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Beneficial effects of the endothelin receptor antagonist bosentan on myocardial and endothelial injury following ischaemia/reperfusion in the rat

Xing-San Li, Qing-Dong Wang, John Pernow *

Department of Cardiology, Karolinska Hospital, S-171 76 Stockholm, Sweden

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Abstract

The effects of bosentan, a nonpeptide endothelin receptor antagonist, on endothelin-induced changes in coronary flow and myocardial ischaemic and reperfusion injury were investigated in the Langendorff perfused rat isolated heart. Endothelin-1 (0.012–0.4 nmol) evoked dose-dependent reduction in coronary flow, which was attenuated by bosentan (1.0–10 μM) in a concentration-related fashion. The inhibitory effect of bosentan lasted more than 30 min. The endothelin ET_B receptor agonist Suc-[Glu^9,$Ala^{11,15}$]endothelin-1-(8–21) (IRL 1620) increased coronary flow in the absence but not in the presence of bosentan. In hearts subjected to 30 min of global ischaemia followed by 30 min of reperfusion, the recoveries of the left ventricular developed pressure, dP/dt_{max}, and coronary flow were significantly larger in a group given bosentan 10 μM at the start of ischaemia ($92 \pm 7\%$, $98 \pm 8\%$ and $83 \pm 5\%$, respectively) than in a vehicle-treated group ($70 \pm 4\%$, $70 \pm 6\%$ and $42 \pm 2\%$, respectively) at the end of the reperfusion period. During the reperfusion period, left ventricular end diastolic pressure was significantly lower in the bosentan group than in the vehicle group. The area of no-reflow in the bosentan group was $7 \pm 3\%$ of left ventricle compared to $21 \pm 2\%$ in the vehicle group ($P < 0.01$). Acetylcholine-induced endothelium-dependent vasodilatation was significantly reduced after ischaemia and reperfusion in the vehicle group but not in the bosentan group. It is concluded that bosentan attenuates the coronary vasoconstrictor effect elicited by endothelin and reduces ischaemia/reperfusion-induced myocardial and endothelial injury in the rat isolated heart. The results suggest that endogenous endothelin may be involved in the pathogenesis of myocardial ischaemic and reperfusion damage and a beneficial effect of bosentan in preventing myocardial and endothelial injury following ischaemia and reperfusion.

Keywords: Endothelin receptor antagonist; Bosentan; Endothelium; Myocardial injury; Heart; (rat)

1. Introduction

Endothelin is a 21-amino acid peptide that is produced by vascular endothelial cells and exerts potent vasoactive properties (Yanagisawa et al., 1988). The endothelin family consists of the isopeptides endothelin-1, endothelin-2, and endothelin-3 (Inoue et al., 1989). So far, two distinct mammalian endothelin receptor subtypes have been identified and cloned, endothelin ET_A and endothelin ET_B receptors. Both subtypes of receptors are widely distributed in cardiovascular and non-cardiovascular tissues (Masaki, 1991). The endothelin ET_A receptor shows higher affinity for endothelin-1 than for endothelin-3 (Arai et al., 1990), is located on vascular smooth muscle cells and mediates vasoconstriction. The endothelin ET_B receptor is unselective with regards to the isopeptides (Sakurai et al., 1990), and mediates endothelium-dependent relaxation or vasoconstriction depending on whether the receptor is located on endothelial or vascular smooth muscle cells (Takayanagi et al., 1991; Clozel et al., 1992). It has been speculated that endogenous endothelin may be of pathophysiological significance in a variety of cardiovascular disorders. Thus, increased plasma levels of endothelin is observed in patients with advanced atheroscleroses (Lerman et al., 1991), angina pectoris (Matsuyama et al., 1991) and myocardial infarct (Miyauchi et al., 1989; Stewart et al., 1991). In experimental animals, endothelin is released locally

* Corresponding author. Tel. 46-8-7295876, fax 46-8-311044.

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during myocardial ischaemia and reperfusion (Brunner et al., 1992; Tønnessen et al., 1993; Wang et al., 1995a). The major release seems to occur during the reperfusion. Furthermore, ischaemia and reperfusion may enhance the production of endothelin-1 (Wang et al., 1995a), and increase the number as well as cause externalisation of endothelin-1 binding sites in myocardial membranes (Liu et al., 1989,1990). These findings all indicate a role for endogenous endothelin in the development of ischaemic/reperfusion injury. Accordingly it has been reported that endothelin monoclonal antibodies reduce the infarct size in rats (Watanabe et al., 1991). The recent development of specific endothelin receptor antagonists has opened up new possibilities to further investigate this matter. However, the use of endothelin receptor antagonists of peptide nature has resulted in conflicting results. Thus, in one study BQ-123, a selective ET_A receptor antagonist, was found to reduce the infarct size in a canine model of coronary occlusion and reperfusion (Grover et al., 1993), whereas in another study no such effect was observed (Krause et al., 1994). A third study using the endothelin ET_A receptor antagonist FR 139317 found no cardioprotective effect in the rabbit (McMurdo et al., 1994). The reason for these conflicting results may be due to species differences and/or that both BQ-123 and FR 139317 lack affinity to ET_B receptors.

Bosentan (Ro 47-0203, 4-tert-butyl-*N*-[6-(2-hydroxyethoxy)-5-(2-methoxy-phenoxy)-2,2′-bipyrimidin-4-yl]-benzenesulfonamide) is a new potent orally active non-peptide antagonist of both endothelin ET_A and ET_B receptors (Clozel et al., 1994). It may be of importance to use a mixed endothelin ET_A and endothelin ET_B receptor antagonist in a vascular bed like the rat coronary bed in which both subtypes of receptors are present (Balwierczak, 1993; Wang et al., 1994). The main objectives of the present study were to characterise the effect of bosentan on coronary flow changes induced by endothelin ET_A and endothelin ET_B receptor activation and to investigate its possible beneficial effects on myocardial and endothelial injury following ischaemia and reperfusion in rat isolated hearts.

2. Materials and methods

2.1. Heart preparation

Male Sprague-Dawley rats (250–350 g) were anaesthetised with a mixture of fluanisonum and fentanylum (Hypnorm; 1 mg and 0.02 mg/100 g, respectively i.m.). After injection of heparin (1000 IU/kg i.v.) the hearts were excised and placed in cold (4°C) Krebs-Henseleit buffer solution of the following composition (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 1.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.2 and glucose 11.1. The hearts were mounted on a column according to the non-recirculating Langendorff perfusion technique and perfused with buffer bubbled with 95% O_2 and 5% CO_2 to achieve a pH of 7.4 at a constant pressure of 85 cm H_2O. The temperature was kept at 37.0 ± 0.5°C by circulating heated water. The buffer was filtered through a 5 μm filter prior to use to remove any contaminating particles. A latex balloon filled with saline was inserted into the left ventricular cavity via the left atrium and connected to a pressure transducer (Gould, USA) to measure the left ventricular isovolumic pressure. The volume of the balloon was adjusted to obtain a left ventricular end diastolic pressure of 5–10 mm Hg during the equilibration period. The maximum value of the first derivative of left ventricular pressure (dP/dt_{max}) was determined by electronic differentiation. A transonic flow probe (Transonic, Ithaca, NY, USA) connected to a Transonic flow meter (model 208) was put on the circuit proximal to the aortic cannula for continuous measurement of coronary flow. All parameters were continuously recorded on a polygraph (model 7D, Grass Instrument, USA). A side arm in the perfusion system just proximal to the aortic cannula permitted local administration of drugs. All hearts were allowed to equilibrate for 30 min before the experiments were started.

2.2. Pharmacological experiments

The pharmacological characterisations were performed in 7 groups. Only one dose-response curve to each agonist was made in one preparation to avoid tachyphylaxis. Group 1 ($n = 7$) received bolus injections (0.4 ml) of endothelin-1 at cumulative doses (0.012–0.4 nmol). At least a 5-min interval was allowed between injections in order to obtain stable preinjection conditions. Group 2 and 3 ($n = 7$) received infusion of bosentan at a final concentrations of 1.0 and 10 μM, respectively. The infusions were started 10 min before and during injections of endothelin-1 (0.012–0.4 nmol). Group 4 ($n = 7$) received injections of endothelin-1 30 min after a 10 min infusion of bosentan (10 μM). Group 5 ($n = 7$), group 6 ($n = 6$) and group 7 ($n = 6$) received bolus injections (0.4 ml) of the endothelin ET_B receptor agonist IRL 1620 (Suc-[Glu^9,$Ala^{11,15}$]endothelin-1-(8–21)) (0.016 and 0.055 nmol) in the absence and presence of bosentan according to groups 1–3. In each experiment, 0.4 ml Krebs-Henseleit buffer was injected before the agonist was given.

2.3. Ischaemia-reperfusion experiments

Three groups of hearts were subjected to 30 min of global ischaemia by clamping the aortic cannula followed by 30 min reperfusion. One group ($n = 8$) re-

ceived a bolus injection (3 ml) of bosentan (10 μM) and the second group ($n = 8$) received vehicle (3 ml Krebs-Henseleit buffer) into the side branch of the aortic cannula just after the aortic clamping. The third group ($n = 6$) subjected to ischaemia and reperfusion received a 10 min infusion of bosentan (10 μM) after 30 min of reperfusion. Acetylcholine (4.5 nmol) and *S*-nitroso-*N*-acetyl-D,L-penicillamine (SNAP, 4.5 nmol) were administered 20 min before ischaemia and after 30 min of reperfusion, to investigate endothelium-dependent and endothelium-independent coronary vasodilatation, respectively. In a separate group ($n = 7$) the responses to acetylcholine and SNAP were investigated after 60 min of non-ischaemic perfusion to serve as a time-matched control group.

2.4. Determination of no-reflow area

At the end of each experiment, 3 ml of 2% Evans blue dye was injected into the aortic cannula in order to determine the no-reflow area (Sjöquist et al., 1992). The hearts were dismounted, and the atria, right ventricle and large vessels were trimmed off. The left ventricle was subsequently sliced into 2 mm thick slabs (5 pieces) from base to apex. The slices were photographed and magnified for planimetric measurement of stained and unstained myocardial tissue.

2.5. Calculations and statistics

Left ventricular developed pressure is the systolic minus the diastolic pressure. The left ventricular developed pressure, dP/dt_{max} and coronary flow are given both in absolute values and the recoveries at the end of the reperfusion period expressed as a percentage of the pre-ischaemic value. The responses to acetylcholine and SNAP are expressed as percent increase from basal coronary flow. The size of the no-reflow area is expressed as the proportion of non-stained myocardial area in relation to the left ventricular area. All results are presented as mean ± S.E.M. The levels of significance were calculated by Kruskal-Wallis analysis of variance, or by the Mann-Whitney *U*-test for unpaired comparisons. A P value of < 0.05 was considered to be significant.

2.6. Drugs

Hypnorm (fluanisonum + fentanylum; Janssen, Belgium), sodium heparin (Kabi Vitrum, Sweden), acetylcholine hydrochloride (Sigma, St. Louis, MO, USA), SNAP, endothelin-1 and Suc-[Glu^9,Ala^{11,15}]endothelin-1-(8–21) (Neosystem or Alexis Corporation, Läufelfingen, Switzerland). Bosentan sodium salt was a kind gift from Dr. M. Clozel, Hoffmann-LaRoche, Basel, Switzerland. Endothelin-1 was dissolved in 5% acetic acid. Suc-[Glu^9,Ala^{11,15}]endothelin-1-(8–21) was dissolved in 1:1 acetonitrile-phosphate buffer. Bosentan was dissolved in distilled water. The other drugs were dissolved in 0.9% NaCl and all drugs were diluted in Krebs solution.

3. Results

3.1. Effects of bosentan on endothelin ET_A and endothelin ET_B receptor-mediated coronary effects

Basal coronary flow in the group that received endothelin-1 was 12.3 ± 1.2 ml/min ($n = 7$) which was

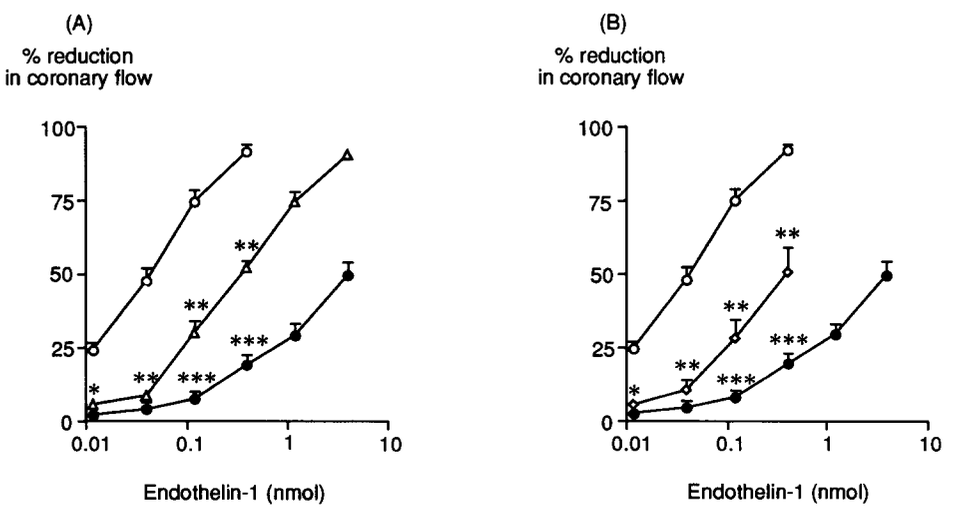

Fig. 1. (A) Effects of endothelin-1 on coronary flow under control conditions (○), in the presence of 1.0 μM (△) and 10 μM (●) bosentan. (B) The effect of endothelin-1 under control conditions (○), in the presence of 10 μM bosentan (●) and 30 min after the administration of 10 μM bosentan (◇). Data (means ± S.E.M.; $n = 7$) are expressed as the percent reduction from basal values. Significant differences compared with the controls are shown: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

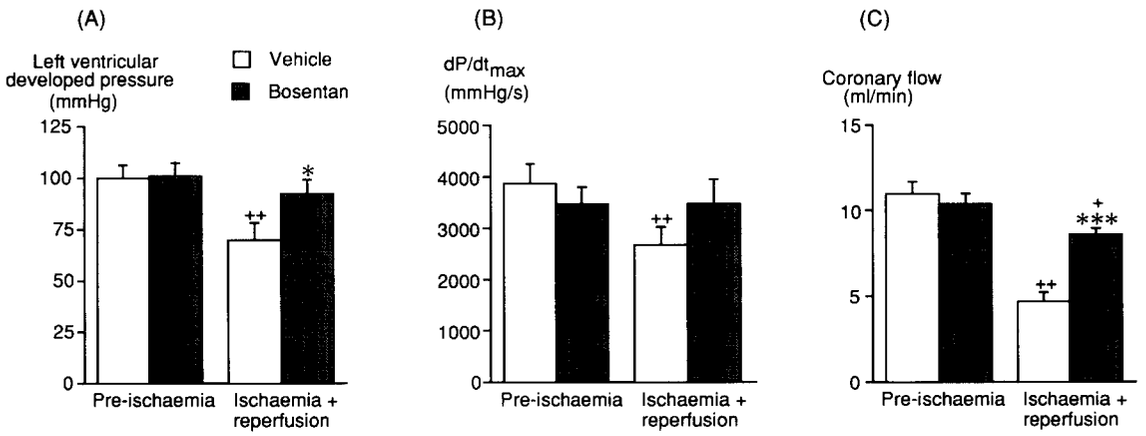

Fig. 2. (A) Left ventricular developed pressure, (B) left ventricular dP/dt_{max} and (C) coronary flow before start of ischaemia and at the end of 30 min of global ischaemia followed by 30 min of reperfusion in hearts given vehicle or bosentan (10 μM) at the start of ischaemia. All values are means ± S.E.M. from eight hearts. Significant differences from the pre-ischaemic values $^{+}$ $P < 0.05$, $^{++}$ $P < 0.01$ and between the bosentan and vehicle groups * $P < 0.05$ and *** $P < 0.001$ are shown.

not significantly different from the other groups. Endothelin-1 caused a dose-dependent reduction in coronary flow (Fig. 1). The lowest endothelin-1 dose (0.012 nmol) induced a decrease in coronary flow by 24% and the highest dose (0.4 nmol) reduced coronary flow by 91% to 1.2 ± 0.3 ml/min. The vasoconstrictor effect induced by endothelin-1 was significantly and dose-dependently attenuated by bosentan and the dose-response curve to endothelin-1 was shifted to the right in a competitive fashion by bosentan (Fig. 1A). Thirty minutes after infusion of the high concentration of bosentan, the vasoconstrictor response to all doses of endothelin-1 was still reduced by approximately 50% (Fig. 1B). Infusion of bosentan (1.0 μM or 10 μM) did not affect basal coronary flow.

The endothelin ET_B receptor agonist Suc-[Glu^9,Ala^{11,15}]endothelin-1-(8–21) induced a dose-dependent increase in coronary flow under control conditions. The highest dose (0.055 nmol) increased coronary flow by 38 ± 4%. The vasodilator effect of Suc-[Glu^9,Ala^{11,15}]endothelin-1-(8–21) was significantly attenuated in the presence of bosentan. Thus, the maximal increase in coronary flow by Suc-[Glu^9,Ala^{11,15}]endothelin-1-(8–21) (0.055 nmol) in the presence of 1.0 μM and 10 μM bosentan was reduced to 7.7 ± 1.6% and 5.1 ± 1.4%, respectively ($P < 0.01$).

3.2. Effects of bosentan on myocardial performance and coronary flow after ischaemia and reperfusion

There were no significant differences between the vehicle and bosentan groups prior to ischaemia. After 30 min of reperfusion, the recoveries of the left ventricular developed pressure, dP/dt_{max}, and coronary flow in the control group were 70 ± 4, 70 ± 6 and 42 ± 2%, respectively, of the pre-ischaemic values. In the group given bosentan at the start of ischaemia all parameters were close to the pre-ischaemic values (83–98% recoveries), and the recoveries were significantly higher than in the control group ($P < 0.05$). When expressed in absolute values left ventricular developed pressure and coronary flow were significantly higher in the bosentan group than in the control group at 30 min reperfusion (Fig. 2). Heart rate was significantly lower at 30 min reperfusion (202 ± 29 beats/min) than prior to ischaemia (282 ± 12 beats/min; $P < 0.01$) in the control group, whereas it was not changed in the bosentan group (271 ± 15 vs. 268 ± 10 beats/min). A 10 min infusion of bosentan (10 μM final concentration) at the end of reperfusion did not significantly affect coronary flow (49 ± 7% and 43 ± 9% of the pre-ischaemic flow before and after bosentan, respectively; $n = 6$).

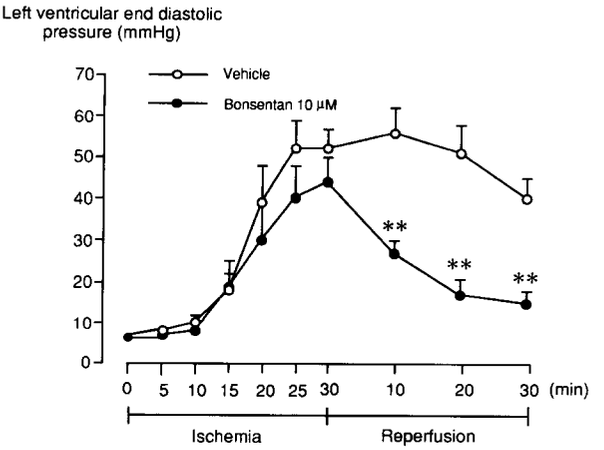

Fig. 3. Left ventricular end-diastolic pressure during the course of 30 min global ischaemia followed by 30 min of reperfusion in hearts given bosentan (10 μM) or vehicle at the start of ischaemia. All values are means ± S.E.M. from eight hearts. Significant differences from the vehicle group are shown: ** $P < 0.01$.

In the group receiving vehicle, the left ventricular end-diastolic pressure increased during the ischaemic period and reached a peak value of 56 ± 6 mm Hg at 10 min of reperfusion. During the course of reperfusion, the left ventricular end-diastolic pressure in the bosentan group decreased significantly and reached nearly the same level as prior to ischaemia at the end of the 30 min reperfusion period. In contrast, the left ventricular end-diastolic pressure in the vehicle-treated group remained high after the onset of reperfusion (Fig. 3).

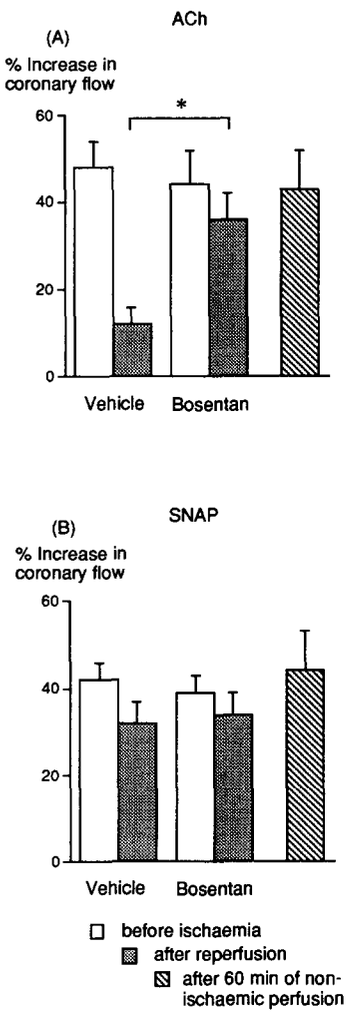

Fig. 4. Increase in coronary flow evoked by (A) acetylcholine (ACh) (4.5 nmol) and (B) the nitric oxide donor SNAP (4.5 nmol) before and after 30 min global ischaemia followed by 30 min of reperfusion in hearts given vehicle or bosentan (10 μM) at the start of ischaemia. The response to acetylcholine and SNAP after 60 min of non-ischaemic perfusion is in addition shown for comparison. The changes in coronary flow are expressed as percent increase from basal flow. All values are means ± S.E.M. from 6–7 hearts. Significant differences from the vehicle group are shown: * $P < 0.05$.

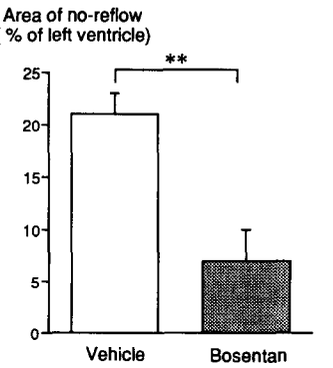

Fig. 5. Area of no-reflow expressed as percent of the left ventricle after 30 min global ischaemia followed by 30 min of reperfusion in hearts given bosentan (10 μM) or vehicle at the start of ischaemia. Data are given as means ± S.E.M. of seven hearts in each group. Significant difference from the vehicle group is shown: ** $P < 0.01$.

3.3. Effect of bosentan on endothelial function after ischaemia and reperfusion

The coronary dilator responses to acetylcholine and SNAP prior to global ischaemia were similar in both groups (Fig. 4). After myocardial ischaemia and reperfusion the response to acetylcholine was significantly reduced in the vehicle group but unaffected in the bosentan group (Fig. 4A). The response to SNAP at the end of 30 min reperfusion was similar to the pre-ischaemic response in both groups with no statistical differences between the two groups (Fig. 4B). The dilator effects of acetylcholine and SNAP were not affected by 60 min non-ischaemic perfusion (Fig. 4A and B).

3.4. Effect of bosentan on the area of no-reflow

There was no difference in the size of the total left ventricle between the groups. The area of no-reflow expressed as percent of the area of the left ventricle was 3 times larger in the vehicle group than in the hearts that received bosentan (Fig. 5).

4. Discussion

The main finding of the present study is that administration of bosentan significantly improves the recovery of myocardial function and coronary flow and prevents development of endothelial dysfunction following global ischaemia and reperfusion in the rat isolated heart.

Endothelin-1 was found to be a potent coronary vasoconstrictor, in line with several previous studies (Yanagisawa et al., 1988; Karwatowska-Prokopczuk and Wennmalm, 1990; Neubauer et al., 1990; Wang et al., 1994). In the rat isolated heart constrictor endothelin

ET_A receptors and dilator endothelin ET_B receptors are present (Wang et al., 1994). Thus, the endothelin ET_A receptor antagonist BQ-123 has been shown to cause inhibition of the endothelin-1-induced coronary constriction, whereas the endothelin ET_B receptor antagonist IRL 1038 enhanced the vasoconstrictor response to endothelin-1 (Wang et al., 1994). In the present experiment, the non-peptide mixed endothelin ET_A-ET_B receptor antagonist bosentan (Clozel et al., 1994) concentration-dependently prevented the vasoconstrictor response to endothelin-1. The vasodilator response induced by the endothelin ET_B agonist Suc-[Glu^9,$Ala^{11,15}$]endothelin-1-(8–21) was also effectively inhibited by bosentan, which indicates that the concentrations used were sufficiently high to block both endothelin ET_A and endothelin ET_B receptors. The finding that the response to ET_B agonist was blocked already by the lower concentration of bosentan despite the fact that the antagonist has higher affinity to endothelin ET_A than to endothelin ET_B receptors (Clozel et al., 1994) is probably related to the fact that it has easier access to endothelial ET_B receptors than to interstitial endothelin ET_A receptors located on vascular smooth muscle cells. The high concentration (10 μM) of bosentan still reduced the vasoconstrictor response to endothelin-1 30 min after the administration of the antagonist, a time period which was similar to the reperfusion period. Based on the high degree of antagonism and the long duration, the higher concentration of bosentan (10 μM) was chosen in the further investigation of the cardioprotective effects of bosentan during myocardial ischaemia and reperfusion.

Administration of bosentan did not influence the basal coronary flow, indicating that endogenous endothelin is not produced in sufficient amounts to regulate coronary flow in rat isolated hearts. However, during ischaemia followed by reperfusion local endothelin biosynthesis and release has been shown to be enhanced in rats and pigs (Brunner et al., 1992; Wang et al., 1995a). These findings may indicate a pathophysiological role for endothelin in ischaemic/reperfusion injury. Accordingly, administration of bosentan protected from ischaemic/reperfusion injury as indicated by the enhanced recovery of the left ventricular developed pressure, dP/dt_{max}, and coronary flow in comparison with the vehicle group. Furthermore, the left ventricular end-diastolic pressure in the vehicle group was significantly higher than that in the bosentan group throughout the reperfusion period. These findings indicate that bosentan not only protected from systolic dysfunction but also improved the diastolic function of the myocardium during reperfusion. The latter finding is in accordance with results showing that endothelin impairs diastolic relaxation (Karwatowska-Prokopczuk and Wennmalm, 1990). Since bosentan is a specific endothelin receptor antagonist (Clozel et al., 1994) and in the presently used concentration effectively blocks both endothelin ET_A and endothelin ET_B receptors for a period that exceeds the reperfusion time, the results suggest that endogenous endothelin is involved in the development of myocardial ischaemic and reperfusion injury. It is therefore surprising to note that Dagassan et al. (1994) recently reported no protective effect of a similar concentration of bosentan in isolated rat hearts subjected to ischeamia and reperfusion. The reason for this is unclear but may possibly be related to differences in experimental protocols. Dagassan et al. (1994) used 20 min global ischaemia compared to 30 min in the present study which causes less severe myocardial damage. This is evident from the complete recovery in coronary flow in the control group (Dagassan et al., 1994) compared to 42% in the present study indicating clear differences in development of the no-reflow phenomenon. In addition the recovery in left ventricular end-diastolic pressure of the control group appears to have been somewhat better in their study than in ours. Finally, Dagassan et al. (1994) administered lidocaine at the start of reperfusion which may exert cardioprotective effects by itself (Hatori et al., 1991). It was also reported that bosentan given to anaesthetised rats (3 mg/kg i.v.) did not protect the myocardium from coronary artery occlusion and reperfusion (Richard et al., 1994). This may be due to completely different experimental conditions, in vivo vs. in vitro. Our results indicating involvement of endothelin in the development of myocardial ischaemic and reperfusion injury are, however, in line with the observations that bosentan given systemically or locally reduced the infarct size in a porcine model of coronary occlusion and reperfusion (Wang et al., 1995b). Furthermore, an endothelin monoclonal antibody given to rats (Watanabe et al., 1991) and the endothelin ET_A receptor antagonist BQ-123 given to dogs (Grover et al., 1993) reduce the infarct size following myocardial ischaemia and reperfusion. According to the discussion above it is possible that various degrees of ischaemia before initiation of reperfusion activate the endothelin system differently and that benificial effects of endothelin antagonists are observed mainly under severe ischaemic conditions.

The phenomenon of underperfused myocardial areas during reperfusion following ischaemia is known as no-reflow (Gavin et al., 1983). The cause of the no-reflow phenomenon is unclear and several factors may contribute including oedema or stiffening of the myocytes and endothelial cell swelling (Powell et al., 1983; Humphrey and Gavin, 1985). The area of no-reflow was markedly smaller and coronary flow was higher in the bosentan group suggesting that endothelin released during myocardial ischaemia and reperfusion may be of importance for the development of the no-reflow during reperfusion. Furthermore, after 4 h of reperfusion following 45 min of coronary artery occlusion in

the pig, coronary blood flow in the reperfused myocardial area was up to 90% higher in bosentan-treated animals than in controls and not significantly different from pre-ischaemic flow (Wang et al., 1995b). It is possible that prevention of no-reflow during the reperfusion period is an important factor by which bosentan protects the myocardium from myocardial ischaemic and reperfusion injury. Accordingly, when the no-reflow phenomenon following a shorter period of ischaemia was absent bosentan exerted no protective effects (Dagassan et al., 1994). Administration of bosentan at the end of the reperfusion period did not improve coronary flow in the present study. This indicates that bosentan has to be present before the development of ischaemia/reperfusion-induced no-reflow.

Other mechanisms by which bosentan protects the myocardium may be by inhibiting direct effects of endothelin on myocytes and the ability of endothelin to increase intracellular calcium levels (Shah et al., 1989; Kelly et al., 1990). Some data have shown that endothelin-1 exerts a pro-ischaemic effect which is independent of coronary vasoconstriction (Grover et al., 1992). The pro-ischaemic effect induced by endothelin may be secondary to its role as a phospholipase C activator (Galron et al., 1990). Phospholipase C induces hydrolysis of inositol phosphates and subsequent release of intracellular calcium (Berridge, 1993), which aggravates myocardial and endothelial injury from myocardial ischaemia/reperfusion (Nayler, 1987). Further studies are needed to determine the possible interaction between phospholipase C, intracellular calcium and bosentan during myocardial ischaemia and reperfusion.

Acetylcholine and SNAP were administered before and after myocardial ischaemia and reperfusion to determine endothelium-dependent and endothelium-independent vasodilatation, respectively. The poor response to acetylcholine after myocardial ischaemia and reperfusion in the vehicle group but not after a similar time period of non-ischaemic perfusion shows that impaired endothelium-dependent relaxation via release of nitric oxide was caused by the ischaemia and reperfusion, which is in line with several previous studies (Tsao et al., 1990; Weyrich et al., 1992). The endothelium-independent vasodilatation was, on the other hand, not affected by myocardial ischaemia and reperfusion as revealed by the unchanged response to the nitric oxide donor SNAP. The endothelium-dependent vasodilator response was significantly larger in the bosentan group than in the vehicle group at the end of reperfusion, suggesting that bosentan preserved endothelial function after myocardial ischaemia and reperfusion. It remains, however, to be established whether this is due to a direct endothelial protective effect of bosentan or if it is secondary to a general cardioprotective effect.

In conclusion, the non-peptide endothelin receptor antagonist bosentan efficiently inhibits both endothelin ET_A and endothelin ET_B receptor-mediated coronary effects and prevents development of myocardial and endothelial injury following ischaemia and reperfusion which suggest that endogenous endothelin is involved in the pathogenesis of myocardial ischaemic and reperfusion injury in the rat isolated heart. Furthermore, bosentan may be an important future therapeutic tool in the management of myocardial ischaemic and reperfusion injury.

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