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SB 211475, a metabolite of carvedilol, reduces infarct size after myocardial ischemic and reperfusion injury in rabbits

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Abstract

The aim of this study was to investigate the effect of SB 211475, a metabolite of carvedilol with weak α_1-adrenoceptor antagonism and antioxidant effect, on myocardial reperfusion injury and infarct size in anesthetized rabbits. The rabbits were subjected to 60 min of regional myocardial ischemia and 180 min of reperfusion. SB 211475 was administered either as 0.3, 1.0 or 3.0 mg/kg and compared to vehicle and carvedilol (1 mg/kg) treated animals. The lowest dose of SB 211475 (0.3 mg/kg) did not reduce infarct size compared to vehicle, whereas SB 211475 1.0 or 3.0 mg/kg reduced infarct size significantly compared to vehicle ($41.2 \pm 2.2\%$ and $40.5 \pm 2.8\%$ vs. $59.1 \pm 3.9\%$, $p < 0.05$). Carvedilol reduced infarct size significantly more than SB 211475 1.0 and 3.0 mg/kg ($28.8 \pm 3.9\%$ vs. $41.2 \pm 2.2\%$ and $40.5 \pm 2.7\%$, $p < 0.05$). Carvedilol and SB 211475 1.0 and 3.0 mg/kg reduced myeloperoxidase activity to the same extent, indicative of reduced inflammation. Rate-pressure product did not differ between doses of SB 211475. In conclusion, SB 211475 in the two highest doses reduced infarct size by protecting from reperfusion injury, possibly by reduced neutrophil accumulation. The superior cardiac protective effect of Carvedilol over SB 211475 are most likely due to its adrenergic pharmacology including non-selective β- and α_1-adrenoceptor antagonism.

Keywords: Myocardial ischemia; Reperfusion; Infarct size; Antioxidant; β-Adrenoceptor antagonist; (Rabbit)

1. Introduction

Carvedilol is a novel non-selective β-adrenoceptor antagonist with vasodilating properties due to α_1-adrenoceptor antagonism (Feuerstein and Ruffolo, 1995). In addition, carvedilol has been shown to be a potent antioxidant (Yue et al., 1992; Feuerstein et al., 1993; Yue et al., 1993). Several studies have demonstrated marked infarct size reduction in 5 different species subjected to myocardial ischemia and reperfusion when carvedilol was administered prior to or following ischemic insults (Hamburger et al., 1991; Bril et al., 1992; Feuerstein et al., 1992). Compared to traditional non-selective β-adrenoceptor antagonists like propranolol or metoprolol, carvedilol has shown significantly better cardioprotective actions (Bril et al., 1992). Recent studies have shown that carvedilol administered immediately prior to reperfusion reduces infarct size by protecting against reperfusion injury partly due to non-selective β- and α_1-adrenoceptor antagonism (Brunvand et al., 1996a,b). However, it has not yet been shown whether the antioxidant property of carvedilol contributes to infarct size reduction. Therefore we found it interesting to examine the effect of a molecule with similar structure to carvedilol, but without significant β-adrenoceptor antagonism, in order to evaluate whether effects other than β-adrenoceptor antagonism may contribute to reduced infarct size in the experimental situation.

Carvedilol is extensively metabolized in vivo with less than 2% of the dose excreted unchanged in the urine. One of the main metabolites is SB 211475 in which a hydroxyl group is introduced at the third position of the carbazole moiety of carvedilol (Fig. 1). The metabolite has α_1-adrenoceptor antagonistic effect similar to carvedilol, but lacks significant β-adrenoceptor antagonistic effect (Hieble,

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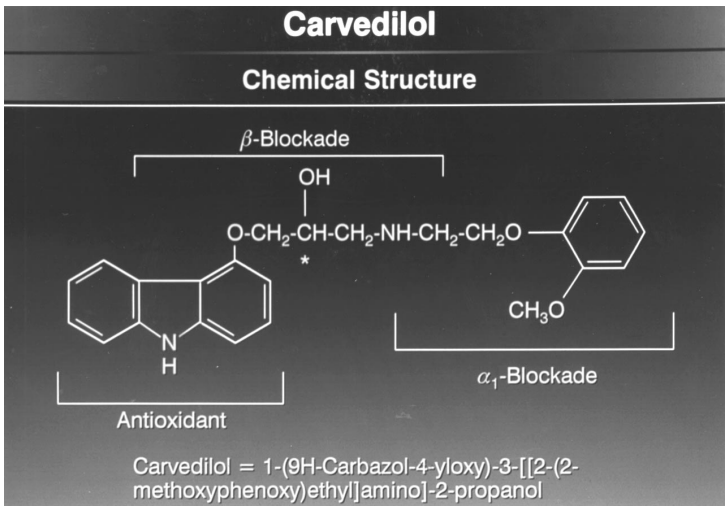

Fig. 1. Molecular structure of carvedilol. Note that SB 211475, one of the main metabolites of carvedilol has a hydroxyl group introduced at the third position of the carbazole moiety of carvedilol (marked by asterisks).

SmithKline Beecham, unpublished results). In addition SB 211475 is a very potent antioxidant in vitro (Yue et al., 1994). The present study was undertaken to examine the hypothesis that SB 211475 could reduce myocardial infarct size after ischemic/reperfusion injury in an in vivo rabbit model. SB 211475 was administered prior to and during reperfusion, thus emphasizing the effect of this substance on reperfusion injury. Since neutrophils are thought to contribute to the development of reperfusion injury (Litt et al., 1989), the study also examined the effect of carvedilol and SB 211475 on myeloperoxidase activity in the postischemic myocardium as an indirect measure of neutrophil invasion.

2. Methods

2.1. Experimental preparation

The experiments were performed in adherence to National Institute of Health Guidelines on the Use of Laboratory Animals and were approved by the Thomas Jefferson University Committee on Animal Care. Male New Zealand White rabbits—not fasting—weighing 2.5–3.5 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.). The animals were tracheotomized and ventilated by positive pressure ventilation via a small animal respirator (Harvard apparatus, S. Natick, MA, USA). Ventilation was regulated according to blood gas measurements. Body temperature was held constant with a heat blanket. A polyethylene catheter was inserted into the left femoral vein for infusion purposes. An additional polyethylene catheter was inserted into the right carotid artery for measurement of arterial blood gases. After a midsternal thoracotomy, the heart was exposed through a pericardiotomy and a 4-0 silk ligature was carefully placed around the major marginal branch of the left circumflex coronary artery located on the dorsolateral surface of the heart, 10–12 mm from its origin. A high-fidelity catheter tip transducer (MPC-500, Millar Instrument) was inserted into the left ventricular cavity through an apical stab wound, and secured with a 4-0 silk suture. Heart rate and left ventricular systolic pressure were continuously recorded on a computer based data recorder. The rate-pressure product, calculated as the product of left ventricular systolic pressure and heart rate, was employed as an approximation of myocardial oxygen demand.

2.2. Experimental protocol

The rabbits were randomly assigned to 5 groups with 8–9 rabbits in each group. After a 30-min stabilization period after instrumentation, myocardial ischemia was initiated by complete ligation of the marginal coronary artery. After 60 min of regional ischemia, the ligature was untied and the ischemic myocardium was reperfused for 180 min. At least 2 h reperfusion is necessary in order to be able to stain necrotic tissue due to washout of dehydrogenase enzymes from necrotic tissue.

The animals were treated with either vehicle, carvedilol 1 mg/kg or SB 211475 in three different dosages (0.3, 1.0 and 3.0 mg/kg), administered as a bolus injection 10 min prior to reperfusion, followed by continuous infusion throughout the experiment. For continuous infusion, we used the same dose as the bolus injection per hour. The dose of carvedilol was based upon previous studies with carvedilol in myocardial ischemia/reperfusion models (Bril et al., 1992; Feuerstein et al., 1992; Brunvand et al., 1996a). All drugs were dissolved in 100 μl 30% dimethyl sulfoxide and diluted in 0.9% saline to a total volume of 10 ml. The same concentration was used as vehicle. Intravenous infusion of 0.9% saline at a rate of 10 ml/kg per hour was continued throughout the experiment.

2.3. Measurement of necrosis

At the end of the experiment, the ligature was retightened. Thirty milliliters of 5% Evans blue dye was injected into the left ventricular cavity to stain the area of the myocardium perfused by the patent coronary arteries. The area at risk was therefore determined by negative staining. Rabbits were then killed by an overdose of sodium pentobarbital, and the heart was quickly removed. The left ventricle was isolated and sliced into sections 3 mm thick parallel to the atrioventricular groove. The unstained portion of the myocardium (i.e., area at risk) was separated from the stained portion (i.e., the area not at risk). Both were weighed. The unstained portion was again sliced into sections of 1 mm thickness and incubated in a 0.1% solution of nitroblue tetrazolium stain in phosphate buffer at pH 7.4 and 37°C for 15 min to detect presence of coenzyme and dehydrogenase in vital tissue. The necrotic portion of the myocardium which did not stain, was separated from the stained portion (i.e., the non-necrotic area at risk) and both were weighed. Infarct size was calculated as the percentage necrotic tissue of the area at risk, based on the respective weights. Samples from all three portions of the left ventricular cardiac tissue (nonischemic, ischemic non-necrotic and ischemic necrotic) were weighed and stored at −70°C for subsequent assay of myeloperoxidase activity.

2.4. Measurement of myeloperoxidase activity in cardiac tissue

Myeloperoxidase, an enzyme that is specific for neutrophils, was determined in cardiac tissue by the method of Bradley et al. (1982), as modified by Mullane et al. (1985), and was used as an index of neutrophil accumulation. Cardiac tissue samples (0.2–0.5 g) were homogenized in ice-cold 0.5% hexadecyltrimethyl ammonium bromide (Sigma Chemical, St. Louis, MO, USA) and dissolved in 50 mM potassium phosphate buffer at pH 6.0 using a PRO 200 homogenizer (PRO Scientific, Monroe, CT, USA). Tissue samples were kept cold on ice during the homogenization. Homogenates were centrifuged at $12\,500 \times g$ at 4°C for 30 min. The supernatants were then collected and reacted with 0.167 mg/ml of *o*-diansidine dihydrochloride (Sigma) and 0.0005% H_2O_2 in 50 mM phosphate buffer at pH 6.0. The change in absorbance was measured spectrophotometrically at 460 nm (Bechman DU 640, Fullerton, CA, USA). One unit of myeloperoxidase was defined as the quantity of enzyme that hydrolyzed 1 mmol of peroxide per minute at 25°C. The enzymatic reaction was linear. Since tissue water content does not differ more than 2–3% between ischemic and non-ischemic myocardium (Andersen et al., 1985), the enzyme activity is presented as U/100 mg wet tissue.

2.5. Statistical analysis

All values in text, table and figures are presented as mean ± S.E.M. Hemodynamics were analyzed by two way analysis of variance (ANOVA) with repeated measurements. Infarct size and myeloperoxidase were analyzed by one way ANOVA. Scheffé correction for post hoc *t*-test comparison was used when appropriate. Probabilities of 0.05 or less were considered statistically significant.

3. Results

3.1. Hemodynamics

Baseline hemodynamic results are presented in Table 1. Heart rate did not change from preocclusion values in any

Table 1
Hemodynamic results

	Preoccl	Occlusion		Reperfusion					
		20 min	60 min	5 min	20 min	40 min	60 min	120 min	180 min
HR (beats/min)									
Control	233 ± 4	235 ± 5	233 ± 5	228 ± 5	227 ± 6	229 ± 6	231 ± 10	228 ± 5	231 ± 5
Carvedilol	236 ± 5	238 ± 6	192 ± 3[ab]	191 ± 4[ab]	190 ± 4[ab]	189 ± 4[ab]	191 ± 4[ab]	190 ± 5[ab]	188 ± 5[ab]
SB 211475 0.3 mg/kg	237 ± 7	242 ± 7	253 ± 8[b]	249 ± 8[b]	251 ± 11[b]	248 ± 10[b]	245 ± 9	241 ± 10	237 ± 9
SB 211475 1.0 mg/kg	253 ± 7[b]	244 ± 9	252 ± 9[b]	251 ± 9[b]	247 ± 9[b]	240 ± 10	240 ± 11	237 ± 10	239 ± 9
SB 211475 3.0 mg/kg	241 ± 5	243 ± 5	249 ± 5[b]	244 ± 6[b]	244 ± 7[b]	246 ± 8[b]	239 ± 8	231 ± 8	231 ± 8
LVSP (mmHg)									
Control	100 ± 1	89 ± 2[a]	94 ± 3	94 ± 3	93 ± 4	96 ± 3	89 ± 4	92 ± 2	90 ± 2
Carvedilol	102 ± 3	92 ± 3[a]	68 ± 2[ab]	68 ± 2[ab]	70 ± 2[ab]	70 ± 2[ab]	71 ± 3[ab]	71 ± 3[ab]	73 ± 3[ab]
SB 211475 0.3 mg/kg	100 ± 2	91 ± 3[a]	79 ± 2[ab]	80 ± 3[ab]	80 ± 3[ab]	80 ± 3[ab]	82 ± 3[a]	80 ± 2[ab]	82 ± 4[a]
SB 211475 1.0 mg/kg	104 ± 2	95 ± 2[a]	83 ± 3[ab]	85 ± 2[ab]	84 ± 2[ab]	83 ± 3[ab]	84 ± 2[a]	84 ± 3[ab]	82 ± 3[ab]
SB 211475 3.0 mg/kg	100 ± 3	91 ± 2[a]	77 ± 2[ab]	82 ± 4[ab]	80 ± 3[ab]	80 ± 3[ab]	79 ± 3[ab]	82 ± 4[ab]	75 ± 7[ab]

[a] $p < 0.05$ vs. preoccl. [b] $p < 0.05$ vs. control. Values are mean ± S.E.M. $n = 8$ for animals treated with carvedilol, $n = 9$ for other groups. Preoccl denotes preocclusion. HR is heart rate, LVSP is left ventricular systolic pressure, Control is control group treated with vehicle, Carvedilol is animals treated with carvedilol, SB 211475 followed by dose in mg/kg denotes animals treated with SB 211475 with the respective doses.

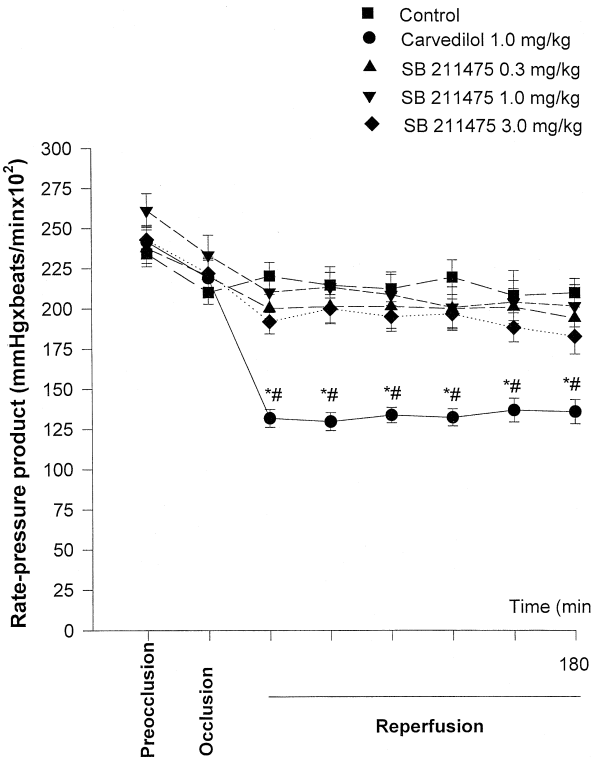

Fig. 2. Time-course of the rate-pressure product in rabbits subjected to 60 min of regional ischemia and 180 min of reperfusion. Comparisons are made between treatment groups and control, and within each group. Values are mean ± S.E.M. $* p < 0.05$ vs. preocclusion, $\# p < 0.05$ vs. control group. $n = 9(8)$ in each group. Preoccl denotes preocclusion (prior to coronary artery occlusion). Occlusion denotes 20 min of coronary artery occlusion.

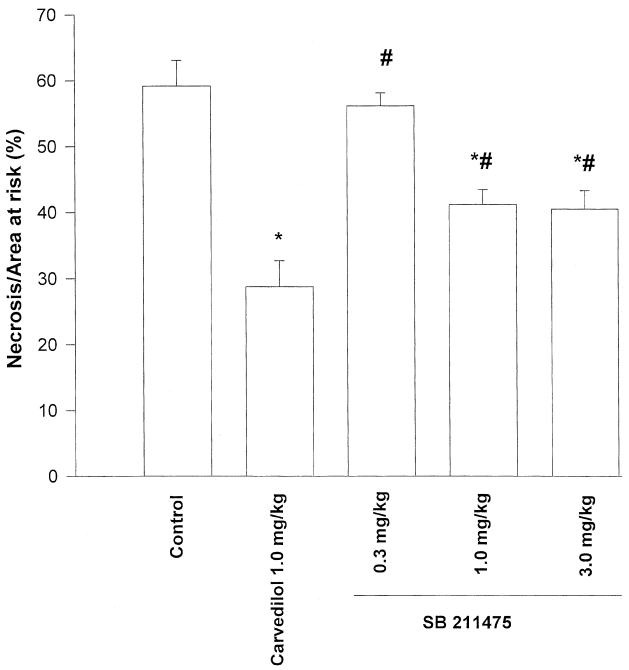

Fig. 3. Infarct size in all 5 groups presented as percent necrosis of area at risk ($n = 9(8)$ in each group). Values are mean ± S.E.M. $* p < 0.05$ vs. control animals. $\# p < 0.05$ vs. carvedilol treated animals.

groups, except in animals treated with carvedilol, where a significant reduction of heart rate occurred after administration of carvedilol. Left ventricular systolic pressure was reduced compared to both preocclusion values and vehicle treated animals in all groups after treatment with carvedilol or SB 211475. Rate-pressure product (Fig. 2), which gives a rough estimate of oxygen demand, was reduced after treatment with carvedilol, but unaffected by vehicle or SB 211475.

3.2. Infarct size

Area at risk did not differ statistically between groups. Infarct size as a percentage of area at risk is presented in Fig. 3. Carvedilol treatment led to a significant reduction in infarct size compared to vehicle. 0.3 mg/kg SB 211475 did not reduce infarct size compared to vehicle, whereas 1.0 and 3.0 mg/kg SB 211475 led to an equal reduction in infarct size compared to vehicle. Carvedilol reduced infarct size significantly more than SB 211475 1.0 and 3.0 mg/kg.

3.3. Myeloperoxidase activity in cardiac tissue

The activity of myeloperoxidase (Fig. 4), which reflects the presence of neutrophils, was very low in non-ischemic tissue, and did not differ between groups. In ischemic tissue increased myeloperoxidase activity was most extensive in vehicle treated animals compared to animals treated with either carvedilol or SB 211475. However, myeloper-

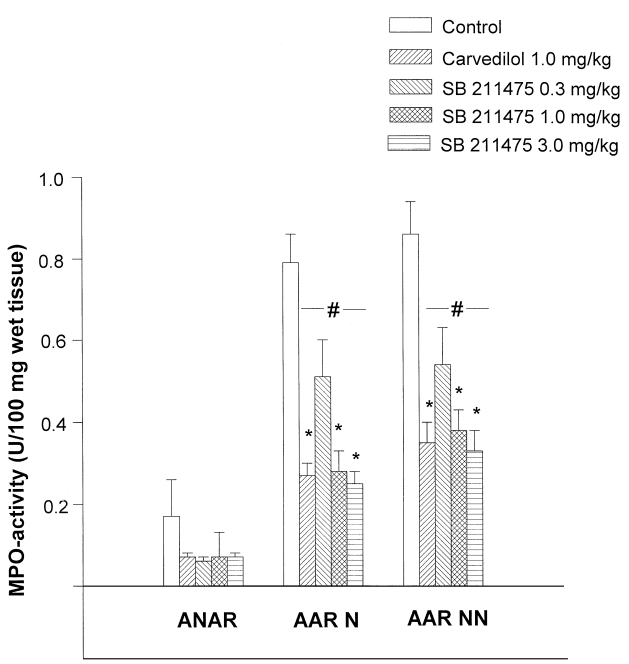

Fig. 4. Tissue myeloperoxidase activity in area not at risk (ANAR), area at risk not necrotic (AAR NN), and area at risk necrotic (AAR N). Values are mean ± S.E.M. $* p < 0.05$ vs. SB 211475 0.3 mg/kg, $\# p < 0.05$ all treatment groups vs. control. $n = 9(8)$ in each group.

oxidase activity was significantly higher in animals treated with 0.3 mg/kg compared to carvedilol and higher doses of SB 211475. There was no difference in myeloperoxidase activity between groups treated with carvedilol or SB 211475 1.0 and 3.0 mg/kg in ischemic tissue.

4. Discussion

The present study showed that SB 211475 1.0 and 3.0 mg/kg, a metabolite of carvedilol without β-adrenoceptor antagonism, reduced infarct size through protection against reperfusion injury. The study confirmed previous findings in this species where carvedilol was able to reduce infarct size significantly by protecting against reperfusion injury (Ma et al., 1996).

Since SB 211475 lacks β-adrenoceptor antagonism but possesses antioxidant properties and α_1-adrenoceptor antagonism, these two effects together or separately might account for the protective effect offered by the highest doses of SB 211475. It is well known that oxygen derived free radicals may lead to myocardial stunning (Jeroudi et al., 1994). A large number of studies have investigated whether antioxidative therapy may reduce infarct size by protecting against reperfusion injury. About half the studies demonstrate a protective effect, whereas the other half do not find any infarct size reduction (Bolli, 1991). Thus it is still unclear whether oxygen derived free radicals play a role in the development of necrosis as a result of reperfusion injury (Jeroudi et al., 1994; Reimer et al., 1993).

Previous studies in vitro have shown that SB 211475 is more potent as an antioxidant than carvedilol (Yue et al., 1994). Thus, if oxygen derived free radicals play a role in the pathogenesis of reperfusion injury, then SB 211475 might exert some of its protective effect through antioxidant properties. Failure of antioxidants to protect against reperfusion injury might be due to short-term administration (Bolli, 1990). It has been shown that continuous administration of antioxidants during reperfusion may enhance protection against reperfusion injury and infarct development (Horwitz et al., 1994). Thus, continuous administration of SB 211475 as in the present study, may enhance an antioxidant effect and protection against reperfusion injury.

The α_1-adrenoceptor antagonism on the other hand has been shown to reduce the incidence of ventricular arrhythmias during myocardial ischemia and reperfusion (Kurz et al., 1991), but has not been shown to reduce infarct size due to reperfusion injury. In the present study, increasing doses of SB 211475 did not lead to differences in left ventricular systolic pressure and rate-pressure product. This indicates a similar degree of α_1-adrenoceptor antagonism in all groups treated with SB 211475. The rate-pressure product has been suggested to give an indirect measure of —and correlate to—oxygen consumption (Baller et al., 1981). Since rate-pressure product did not differ from control experiments, oxygen consumption was most likely not reduced after administration of SB 211475. Furthermore, SB 211475 offered protection against reperfusion injury and led to reduced infarct size only in the groups treated with 1.0 and 3.0 mg/kg. These findings suggest that hemodynamic effects of α_1-adrenoceptor antagonism was not responsible for the infarct size reduction seen in the groups treated with SB 211475.

The only difference in the molecular structure between carvedilol and its metabolite, SB 211475, is a substitution of a hydroxyl group at the third position of the carbazole moiety (Fig. 1). However, functionally, this leads to lack of significant β-adrenoceptor antagonism in SB 211475. Treatment with carvedilol led to significantly smaller infarct size compared to the effect of 1.0 and 3.0 mg/kg SB 211475. This finding was paralleled by a significant reduction of rate-pressure product in the group treated with carvedilol compared to the other 4 groups. Thus, in contrast to SB 211475, carvedilol treatment led to reduced oxygen consumption. It is well documented that β-adrenoceptor antagonism during myocardial ischemia can reduce infarct size by reducing oxygen consumption in the myocardium through reduction of cardiac work and wall tension. Furthermore we have previously suggested that reduced oxygen consumption by carvedilol during reperfusion may play a role in the protection against reperfusion injury and thereby lead to smaller infarcts (Brunvand et al., 1996a,b). Together, these findings strongly suggest that β-adrenoceptor antagonism contributes to the protection offered by carvedilol. It is therefore of great interest that carvedilol reduced infarct size to a greater extent than SB 211475. Thus the non-selective β-adrenoceptor antagonism offered by carvedilol is an important protective mechanism against reperfusion injury. Previous findings that carvedilol reduces infarct size more than a combination of non-selective β-adrenoceptor antagonism and α_1-adrenoceptor antagonism (Brunvand et al., 1996b) further support the notion that carvedilol possesses pharmacological properties which can offer additional protection against reperfusion injury, beyond non-selective β- and α_1-adrenoceptor antagonism.

Neutrophil invasion during myocardial ischemia and reperfusion has been suggested to play a role in reperfusion injury and development of necrosis (Litt et al., 1989). In the reperfused ischemic myocardium the rate of neutrophil infiltration was found to be greatest after 1 h following reperfusion (Dreyer et al., 1991). A number of mechanisms have been suggested to explain how neutrophils may induce reperfusion injury. Neutrophils contribute to the generation of oxygen derived free radicals (Jeroudi et al., 1994), and myeloperoxidase is thought to play a major role in the production of oxygen derived free radicals from neutrophils, through the reaction with hydrogen peroxide (Williams, 1996). An increased amount of myeloperoxidase in the reperfused myocardium as seen in the present study, may contribute to increased production

of oxygen derived free radicals and thus reperfusion injury. In addition proteolytic enzymes liberated from neutrophils may directly injure the myocytes (Williams, 1996). Both carvedilol and SB 211475 induced a reduction of myeloperoxidase activity indicative of reduced invasion of neutrophils in the reperfused myocardium. The reduction in myeloperoxidase was most extensive in animals treated with carvedilol and animals treated with SB 211475 1.0 and 3.0 mg/kg. Thus both carvedilol and SB 211475 led to reduced neutrophil invasion. This effect may partly explain some of the protective effects of these substances beyond β- and α_1-adrenoceptor antagonism. The reduced activity of myeloperoxidase cannot differentiate whether reduced neutrophil invasion led to reduced myocardial injury or was a consequence of reduced infarct size. However, since both carvedilol and SB 211475 was administered immediately prior to reperfusion and not during ischemia, it is possible to suggest that both substances actually reduce neutrophil invasion and thereby protect against reperfusion injury. This mechanism may add to the protective effect of β- and α_1-adrenoceptor antagonism. This study, however, does not answer through which mechanisms such an effect would work, and further studies are required.

In conclusion, SB 211475 protected against reperfusion injury and led to a significant infarct size reduction with appropriate doses (1.0 mg/kg and beyond). This study further suggested that the infarct size reduction by carvedilol reflect additional effects beyond β-adrenoceptor antagonism. Both substances inhibit neutrophil invasion in the postischemic myocardium, an effect which may contribute to infarct size reduction.

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