechanisms underlying the inotropic response to ET-1 and to its ET_B-selective fragment (8-21)ET-1 in the isolated rat heart. The hearts were subjected to (1-21)ET-1 and to (8-21)ET-1, or to 30 min of stop-flow ischemia followed by 40 min of reperfusion, both before and after selective blockade of endothelin receptors.The present study revealed that both peptides, ET-1 and its (8-21)ET-1 fragment, significantly reduced coronary blood flow in nmolar and higher concentrations. The concomitant negative inotropy and chronotropy were marked after ET-1, while the infusion of the ET-1(8-21) fragment produced a slight but significant positive inotropic effect. Among the four endothelin antagonists tested in continuous infusion only the non-selective PD145065 and $ET_{B1/B2}$-selective BQ788 (in μmolar concentrations) slightly reduced the early contractile dysfunction of the heart induced by ischemia, whereas ET_A-selective PD155080 partially protected the rat heart on reperfusion.

Key words

Endothelin-1 • ET_A and ET_B receptors • IRL1620 • endothelin antagonists • BQ788 • PD151242 • PD142893 • PD155080

Introduction

The concentration of human vasopressor and proliferative peptide endothelin-1 (ET-1) in human plasma is low under basal conditions, however, the expression of ET-1 increases with the development of heart failure (Kobayashi *et al.* 1999). Circulating ET-1 is cleared by proteases and specific endopeptidases

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(Sokolovsky *et al.* 1990, Deng *et al.* 1994, Grantham *et al.* 2000). The proteolytic fragments of human ET-1 have been considered to be biologically inactive, but recent evidence suggests that peptide fragments sustain most of the homeostatic response produced by the parent peptide. The increased expression of ET-1 as well as its effect on vascular smooth muscle and resultant cardiac hypertrophy are presumably mediated by two subtypes of endothelin receptors (ET_A and ET_B), which are mostly disproportionally (ET_A>>ET_B) distributed in human, porcine, canine and rat fibroblasts, vascular endothelial and myocardial cells (Gu *et al.* 1992, Molenaar *et al.* 1993, Endoh *et al.* 1996, Modesti *et al.* 1999, Dřímal *et al.* 1999, 2000, 2002). There is a considerable evidence supporting the negative role of ET_A receptors in the failing heart (Brunner and Doherty 1996, Peter and Davenport 1996, Kobayashi *et al.* 1999, McCarthy *et al.* 2000), myocardial ischemia and infarction (Stewart *et al.* 1991, Tomoda 1993), cardiac hypertrophy and remodeling (Adams *et al.* 1996, Takeichi *et al.* 2000, Zhu *et al.* 2000), and in congestive heart failure (Zolk *et al.* 1999, Mishima *et al.* 2000).

The present study was therefore carried out a) to examine the mode of action of human ET-1 and its ET_B-selective fragment in the isolated heart, b) to establish whether ET-1 or its fragment may be involved in the maintenance of cardiac inotropy in control and ischemic hearts both before and after pretreatment with selective endothelin antagonists, and c) to assess the cardiac protective effects of endothelin antagonists.

Methods

All experimental procedures were performed in compliance with Principles of Laboratory Animal Care, formulated by the National Society for Medical Research. Experiments were realized in the Laboratory of Cardiovascular Pharmacology at the Institute of Experimental Pharmacology of the Slovak Academy of Sciences. The model used in this study was an isolated, spontaneously beating, isovolumetric rat heart according to Langendorff. Briefly, male Wistar rats (body weight 230-250 g) were anesthetized with ether, heparinized (Heparin Spofa, 550 IU/kg), and the ascending aorta was cannulated *in situ* and perfused in the Langendorff mode with a special cannula. The heart was then quickly isolated, mounted in a heart chamber and maintained at constant pressure perfusion. The heart was perfused with Krebs-Henseleit solution (in the modification of Dohring and Dehnert 1986), (pH 7.4), containing (in mmol/l): NaCl 118, KCl 4.79, $CaCl_2$ 1.75, $NaHCO_3$ 24.88, glucose 11.1, KH_2PO_4 1.18, $MgSO_4$ 1.6. The solution was equilibrated with 95 % O_2 and 5 % CO_2 at 37 ^oC. Perfusion pressure was maintained at 85 mm Hg. The following parameters of the isolated heart were measured: coronary flow, two parameters of left ventricular contraction (LV contraction), the force of isovolumetric contraction and the first derivative (dp/dt) of the left ventricular end-systolic (LVESP) and end-diastolic pressure (LVEDP) were continuously monitored by means of a latex balloon in the left ventricle connected to a pressure transducer (Siemens-Elema), electronic circuits and Cardiovascular Analyzer (Statham). All variables together with two bipolar ECG leads were continuously recorded on a 6-channel NEK-4T recorder (Germany). Baseline data were recorded after 30-min stabilization of the heart in a water-jacketed organ bath. Control group I (n=6) was used for basic measurements of heart rate, left ventricular contractile force, left ventricular systolic and diastolic pressure, coronary blood flow and ECG (60 min). In the treated groups II and III, two endothelin agonists (Fig. 1), human (1-21)endothelin-1 (ET-1), and its (8-21)ET-1 fragment [IRL1620] were continuously (0.5 min) infused into the inflow cannula in the concentration 1.0, 10.0 or 100.0 nmol/l. In group IV (n=6), the $ET_{B1/B2}$ selective endothelin antagonist (BQ788), in groups V and VI nonselective endothelin antagonists (PD142893 and PD145065) and in groups VII and VIII the peptide-type endothelin-ET_A selective antagonist (PD151242) and butenolide (PD155080) were continuously infused (10 min) in concentrations of 10^{-8},10^{7},10^{6} mol/l). Two minutes after beginning of the endothelin antagonist infusion, both perfusion and infusion of the heart were stopped (30 min stop-flow ischemia). After the end of ischemia the heart was reperfused and infusion continued for 8 min. The results were expressed as means ± S.E.M. Data were evaluated using the ANOVA test. Differences between groups were considered significant at $p<0.05$.

Chemicals

BQ788(RBI), human endothelin-1 (1-21), (Sigma, USA), IRL1620 (ICN), PD142893, PD145065, PD151242, PD155080 were employed.

Results

After 20 min of stabilization of the isolated rat heart, the values of basal coronary flow were 15.8±0.75 ml/g/min, the heart rate was 308±9 beats/min and the left

ventricular systolic pressure was 97±5 mm Hg. After further 40 min of perfusion, control hearts showed a reduction of coronary flow (–30±3 %, $p<0.05$) and a decline in the heart rate (–16±5 %), while the left ventricular systolic and diastolic pressure remained unchanged.

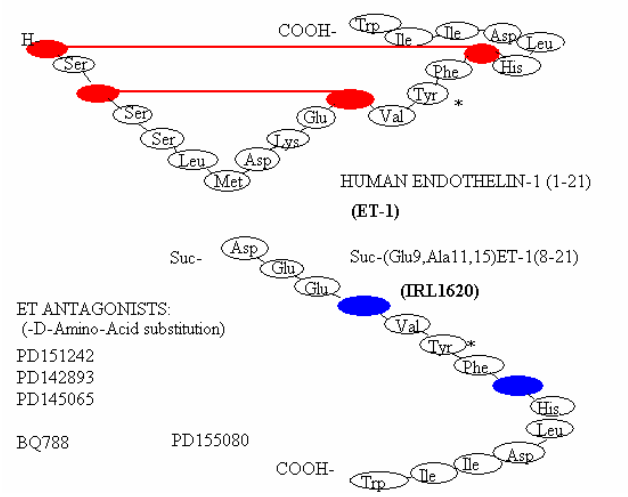

Fig. 1. *Schematic simplification of the structure of human endothelin-1(1-21) with its four -Cys- residues in the peptide moiety ($Cys^{1,3,11,15}$) forming two disulfidic bridges and thus supporting the helical structure of the peptide. Below is the structure of the C-terminal peptide fragment, linear peptide (8-21)ET-1, (IRL1620) and six endothelin antagonists, nonselective (PD142893, PD145065), endothelin ET_A–selective (PD151242), and endothelin ET_B-selective (BQ788), mostly short peptide fragments with –D-amino acid substitution. The butenolide PD155080 is the ET_A-selective endothelin antagonist.*

Effect of (1-21)ET-l and its (8-21)ET-1 fragment (IRL1620) on cardiac function

Two pluripotent peptide agonists, human ET-1 and its linear amino-terminally truncated analog, (8-21)ET-1 fragment (Fig. 1), were infused in a short continuous infusion into the aorta of isolated rat heart to reveal the possible role played by human helical ET-1 and its fragments in a modification of basal cardiac function. Fortunately, the peptide fragment IRL1620 is the most potent and specific agonist for subtype ET_B receptors (Takai *et al.* 1992). Figure 2 summarizes the effects of both endothelin agonists on coronary flow. The native ET-1 produced a concentration-dependent significant reduction of coronary blood flow in isolated perfused rat hearts. Surprisingly, the amino-terminally truncated C-terminal peptide fragment (8-21)ET-1 significantly reduced coronary flow in the concentration range from l.0 to 100 nmol/l. The small increase in coronary flow observed with the high (micromolar) concentration of (8-21)ET-1 is apparently beyond physiological relevance. The effects of the peptides on inotropy of the heart exhibited individual differences (Figs 3 and 4). The ET_A selective ET-1 significantly reduced the heart rate and also the left ventricular systolic force in the whole range of concentrations. On the contrary, the effect of the ET_B selective fragment (8-21)ET-1 was positively inotropic and chronotropic in the whole range of concentrations.

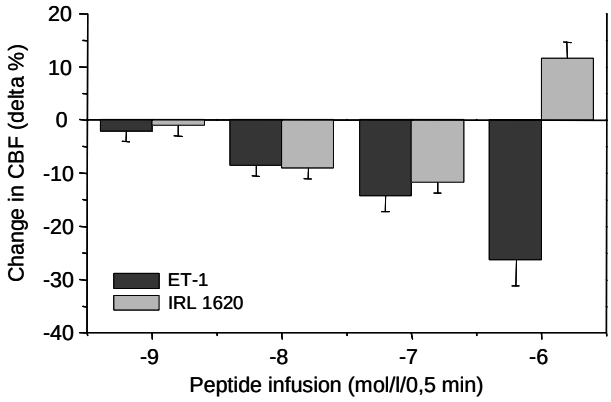

Fig. 2. *Changes in coronary blood flow (CBF, in % of control) induced by short (0.5 min), constant infusion of two endothelin agonists (nonselective human endothelin-1 (ET-1) and ET_B-selective ET-1 (8-21) fragment IRL1620 (concentration in mol/l) in spontaneously beating rat heart perfused at constant pressure (90 mm Hg) (n=15). Control CBF = 15.8±0.7 ml/min. Note: Significant reduction in coronary blood flow was induced by ET-1 and IRL1620. Small but significant increase in coronary blood flow was observed only after the highest concentration of IRL1620.*

Effects of endothelin antagonists in myocardial ischemia

Our additional objective was to reveal whether fragmentation of peptide chains and/or its substitution with one or two –D-amino acid(s) may modify cardiac function in the compromised heart. In the second part of our study we therefore analyzed the possible protective action of peptide fragments, mostly potent endothelin antagonists, in the early and late phase of ischemia. Six selected endothelin antagonists were tested in a classical

experimental model of stop-flow ischemia/reperfusion (30 min/40 min) in the isolated Langendorff rat heart. The antagonists were infused by a slow continuous (10 min) infusion two minutes before induction of stop-flow ischemia and after reperfusion of ischemia. In control experiments stop-flow ischemia of the isolated rat heart produced abrupt and significant reduction in systolic contractile force in the left ventricle. The contractile force declined to 51±14 % after 30 s, to 32±16 % after 60 s, and was abolished 120 s after the beginning of ischemia. Proportionally to the early contractile dysfunction, the left ventricular end-diastolic pressure (LVEDP) and the left ventricular end-diastolic stiffness (LVEDS) increased. The increase in LVEDP was significant very early after induction of ischemia: +12±3 mm Hg ($p<0.05$) after 10 s and +25±8 mm Hg ($p<0.05$) after 20 s.

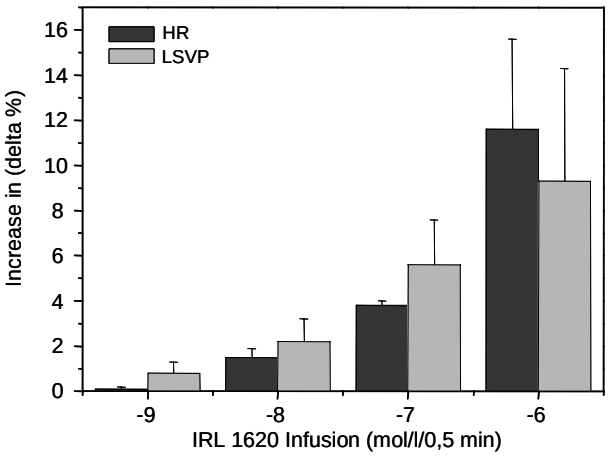

Fig. 3. *The positive chronotropic and positive inotropic effects of infusion of IRL 1620 [(8-21)ET-1 fragment] in isolated perfused rat heart (n = 12). The positive inotropic and chronotropic action of IRL1620 is low when compared on a micromolar basis with the positive inotropy of norepinephrine, phenylephrine or angiotensin-II.*

Discrete but significant protection of early contractile dysfunction (15-60 s after stop-flow ischemia) was seen shortly after beginning of ischemia with infusion of endothelin antagonists PD145065 and BQ788 (Fig. 5).

In control experiments with isolated rat hearts after stop-flow ischemia, both the left ventricular end-diastolic pressure (LVEDP) and also the left ventricular end-systolic pressure (LVESP), which is expressed as increase in percentages of active increase in LVEDP. These two parameters were significantly increased 3 min after ischemia (+2.2±0.8 %, $p<0.05$). A maximal increase in LVEDP (+12±2 mm Hg) was observed 15 min after stop-flow and remained at this value till the end of ischemia

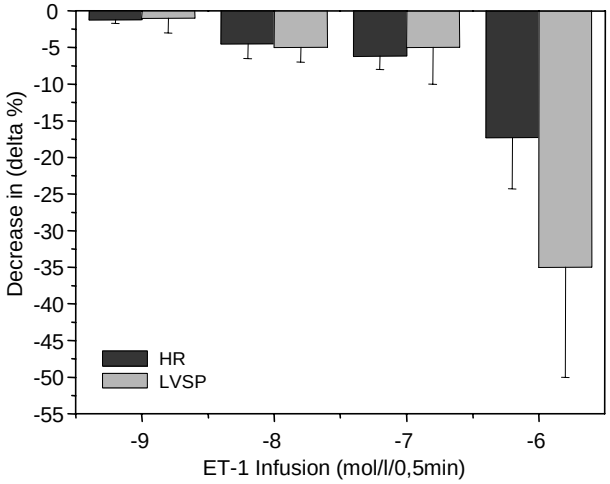

Fig. 4. *Negative inotropic and chronotropic effects of endothelin-1 infusion observed in spontaneously beating perfused rat hearts (n = 15). Control HR = 308± 8 beats/min, LVSP = 95±4 mm Hg. Explanation and symbols as in Fig. 2.*

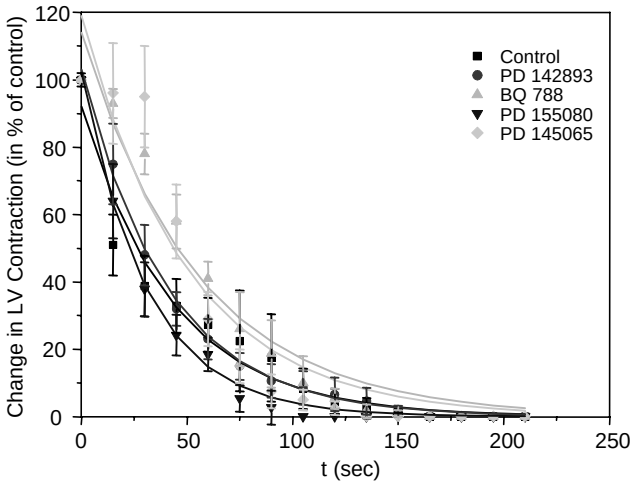

Fig. 5. *Effects of four endothelin antagonists on early contractile dysfunction of isolated, perfused rat hearts induced by stop flow ischemia. Endothelin antagonists were injected directly into the inflow cannula by constant infusion. The infusion started 3 min before ischemia (n= 18).*

During the reperfusion after ischemia, there was a characteristic response of left ventricular pressure in control hearts. LVEDP and LVESP, which already increased after the period of stop-flow ischemia, showed a further increase on reperfusion (17.5±3.9, 22.5±2.2 and 25.3±4.6 mm Hg 0.5, 1 and 3 min after reperfusion, $p<0.05$). Subsequently, LVEDP and LVEDS gradually declined approximately to their increased pre-reperfusion values, with LVEDP maintained at 11.2±2.8 mm Hg. The subype-ET_A selective endothelin antagonist PD155080 and a nonselective endothelin antagonist BQ788 showed a further slight but significant reduction in LVEDP and LVESP after reperfusion. The protective effects of endothelin antagonists on cardiac function were manifested here as discrete restitution of left ventricular contractions during reperfusion of cardiac ischemia (Fig. 6).

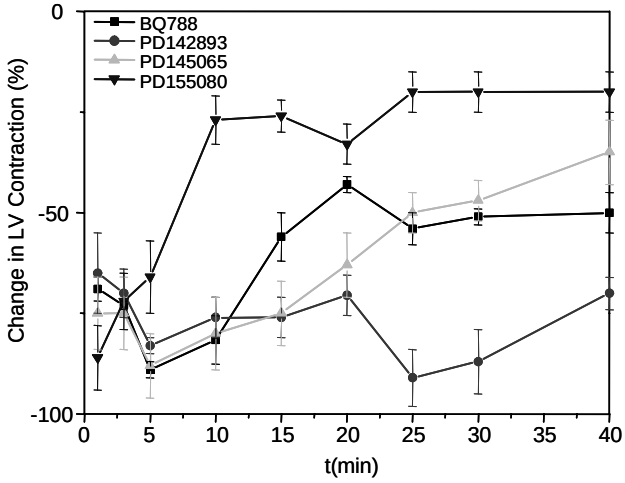

Fig. 6. *Effects of infusion of endothelin antagonists on late response of left ventricular contractile force (LVC, in % of control) after reperfusion of ischemia (stop-flow ischemia lasted 30 min). Infusion started 3 min before ischemia and lasted 10 min (n = 19). Note: Significant protective effect on LV contraction with infusion 1.0 μmol/l of PD155080 and partial protection with BQ788.*

Discussion

Early *in vitro* studies on cardiac myocytes reported a positive inotropic effect of porcine endothelin and they postulated that members of the endothelin family may produce significant positive inotropic effects on the heart (Masaki *et al.* 1992, Hilal-Dandan *et al.* 1997, Katoh *et al.*1998). In the present study we tested two endothelin agonists, the human ET-1 and its ET_B selective fragment (IRL1620), and selective endothelin-ET_A and -ET_B antagonists in isolated rat hearts to demonstrate intrinsic cardiac effects of endothelin, the subtype-selectivity of its inotropic response, and also the possible protective action of endothelin antagonists in myocardial ischemia. Both peptides, ET-1 and its N-terminally truncated fragment IRL1620, reduced coronary flow significantly and in a concentration-dependent way. The human ET-1 significantly and concentration-dependently also reduced the heart rate and left ventricular force of contraction. The threshold for the reduction of coronary flow and cardiac negative inotropy in our study was in the nanomolar concentration range of ET-1. Our results are in agreement with the findings of a significant negative inotropic effect of ET-1 in canine ventricular trabeculae (Zhu *et al.* 1997) and with the reported active coronary vasoconstriction induced by ET-1 in anesthetized rats (Beyer *et al.* 1996). In the present study, the peptide fragment (8-21)ET-1, infused in the same concentration range, produced small but significant positive chronotropic and significant positive inotropic effects in the whole range of concentrations used. A high affinity and ET_B selectivity of (8-21)ET-1 was clearly demonstrated (Takai *et al.* 1992). With the exception of the highest concentrations, the short infusion of IRL1620 in the present study significantly reduced coronary blood flow and only slightly increased left ventricular contractile force. When a compound increases the rate and the force of contraction, there is a possibility that force effects may only be secondary to the rate changes. Except the high IRL1620 concentration, the increase in left ventricular contractile force, observed in the present study, always exceeded the increase in heart rate. The significant reduction in coronary blood flow seen in our study with both peptide agonists, i.e. human ET-1 and the (8-21)ET-1 fragment, may indicate that a significant part of vasoconstrictor endothelin receptors (possibly ET_{B2} subtype) in rat coronary arteries and/or the preference of N-terminally truncated peptide for the presumably vasoconstrictive endothelin-ET_{B2} receptors is expressed abundantly in rat arteries. The ligand-binding experiments on the rat heart showed that cardiac membranes of normal rats contained ET_A and ET_B receptors in a ratio of 91:9 (Sakai *et al.* 1996). It thus appears that the small cardiac positive inotropic and chronotropic response observed in the present study in the whole concentration range of IRL1620 may have

resulted from the activation of a minor form of the myocardial endothelin receptor (possibly ET_{B1} subtype) induced by ET-1, released after ET_B receptor activation. To our knowledge, there are no other reports indicating which subtype of ET receptor may be involved in the cardiac positive inotropic and chronotropic response. The rare amino acid substitution studies of endothelin agonists have indicated that -Ser at the N-terminus may be important for ET-1 binding (Wallace and Janes 1995) and although it cannot account for the entire binding affinity of human ET-1 and its (8-21)ET-1 fragment, it may have an impact on the surface accessibility and on the pocket fit of human ET-1. With respect to the consistent coronary vasoconstriction induced by ET-1 and its linear fragment IRL1620 in our study, we would like to draw attention to the fact that a variety of C-terminal amino-acid substitution studies were carried out on endothelin peptides, and that most of them did not note any dramatic changes in the vasoactivity of the C-terminal peptide-mutants. Concerning the pharmacology of the endothelin family of peptides, it has been assumed that the unique structural motif called a "cysteine noose", formed by "intact N-terminus and the middle part of endothelin molecule" may be important for the "vasoconstrictor activity of peptides" (Lapthorn *et al.* 1995). The present study has clearly shown that the linear peptide fragment which is devoid of a "cysteine noose" also induced significant coronary vasoconstriction and small but significant positive inotropy. In a previous study we have clearly demonstrated that as far as the induction of the ET_B receptor down-regulation is concerned, the potency of an ET_B-selective agonist, linear-type (6-21)[$Ala^{11,15}$]-ET-1 derivative (BQ3020), which is also devoid of the amino-terminus, was significantly reduced. One possibility of explaining these differences in the pharmacology of endothelin peptides is that the myocardial ET_{B1}-subtype may be differently regulated and/or that stimulation of myocardial endothelin receptors with a truncated peptide fragment (see above) may induce an overexpression and release of the vasoconstrictor, mature peptide ET-1. It follows then that the excess of circulating peptide endothelin-1 and possibly also of some of its metabolic fragment(s) could directly stimulate the cardiac tissue and thus control the positive inotropic response of the heart muscle.

In conclusions, our results obtained with ET-1 and with its C-terminal fragment (8-21)ET-1 demonstrate their potency to induce effective coronary vasoconstriction in the isolated perfused rat heart. Moreover, the (8-21)ET-1 fragment also exhibited the ability to stimulate the cardiac muscle. However, the intensity of the direct positive inotropic effect of the (8-21)ET-1 fragment was minimal. The discrete cardiac protection was demonstrated in the present study in preventive tests with two different types of endothelin antagonists, with the $ET_{B1/2}$ selective antagonist BQ788 and with the ET_A selective antagonists PD155080 and PD151242. The protective effect was seen both in the early response of the isolated heart to ischemia and after reperfusion following ischemia. Thus, the ET_A-selective tripeptide PD151242 or non-peptide PD155080 and the $ET_{B1/B2}$-selective BQ788 may provide certain therapeutic benefits in cardiac ischemia.

Acknowledgements

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