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Combined blockade of endothelin-1 and thromboxane A_2 receptors against postischaemic contractile dysfunction in rat hearts

[1]Pius S. Hornstein, *,[1]Christian E. Zaugg, [1]Peili Zhu, [2]Peter R. Allegrini & [3]Peter T. Buser

[1]Cardiovascular Research Group, Department of Research, ZLF 319, Hebelstrasse 20, University Hospital, CH-4031 Basel, Switzerland; [2]Biology Research Laboratory, NOVARTIS Ltd., H-4002 Basel, Switzerland and [3]Division of Cardiology, Department of Internal Medicine, ZLF 319, Hebelstrasse 20, University Hospital, CH-4031 Basel, Switzerland

1 Endothelin-1 (ET-1) may play a role in myocardial ischaemia/reperfusion injury because both the release and vasoconstrictor effect of ET-1 are increased after ischaemia. Since the increased vasoconstrictor effect of ET-1 can be mediated by ET-1-induced release of thromboxane A_2 (TXA_2), the aim of this study was to test whether combined blockade of ET and TXA_2 receptors protects the coronary flow, contractile performance, and cardiac energy metabolism during ischaemia and reperfusion.

2 Bosentan (antagonist for ET_A and ET_B receptors, 1 μM based on concentration-response curves of ET-1), SQ 30,741 (antagonist of TXA_2 receptors, 0.1 μM), or the combination thereof was administered to isolated perfused rat hearts undergoing 15 min of global ischaemia and 60 min of reperfusion.

3 Neither bosentan or SQ 30,741 alone, nor the combination thereof, improved the incomplete postischaemic recovery of coronary flow, left ventricular developed pressure, phosphocreatine, or ATP. However, they attenuated ischaemia-induced acidosis but this did not translate into a measurable effect on haemodynamic or metabolic variables.

4 Thus, combined blockade of ET and TXA_2 receptors does not protect the coronary flow, contractile performance, and cardiac energy metabolism during ischaemia and reperfusion in isolated perfused rat hearts. This finding suggests that neither ET-1 nor ET-1-induced release of TXA_2 play a major role in the postischaemic recovery of the cardiac contractile function and energy metabolism. *British Journal of Pharmacology* (2001) **132,** 234–240

Keywords: Endothelin-1; endothelin receptor antagonist; bosentan; thromboxane A_2 receptor antagonist; ischaemia; reperfusion; cardioprotection; vasoconstriction; left ventricular function; coronary flow

Abbreviations: ATP, adenosine triphosphate; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; ET-1, endothelin-1; $[H^+]_i$, intracellular H^+ concentration; pH_i, intracellular pH; TXA_2, thromboxane A_2

Introduction

The vasoconstrictor peptide endothelin-1 (ET-1) has been implicated in the pathogenesis of myocardial ischaemia/reperfusion injury (Pernow & Wang, 1997; Watanabe *et al*., 1990; Zaugg *et al*., 1996). This implication has been based on both increased ET-1 plasma levels after myocardial ischaemia (Miyauchi *et al*., 1989; Watanabe *et al*., 1991) and increased vasoconstrictor effects of ET-1 during postischaemic reperfusion (McMurdo *et al*., 1991; Neubauer *et al*., 1991; Zaugg *et al*., 1993). As a consequence, ET-1 receptor antagonists have been proposed to be cardioprotective and to limit ischaemia/reperfusion injury.

However, experimental studies using either selective antagonists against the ET_A receptor subtype or mixed antagonists against both ET_A and ET_B receptors subtypes have produced controversial results in terms of both reduction of infarct size and improved recovery of myocardial performance and coronary flow (reviewed by Pernow & Wang, 1997). In their detailed analysis, the authors conclude that the controversial results of ET antagonists may be partially explained by differences in animal species, experimental protocol, route of administration, and chemical nature of the ET antagonists (Pernow & Wang, 1997). Nevertheless, additional studies are needed to further elucidate the role of ET-1 in myocardial ischaemia/reperfusion injury.

We have previously demonstrated that the increased vasoconstrictor effect of ET-1 during postischaemic reperfusion in rat hearts can be mediated by ET-1-induced release of another vasoconstrictor agent, thromboxane A_2 (TXA_2) (Zaugg *et al*., 1996). This interaction of ET-1 and TXA_2 may be an interesting target for pharmacological interventions reducing myocardial ischaemia/reperfusion injury. In the present study, we therefore tested whether combined blockade of ET and TXA_2 receptors protects the coronary flow, contractile performance, and cardiac energy metabolism during ischaemia and reperfusion. For this purpose, we administered bosentan (a nonpeptide antagonist for both ET_A and ET_B receptors (Clozel *et al*., 1994)), SQ 30,741 (a selective antagonist of TXA_2 and prostaglandin endoperoxides receptors (Grover *et al*., 1988)), or the combination thereof to isolated perfused rat hearts undergoing 15 min of global ischaemia and 60 min of reperfusion. This preparation has previously been shown to release meaningful (i.e. close

*Author for correspondence:

(patho)-physiological plasma) concentrations of ET-1 and TXA_2 into the coronary circulation during and after reversible ischaemia (Brunner *et al*., 1992; Zaugg *et al*., 1996) and can thus be used to test the present hypothesis. Cardioprotection was assessed as postischaemic recovery of coronary flow, left ventricular developed pressure, phosphocreatine, adenosine triphosphate (ATP), and intracellular pH (pH_i). The nonspecific ET antagonist bosentan was preferred to a selective ET_A antagonists because both ET_A and ET_B receptors have been reported to mediate constriction of coronary arteries in pigs and human beings (Seo *et al*., 1994; Wang *et al*., 1995a).

Methods

Isolated heart preparation

The animal use protocol was approved by the veterinary department of Basel (Switzerland) and was in compliance with the rules of the Swiss Animal Welfare Law (Swiss Veterinary Legislation publication, 1981). Sprague-Dawley rats (RCC Ltd, Füllinsdorf, Switzerland; only male gender to reduce potential variability of postischaemic recovery) weighing 250–330 g were anaesthetized with sodium pentobarbital (30 mg kg^{-1} intraperitoneally), and heparinized (1000 IU kg^{-1}). After midline sternotomy, hearts were excised rapidly and the aorta was cannulated within 30 s. Retrograde perfusion was performed at 37°C from a reservoir 900 mm above the aortic cannula (Zaugg *et al*., 1996). All hearts were perfused with a nonrecirculating modified Krebs-Henseleit solution containing (in mM); NaCl 117.0, KCl 4.3, $CaCl_2$ 1.5, $MgCl_2$ 1.2, KH_2PO_4 0.1, $NaHCO_3$ 25.0, glucose 15.0, and insulin 10 IU l^{-1} (to warrant energy provision (Lopaschuk, 1997; Rosen *et al*., 1984)), saturated with a mixture of 95% O_2 and 5% CO_2, at pH 7.4. To avoid inhibition of endogenous ET-1 generation by the ET converting enzyme, NaEDTA was omitted in this perfusate (Ashizawa *et al*., 1994). A pair of platinum pacemaker wires from a pulse generator (Grass SD 5, Grass Instruments, Quincy, MA, U.S.A.) was implanted in the right ventricular wall and the right atrium. Throughout the experiment, hearts were paced at a constant frequency which was 120% of the spontaneous heart rate (Zaugg *et al*., 1996; Zhu *et al*., 1995). During all experiments, the hearts were immersed in perfusate that was maintained at exactly 37°C.

Experimental protocol

After a 20-min stabilization period, hearts underwent 10 min preischaemic treatment, 15 min of global isothermic ischaemia, and 60 min of postischaemic reperfusion. This protocol did not cause any myocardial necrosis in our experiments as evaluated by 2,3,5-triphenyltetrazolium chloride staining (Barbosa *et al*., 1996) in six hearts after 15 min ischaemia and prolonged reperfusion (120 min).

The hearts were randomly assigned to one of four groups: (I) control (pre- and postischaemic perfusion without drugs, $n=9$); (II) bosentan (pre- and postischaemic perfusion with bosentan, $n=6$); (III) SQ 30,741 (pre- and postischaemic treatment with SQ 30,741, $n=6$); and (IV) bosentan combined with SQ 30,741 (pre- and postischaemic treatment with bosentan and SQ 30,741, $n=8$). The concentration of bosentan was determined in dose-response curves of ET-1 in our experimental system in isolated perfused rat hearts. For this purpose, cumulative 1-ml boli of 1, 3, 10, 30, and 100 pmol ET-1 were injected into the perfusion line 10 cm proximal to the aortic cannula. Moreover, the selected concentration was tested during reperfusion to antagonize the effect of an ET-1 bolus (10 pmol) that has been shown to elicit strong vasoconstriction during reperfusion in isolated rat hearts (Zaugg *et al*., 1993; 1996). The concentration of SQ 30,741 (0.1 μM) has previously been used in similar concentrations and pretreatment periods to effectively block TXA_2 and prostaglandin endoperoxides receptors in perfused rat hearts (Grover *et al*., 1990; Zaugg *et al*., 1996). Bosentan and SQ 30,741 were administered preischaemically to antagonize potential accumulation of ET-1 and TXA_2 in the coronary circulation and in cardiac tissue during ischaemia (due to reduced coronary washout) and early reperfusion (Brunner, 1995; Zaugg *et al*., 1996). Bosentan was dissolved in 1 ml of distilled water and then diluted with Krebs-Henseleit solution to the final concentration. SQ 30,741 was dissolved in 100 μl ethanol and then diluted with Krebs-Henseleit solution to a final ethanol concentration of 0.01% (v v^{-1}). Bosentan (Ro 47-0203, sodium salt; solutions protected from light) was donated by Hoffmann-La Roche (Basel, Switzerland) and SQ 30,741 was from Squibb (Princeton, U.S.A.).

During all experiments, left ventricular pressure was measured continuously and coronary flow was measured at 5-min intervals. Phosphocreatine, ATP, and pH_i were recorded during 5-min periods.

Measurements of coronary flow and left ventricular pressure

Coronary flow was measured by collecting the effluent from the right ventricular outflow tract in graduated cylinders. Left ventricular developed pressure was measured by a fluid-filled polyethylene catheter inserted through the left atrial appendage into the left ventricle (Brunner *et al*., 1992; Zaugg *et al*., 1996). A balloon-tipped catheter was not used to avoid inconsistent myocardial stretch during reperfusion that can cause arrhythmias due to stretch-induced depolarizations (Rosen *et al*., 1991; Zabel *et al*., 1996). The catheter was connected to a Statham P23Db pressure transducer (Gould, Cleveland, OH, U.S.A.) which was outside the magnet at the same height as the heart. Left ventricular developed pressure was defined as the difference between systolic and diastolic values of left ventricular pressure.

^{31}Phosphorus magnetic resonance spectroscopy

^{31}Phosphorus magnetic resonance spectroscopy of the beating isolated perfused rat heart was performed on a 4.7 Tesla horizontal 300 mm bore magnet (Bruker Spectrospin B-C 47/30, Fällanden, Switzerland), as previously described (Zaugg *et al*., 1996). Spectra were obtained at 81.0 MHz with a bandwidth of 5000 Hz. The pulse angle was 45° and the pulse duration was 50 μs with a repetition time of 1.0 s. Three hundred transients were accumulated at 5-min intervals. The signal to noise ratio was approximately 20:1. For each spectrum the characteristic peaks for phosphocreatine, the

three phosphate groups of ATP (β-ATP used for quantification purposes) were identified. Each spectrum and the corresponding areas were numerically integrated after definition of the baseline and expressed as percentage of the baseline values. The intracellular pH was calculated from the chemical shift of the inorganic phosphate relative to phosphocreatine (Gaddian *et al*., 1979).

Statistical analysis

Results are expressed as mean ± standard deviation (s.d.) after normality testing using the Kolmogorov-Smirnov test ($P>0.1$ for coronary flow, left ventricular pressure, and pH_i at baseline). Statistical analysis between groups was performed by one-way ANOVA followed by Student-Newman Keuls test. For all statistical analyses, the null hypothesis was rejected at the 95% level, considering $P<0.05$ significant.

To avoid type II errors (missing significant differences when the sample size is too small) we determined the required sample size for the present study using an approximation for α equal to 0.05 and power to 0.90 (Dawson-Saunders & Trapp, 1990). Accordingly, six rats per group was the minimal sample size to detect differences of 2.5 ml min^{-1} in coronary flow and of 10 mmHg in left ventricular pressure with s.d. of 1.3 ml min^{-1} and 5.7 mmHg, respectively (s.d. taken from previous data recorded in identical experimental conditions Zaugg *et al*., 1993; 1996; Zhu *et al*., 1995)).

Results

Dose-response curves

In dose-response curves of ET-1 (1 – 100 pmol), we evaluated the effects of bosentan (0.1, 1, and 10 μM) on the coronary flow of isolated perfused rat hearts (Figure 1). Based on these curves, we then selected a concentration of bosentan (1 μM) that could effectively antagonize the vasoconstrictory effects of ET-1 up to 10 pmol (corresponding to ≈1 nM ET-1 because after injection the bolus diluted ≈1 : 10 in perfusate before acting on the heart in our experiments). This concentration was considered sufficient to antagonize circulating ET-1 levels in myocardial ischaemia and reperfusion (0.1 – 1 pM) (Battistini *et al*., 1993). To test the selected concentration of bosentan in our system, an ET-1 bolus (10 pmol) was administered after 30 min of reperfusion to elicit strong vasoconstriction as previously shown (Zaugg *et al*., 1993; 1996). Accordingly, 1 μM bosentan prevented the reduction of coronary flow induced by the ET-1 bolus during reperfusion (Figure 2). Based on the foregoing, 1 μM bosentan was considered sufficient to antagonize endogenous ET-1 in further experiments during ischaemia and reperfusion.

Effects of bosentan and SQ 30,741 during ischaemia/reperfusion

At baseline, no significant differences of coronary flow, left ventricular developed pressure, or pH_i could be detected among the four groups. Similarly, after 10 min of preischaemic treatment with or without drugs, no significant differences of coronary flow, left ventricular developed pressure, or pH_i could be detected among the groups (Figures 3 – 5).

During 15 min of global ischaemia, both coronary flow and developed pressure rapidly decreased to zero in all groups (Figure 3). Furthermore, phosphocreatine, ATP, and pH_i gradually decreased to ≈8%, ≈38%, and ≈5.5 respectively (Figures 4 and 5). Moreover, no differences of phosphocreatine or ATP levels could be detected among the four groups throughout the ischaemic period (Figure 4). However, in hearts treated with the combination of bosentan and SQ 30,74, pH_i decreased temporarily less than in control hearts (Figure 5).

During 60 min of postischaemic reperfusion, all variables recovered incompletely in the four groups (Figures 3 – 5). Furthermore, most variables did not differ among the four groups during or at the end of the reperfusion period.

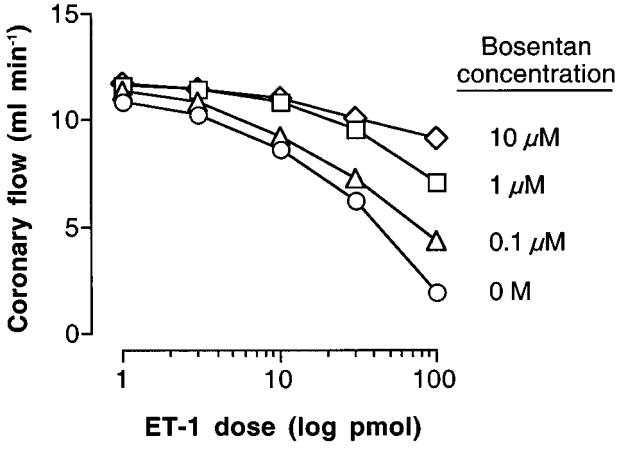

Figure 1 Dose-response curves of ET-1 (1, 3, 10, 30, and 100 pmol) with bosentan (0, 0.1, 1, and 10 μM) on coronary flow in isolated perfused rat hearts ($n=3$ hearts per concentration of bosentan). Values are means normalized at baseline (before ET-1 administration) to the average coronary flow (11.7 ml min^{-1}) of all groups. Average s.d. of coronary flow = 1.5 ml min^{-1}.

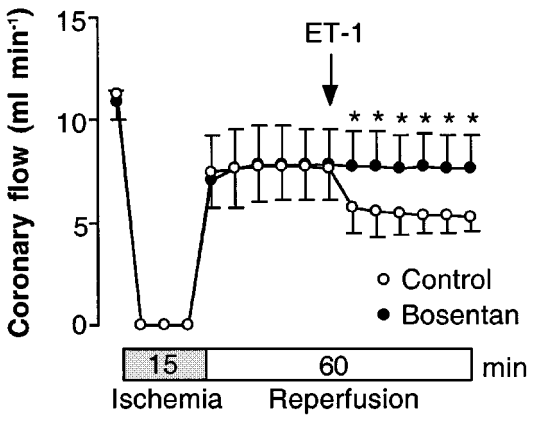

Figure 2 Effect of bosentan (1 μM) on the vasoconstrictory effect of ET-1 (10-pmol bolus) evaluated as coronary flow reduction in isolated perfused rat hearts during postischemic reperfusion. Bosentan was administered at the beginning of reperfusion and ET-1 was injected 30 min later. Values are mean ± s.d. of six hearts in each group. *$P<0.05$ vs untreated control. Note that 1 μM bosentan prevented the ET-1-induced reduction of coronary flow.

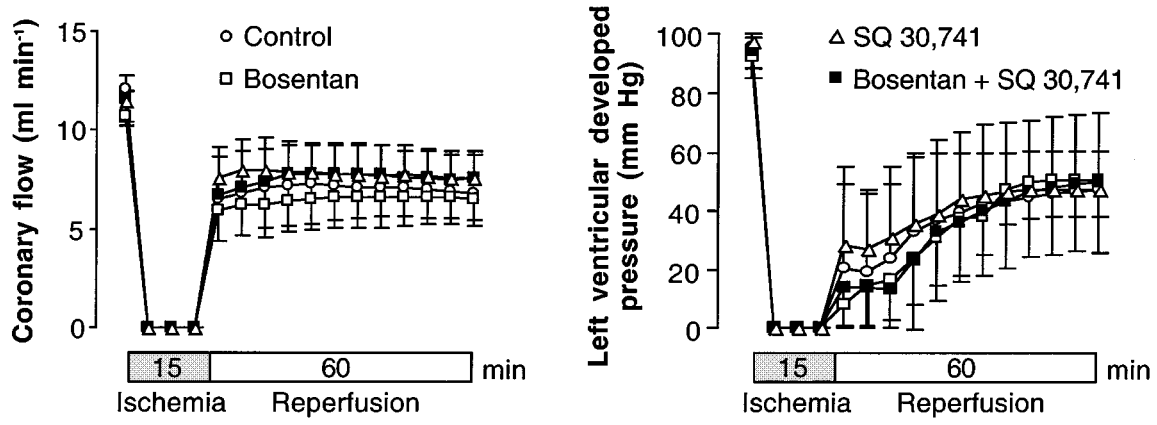

Figure 3 Effects of bosentan (1 μM), SQ 30,741 (0.1 μM), or bosentan (1 μM) combined with SQ 30,741 (0.1 μM) on coronary flow (left) and left ventricular developed pressure (right) of isolated perfused rat hearts during ischaemia and reperfusion in comparison to untreated controls. Values are mean ± s.d. of six, eight, six and nine hearts, respectively. Note that bosentan alone or combined with SQ 30,741 did not improve postischemic recovery of coronary flow or developed pressure.

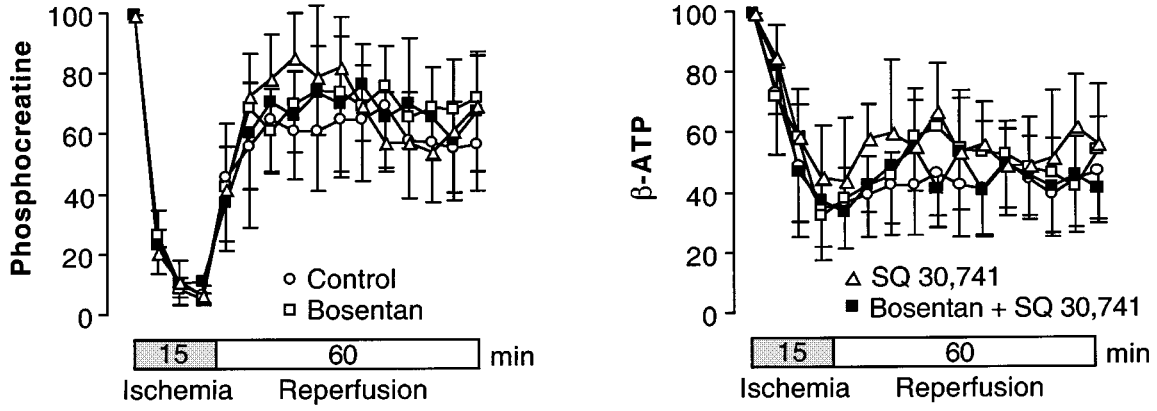

Figure 4 Effects of bosentan (1 μM), SQ 30,741 (0.1 μM), or bosentan (1 μM) combined with SQ 30,741 (0.1 μM) on phosphocreatine (left) and β-ATP (right) of isolated perfused rat hearts during ischaemia and reperfusion in comparison to untreated controls. Values are mean ± s.d. of six, eight, six and nine hearts, respectively. Note that bosentan alone or combined with SQ 30,741 did not improve postischaemic recovery of phosphocreatine or β-ATP.

Specifically, coronary flow, developed pressure, phosphocreatine, and ATP did not differ during or at the end of reperfusion (Figures 3 and 4). However, in hearts treated with bosentan, SQ 30,741, or the combination of bosentan and SQ 30,741, pH_i recovered temporarily better than in control hearts (Figure 5).

Discussion

The present study showed that combined blockade of ET and TXA_2 receptors does not protect the coronary flow, contractile performance, and cardiac energy metabolism during ischaemia and reperfusion in isolated perfused rat hearts. Specifically, neither the nonselective ET receptor antagonist bosentan, nor the TXA_2/prostaglandin endoperoxides receptor antagonist SQ 30,741, nor the combination thereof improved the postischaemic recovery of coronary flow, left ventricular developed pressure, phosphocreatine, or ATP. However, they slightly increased pH_i during ischaemia and reperfusion, indicating attenuation of ischaemia-induced acidosis. Nevertheless, this did not translate into a measurable effect on haemodynamic or metabolic variables in our experiments.

This negative finding is in accordance with most previous reports demonstrating no protective effects of ET antagonists on the contractile function after ischaemia and reperfusion. Specifically, in nine out of 15 reports, the selective ET_A antagonists BQ-123 (Grover *et al*., 1992; 1993; Tiefenbacher *et al*., 1997; Wang *et al*., 1995b) or FR 139317 (McMurdo *et al*., 1994), or the selective ET_B antagonist sarafotoxin S6c (Sargent *et al*., 1994), or the mixed antagonist bosentan (Dagassan *et al*., 1994; Krause *et al*., 1994; Richard *et al*., 1994) did not improve the postischaemic recovery of contractile variables in rats, dogs, pigs, or rabbits after regional or global zero-flow ischaemia. Our findings extend these reports in a manner that combining bosentan with the TXA_2 receptor antagonist SQ 30,741 is not superior to bosentan alone and still confers no protection against postischaemic contractile dysfunction in isolated rat hearts. In addition, bosentan and SQ 30,741 alone or in combination also confer no protection against depletion of phosphocreatine and ATP during ischaemia.

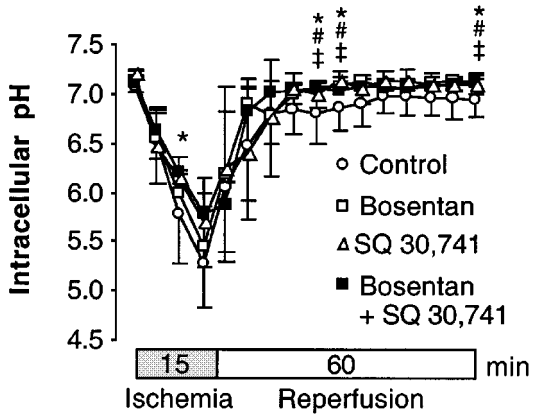

Figure 5 Effects of bosentan (1 μM), SQ 30,741 (0.1 μM), or bosentan (1 μM) combined with SQ 30,741 (0.1 μM) on intracellular pH of isolated perfused rat hearts during ischaemia and reperfusion in comparison to untreated controls. Values are mean $\pm$ s.d. of six, eight, six and nine hearts, respectively. $^{*}P<0.05$ bosentan + SQ 30,741 vs control; $^{\#}P<0.05$ bosentan vs control; $^{\ddagger}P<0.05$ SQ 30,741 vs control. Note that bosentan and SQ 30,741 alone, and particularly in combination, partially attenuated acidosis during ischaemia and improved postischaemic recovery of intracellular pH.

However, our findings contrast with reports from three laboratories that found protective effects of ET antagonists (ET_A or mixed antagonists) on the postischaemic contractile function (Brunner, 1997; Brunner & Kukovetz, 1996; Han *et al*., 1995; Illing *et al*., 1996; Li *et al*., 1995; Wang *et al*., 1995b). In one of these reports, protective effects of ET_A antagonists were found against early but not late (>10 min) contractile dysfunction during reperfusion (Illing *et al*., 1996).

Furthermore, we noted a discrepancy between the effects of ET antagonists against contractile dysfunction and their effects against extension of myocardial infarction. Although most studies failed to demonstrate protection against postischaemic contractile dysfunction, several studies found ET antagonists or antibodies against ET-1 to reduce infarct size in various animal models (Grover *et al*., 1993; Kojima *et al*., 1995; Lee *et al*., 1994; Wang *et al*., 1995b; Watanabe *et al*., 1991). In two *in vivo* studies assessing both endpoints, BQ-123 or bosentan even reduced the infarct size in dog and pig hearts without affecting the contractile function (Grover *et al*., 1993; Wang *et al*., 1995b) thus, supporting the notion that ET antagonists may confer better protection against extension of myocardial infarction than against postischaemic contractile dysfunction. In three other reports, however, bosentan (Krause *et al*., 1994; Richard *et al*., 1994) or FR139317 (McMurdo *et al*., 1994) did not reduce both infarct size and postischemic contractile dysfunction in dogs, rabbits, or rats. Similar to ET antagonists, the TXA_2 receptor antagonist SQ 30,741 reduced the infarct size in models of ischaemia and reperfusion in dogs and monkeys (Grover *et al*., 1988; Schumacher & Grover, 1990) but failed to affect the postischaemic contractile recovery of perfused rat hearts (Grover *et al*., 1990; Zaugg *et al*., 1996). In the present study focusing on functional and metabolic recovery, we did not prolong the ischaemia time to assess protection against extension of myocardial infarction.

Thus, despite these conflicting results, ET antagonists (and maybe also TXA_2 antagonists) appear to confer better protection against extension of myocardial infarction than against postischaemic contractile dysfunction. This differential protection may be due to different degrees of ischaemic injury inflicted. As previously hypothesized (Pernow & Wang, 1997) the importance of ET-1 for the development of ischaemia/reperfusion injury may be related to the degree of ischaemic injury, and ET antagonists may be beneficial when the ischaemia is severe. However, analysing the ischaemia time of previous studies of ET antagonists against myocardial ischaemia/reperfusion injury does not support this hypothesis. Specifically, the duration of ischaemia in studies that found protection was not longer than in those that did not (disregarding end-points, models, and species). Still, the differential protection and contradictory results of ET antagonists may be partially explained by differences in animal species, experimental protocol, route of administration and chemical nature of the ET antagonists, as well as preconditioning effects of ET-1 (Bugge & Ytrehus, 1996; Pernow & Wang, 1997).

Furthermore, it could be speculated that the differential protection is due to effects of ET antagonists on pH_i during ischaemia and reperfusion. ET-1 has been previously reported to stimulate the sarcolemmal Na^+/H^+ exchange in rat cardiac cells (Krämer *et al*., 1991). This stimulation may decrease the intracellular proton concentration ($[H^+]_i$) resulting in intracellular alkalization and increased Ca^{2+}-sensitivity of the myofilaments during acidosis (Wang & Morgan, 1992). This is, however, at the expenses of increased intracellular free Ca^{2+} concentrations ($[Ca^{2+}]_i$) *via* Na^+/H^+ exchange and Na^+/Ca^{2+} exchange, and may so contribute to irreversible ischaemic myocardial injury (Silverman & Stern, 1994; Steenbergen *et al*., 1993). Accordingly, ET-1-stimulated Na^+/H^+ exchange aggravated ischaemia/reperfusion injury in rat hearts (Khandoudi *et al*., 1994). ET antagonists may thus be beneficial by inhibition of the ET-1-stimulated Na^+/H^+ exchange. However, this implies that pH_i in bosentan treated hearts is lower than in untreated hearts during ischaemia and reperfusion. This could not be detected in our experiments. In contrast, pH_i was slightly elevated in bosentan treated hearts. It is unclear whether this was due to an unspecific effect of bosentan. Still, as long as pH_i is near normal (as during reperfusion in our experiments), slightly elevated pH_i may not translate into a large effect on contractile function. Nevertheless, if this elevation is sustained, it might reduce the rise of $[Ca^{2+}]_i$ and so translate into limitation of myocardial infarction.

Finally, it should be noted that the negative findings of the present study were not due to type II errors (see Statistical analysis) or to insufficient concentrations of bosentan or SQ 30,741. Specifically, the concentrations of bosentan (1 μM) and SQ 30,741 (0.1 μM) were based on previous reports and on dose-response curves. This way, the bosentan concentration was most likely sufficient to antagonize endogenous ET-1 concentrations in myocardial ischaemia and reperfusion (0.1 – 1 pM) (Battistini *et al*., 1993) as well as to antagonize ET-1 concentrations necessary to occupy ET receptors (50 pM – 1 nM) or to elicit contractions in isolated vessel preparations (0.1 – 50 nM) (Frelin & Guedin, 1994). Accordingly, 1 μM bosentan prevented the effect of a 10-pmol ET-1 bolus ($\approx$1 nM; Figure 2) that elicits strong vasoconstriction (Zaugg *et al*., 1993; 1996).

Similarly, the negative results of the present study were most likely not due to reduced formation and/or release of ET-1 or

TXA_2 in the isolated rat heart. The endothelium of perfused rat hearts has been shown to produce ET-1 and to release it to the coronary circulation providing similar ET-1 concentrations ($\approx$0.1 pM (Brunner, 1995)) as observed in plasma of healthy rats (1–2 pM (Frelin & Guedin, 1994)). Moreover, ET-1 concentrations increased nearly 5 fold during and after 90 min low-flow ischaemia or 20 min global ischaemia (Brunner *et al*., 1992) to near (patho)-physiological plasma concentrations of ET-1 during ischaemia/reperfusion *in vivo* (Battistini *et al*., 1993). Similarly, TXA_2 concentrations in the coronary circulation of perfused rat hearts were $\approx$14 pM and increased 4 fold upon postischaemic reperfusion (Zaugg *et al*., 1996) to similar levels as observed in angina pectoris patients (Lewy *et al*., 1980). Thus, the crucial question is not whether isolated hearts produce insufficient ET-1 and TXA_2 levels but whether these levels are sufficiently high at the site(s) of action to play a role in ischaemia/reperfusion injury. Circulating ET-1 concentrations are approximately 100–1000 times lower than the concentration necessary to elicit vasoconstriction *in vitro*. Circulating ET-1 levels may however not represent active ET-1 because of high ET-1 levels bound to receptors (Frelin & Guedin, 1994). Even if bound ET-1 levels fall short of directly promoting coronary vasoconstriction, threshold concentrations of ET-1 may still potentiate the effects other vasoconstricting agents such as norepinephrine, serotonin (Yang *et al*., 1990) or TXA_2 (Zaugg *et al*., 1996). The present study is the first to address such an interaction as a target for pharmacological interventions reducing myocardial ischaemia/reperfusion injury. Our results suggest, however, that neither endogenous ET-1 nor ET-1-induced release of TXA_2 play a major role in the postischaemic recovery of the cardiac contractile function and energy metabolism in isolated perfused rat hearts. It remains to be tested in *in vivo* models whether combined blockade of ET and TXA_2 receptors confers better protection against extension of myocardial infarction than ET blockade alone.

In conclusion, the present study showed that combined blockade of ET and TXA_2 receptors does not protect the coronary flow, contractile performance, and cardiac energy metabolism during ischaemia and reperfusion in isolated perfused rat hearts. This finding suggests that neither ET-1 nor ET-1 induced release of TXA_2 plays a major role in the postischaemic recovery of the contractile function and energy metabolism.

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