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The novel guanidine ME10092 protects the heart during ischemia–reperfusion

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Abstract

The novel guanidine *N*-(3,4-dimethoxy-2-chlorobenzylideneamino)-guanidine [ME10092; a metabolite to the strongly cardioprotective hydroxyguanidine *N*-(3,4-dimethoxy-2-chlorobenzylideneamino)-*N'* -hydroxyguanidine (PR5)] was administered intravenously to rats subjected to left coronary artery clamping followed by reperfusion. Administration of 1–10 mg/kg of ME10092 1 or 5 min before 10 min of coronary artery occlusion followed by 20 min reperfusion significantly and dose-dependently inhibited the reperfusion-induced burst of arrhythmia, and markedly improved the survival of the animals. This dose schedule also dose-dependently and significantly inhibited the ST-segment elevation seen on the ECG during the artery occlusion, and attenuated the secondary rise in ST-segment during the reperfusion. Even when ME10092 was administered 5 min after the start of the reperfusion, the ST-segment elevation became significantly attenuated. Administration of ME10092 (3 plus 1.5 mg/kg) to animals subjected to 1 h left coronary occlusion followed by 2 h reperfusion reduced the heart infarction size by about 40%. ME10092 also dose-dependently reduced the heart rate, both during normal conditions and during ischemia and reperfusion. Moreover, the highest dose of ME10092 used (10 mg/kg) strongly attenuated the reduction in blood pressure seen during 10 min left coronary occlusion, as well as it attenuated the rebound rise in blood pressure seen during the 20 min reperfusion phase; that is, resulting in a normalisation of the blood pressure disturbances caused by the ischemia–reperfusion. We also showed that after its p.o. administration, the PR5 hydroxyguanidine became completely metabolised to its guanidine ME10092, with no detectable traces of PR5 being present 30 and 60 min after the administration. Moreover, after the p.o. administration of ME10092, no signs of the formation of PR5 were seen on analysis of the rats' plasma. In view of the practically indistinguishable pharmacological effects of ME10092 and PR5, we suggest the strong cardioprotective effects of these compounds to be mediated by a direct effect by ME10092 per se.

Keywords: ME10092; Cardioprotection; Ischemia–reperfusion, rat

1. Introduction

The pathophysiology of cardiac ischemic disease is complex and may involve damage caused by the ischemia, as well as by events associated with the eventual reperfusion of the hypoxic myocardium. When the supply of oxygen to a tissue is restricted or completely blocked, the tissue ATP-pools become depleted initiating a series of events that threatens cell survival (Depre and Taegtmeyer, 2000). Thus, the ion gradients over cell membranes decrease and the cellular concentrations of Na^+, Ca^{2+} and H^+ increase, ultimately leading to irreversible damage due to acidification, electric imbalance and secondary damages caused by the calcium accumulation (Flitter, 1993; Piper et al., 1998). If the blood flow is then restored, the re-oxygenation is thought to result in further tissue damage from the generation of oxygen free radicals (McCord, 1985; Ar'Rajab et al., 1996). The complete mechanism of cell death and the events leading to a point of irreversibility is still under investigation, but free radical generation seem to play an important role for the cause of tissue damage. The oxygen radicals are being generated by several mechanisms. Firstly,

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perturbations of the respiratory electron transport chain of the mitochondria contribute to the generation of reactive oxygen species (O'Rourke, 2000). Secondly, activated neutrophils also constitute a source (Welbourn et al., 1991; Black, 2000). Thirdly, at least in some species, xanthine oxidase formed during the ischemic process catalyses the generation of superoxide from oxygen (Ambrosio and Tritto, 1999; Nishino, 1994).

Different strategies have been tried for the preventive treatment of ischemia and reperfusion injury. One of them is the use of radical scavenging drugs such as lipid peroxidation inhibitory aminosteroids, the so-called lazaroids (Campo et al., 1996, 1997), or to use superoxide dismutase for quenching superoxide (Vanhaecke et al., 1991; Watanabe et al., 1993). The xanthine oxidase inhibitor allopurinol was reported to improve cardiac performance following coronary bypass in a clinical study (Castelli et al., 1995). Yet another approach tried is the use of Na^+/H^+ exchange inhibitors such as cariporide (Kusumoto et al., 2001).

In a recent study, we reported that *N*-(3,4-dimethoxy-2-chlorobenzylideneamino)-*N*′-hydroxyguanidine (PR5), which is the hydroxyguanidine analogue of *N*-(3,4-dimethoxy-2-chlorobenzylideneamino)-guanidine (ME10092), possesses marked cardioprotective properties (Veveris et al., 1999). In another recent study, we showed that PR5 is metabolically reduced by the enzyme xanthine oxidase yielding ME10092 (Dambrova et al., 2000). In the process, PR5 acts as an alternative electron acceptor during the oxidation of xanthine by the xanthine oxidase, thereby preventing the formation of superoxide from molecular oxygen. On top of that, we found in that the compound of the present investigation, ME10092 inhibits the aerobically sustained oxidation of xanthine by xanthine oxidase (Dambrova et al., 2000). It is conceivable therefore that the pharmacological mechanism of action of PR5 and ME10092 might be closely related and similar. We therefore undertook the present study to investigate the effects of ME10092 in the ischemia–reperfusion rat heart model previously used in the evaluation of PR5.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 330–420 g were housed under standard conditions (21–23 °C, 12 h light–dark cycle) with unlimited access to food and water. All experimental procedures were performed in accordance with the regulations of the Animal Ethical Committee of BaltLASA.

2.2. Drugs

The following drugs were used: pentobarbital sodium (Richter Pharma); ME10092 (previously also termed PR9 in Dambrova et al., 2000) was synthesised at the Department of Medicinal Chemistry, Latvian Institute of Organic Synthesis Riga and by Nordic Synthesis, Karlskoga, Sweden.

2.3. In vivo metabolism of PR5 and ME10092

Blood samples were drawn 30 or 60 min after the oral administration of PR5 or ME10092 (30 mg/kg), and serum was prepared and placed into test tubes together with a stirring bar. After the addition of an equal volume of methanol, the suspension was stirred at ca. 200 rpm for 15 min until a homogeneous cloudy precipitate formed. The samples were then centrifuged for 20 min, the supernatants collected and diluted with water up to a final volume of 5 ml and analysed by HPLC using a WATERS LC1 Plus module, Nucleosil-5 C_8 column and a UV detector. The mobile phase was 35% MeOH in 0.05 M phosphate buffer, pH 2.5; flow rate: 0.7 ml/min. Oral administration of PR5 and ME10092 was performed by infusion with 1% solutions via a gastric catheter. The recoveries were 62% and 73% for PR5 and ME10092, respectively, in the range 0.25–1 μg/ml.

2.4. Ischemia–reperfusion induced heart failure

The experimental procedure was a modification of the method described by Kane et al. (1984). In brief, rats were anaesthetised with sodium pentobarbital (60 mg/kg i.p.). The femoral vein was cannulated to allow administration of drug or saline. Systemic blood pressure was monitored from the left carotid artery by a pressure transducer P23 DB (Gould Statham, USA) and continuously registered on a physiograph DMP-4B (Narco Bio-Systems, USA) using electrocardiogram (ECG) II standard leads. Rats were intubated through a tracheotomy and ventilated with room air by a V5kG respirator for small animals (Narco Bio-Systems, USA) using an inspiration pressure of 15 cm H_2O and a rate of 55 strokes/min to maintain blood gases and pH within the normal limits. The chest was opened using a left thoracotomy, followed by sectioning of the fourth rib. The pericardium was incised and a sling (6/0 silk Ethicon) was placed around the left coronary artery close to its origin without externalisation of the heart. Both ends of the ligature were passed through a small plastic tube; the chest was partially closed and the animal was allowed to recover for 10 min. The coronary artery was occluded by applying tension to the plastic tube–silk string arrangement. Tension was maintained by clamping the tube; the successful occlusion being confirmed by a decrease in arterial pressure and ischemia-induced alterations in the ECG. Reperfusion was initiated by removing the clamp and releasing the tension to the ligature. Two timing schedules for occlusion and reperfusion were applied. During short-term ischemia–reperfusion, the occlusion was applied for 10 min followed by 20 min of

reperfusion. During long-term ischemia–reperfusion, the occlusion was applied for 60 min followed by 120 min of reperfusion.

2.5. Exclusion criteria

Experiments were terminated and data were excluded from the final analysis if arrhythmia occurred before the coronary artery occlusion; if the mean arterial pressure was less than 60 mm Hg before occlusion (drug or saline administration), or if severe arrhythmia or atrio-ventricular block occurred during the first 5 min of ischemia (which were then probably caused by the ligature occluding the septal branch of the left coronary artery).

2.6. Classification of arrhythmia

Classification of arrhythmia was based on criteria described in the Lambeth Conventions (Walker et al., 1988). Ectopic activity was categorised as a single ventricular premature beat, ventricular tachycardia (i.e. four or more consecutive ventricular premature beats) or as ventricular fibrillation (i.e. inability to distinguish individual QRS complexes or inability to measure a rate). In all experiments, the incidence of ventricular tachycardia, ventricular fibrillation and mortality (due to terminal ventricular fibrillation sustained for at least 3 min) was noted.

2.7. Quantification of myocardial morphological parameters

At the end of the reperfusion of the animals subjected to long-term ischemia–reperfusion, the ligature was re-tied around the coronary artery and Evans blue (5 mg/ml) was quickly infused into the left atrium. The heart was then isolated and soaked in ice-cold saline. The heart was subsequently weighed and the left ventricle was dissected free from other structures and weighed. The non-stained myocardium (i.e. the risk region) was isolated and weighed and cut into 1 mm sections from apex to base. The sections were incubated in a 0.5% solution of triphenyltetrazolium chloride (10 min at 37 °C), which stained the viable myocardium red, allowing the necrotic tissue to be isolated and weighed.

2.8. Statistics

The results are presented as the mean ± S.E.M. Statistical analysis was performed using independent samples *t*-test, one-way analysis of variance (ANOVA) or Chi-square tests. Results were considered significant when $p < 0.05$. The incidence ventricular tachycardia, ventricular fibrillation and lethality were calculated in all animals, whereas only the data obtained for the surviving animals were used in the evaluation of the blood pressure, heart rate and duration of arrhythmia data.

3. Results

3.1. Evaluation of the dose–effect relations of ME10092 on heart rate during short-term ischemia–reperfusion

In the short-term ischemia–reperfusion experiments, the rats were subjected to a 10-min occlusion of their left coronary artery, followed by 20 min of reperfusion. ME10092 was administered intravenously at doses of 1, 3 or 10 mg/kg, either 5 min before the application of the coronary artery clamp, 1 min before the start of the reperfusion or 5 min after the start of the reperfusion. Fig. 1 shows the effects on heart rate. The ischemia–reperfusion per se did not induce any appreciable change in the heart rate. At whichever time-point administered, the ME10092 induced a reduction in the heart rate that peaked during 1–2 min following the injection and then gradually declined; at the highest dose tested, the effect amounted to maximally an about 25% reduction of the heart rate (Fig. 1).

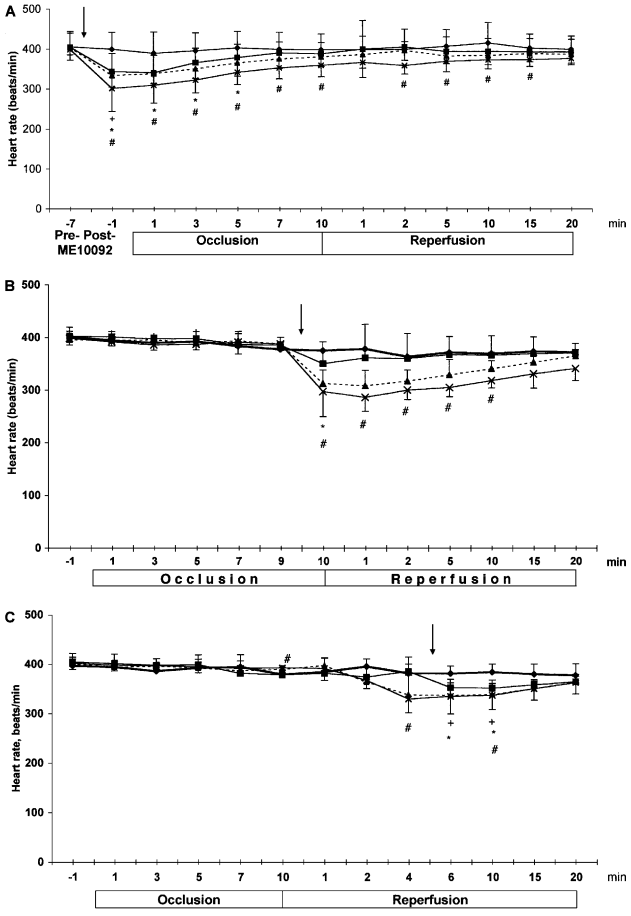

Fig. 1. Effect of ME10092 on heart rate in rats subjected to 10 min coronary occlusion and 20 min reperfusion. The compound was administered in doses 1 (■), 3 (▲) or 10 (×) mg/kg i.v. either 5 min before the occlusion of the left coronary artery (A), 1 min before the reperfusion (B) or 5 min after the onset of the reperfusion (C) (indicated by the arrows). Controls (◆) received the i.v. injection of the equal volume of saline. Significant differences from the control at $p < 0.05$ indicated by +, * and # for, respectively, 1, 3 and 10 mg/kg ME10092.

3.2. Evaluation of the dose–effect relations of ME10092 on blood pressure during short-term ischemia–reperfusion

The time course for mean arterial blood pressure is illustrated in Fig. 2. In the control animals, the blood pressure dropped about 20 mm Hg 1 min after the coronary occlusion, and then regained somewhat. One minute after the start of the reperfusion, a major reduction of the blood pressure then occurred, which was followed by a rebound in the pressure, during the 5–10 min following reperfusion. The effect of ME10092, when administered 1 min before the occlusion, is illustrated in Fig. 2. Before the occlusion, the compound induced a dose-dependent reduction in blood pressure. This blood pressure drop was significant after 3 and 10 mg/kg of ME10092. However, the pressure did not then drop further at the time of clamping of the coronary artery, something that resulted in that the blood pressure levels of both the treated and control groups became essentially the same. Similarly as for the controls, the blood pressure then regained somewhat for the ME10092-treated animals, during the occlusion period. During the reperfusion, a large drop in blood pressure thereafter occurred, during the first reperfusion minute. The magnitude of this drop was similar for the controls and 1 and 3 mg/kg ME10092 treatment groups, but for the group of rats receiving 10 mg/kg ME10092, the blood-pressure drop was markedly and significantly attenuated. Moreover, the rebound in the blood pressure otherwise seen at the 5–10 min post-reperfusion period was completely attenuated in the 10 mg/kg ME10092 group (Fig. 2).

3.3. Evaluation of the effect of ME10092 on the ST-segment during short-term ischemia–reperfusion

Effects on the ST-potential from the ECG recordings are shown in Fig. 3. During the pre-occlusion, the ST-segment was 0.1 mV above the baseline. During the occlusion, the ST-segment became elevated maximally to about 0.5 mV above the baseline in the controls. During the first minute of the reperfusion, the ST-segment then rapidly diminished to about 0.2 mV above the baseline, which was then again followed by another rise to about 0.3 mV above the baseline during about the second to sixth minute of the reperfusion (Fig. 3A,B). This was then again followed by a slight reduction in the ST-segment throughout the remaining time of the experiment. However, during the course of the experiment, the ST-segment never regained the same level that it had had before the occlusion, and in the control animals, it ended at about 0.2 mV above the baseline.

When ME10092 was administered 5 min before the occlusion, the rise in the ST-potential during the occlusion period became dose-dependently and significantly reduced (Fig. 3A). Moreover, during the reperfusion period, the second phase of ST-potential elevation also became attenuated by the compound; the effect being significant at 3 and 10 mg/kg of ME10092 (Fig. 3A). For the highest dose of

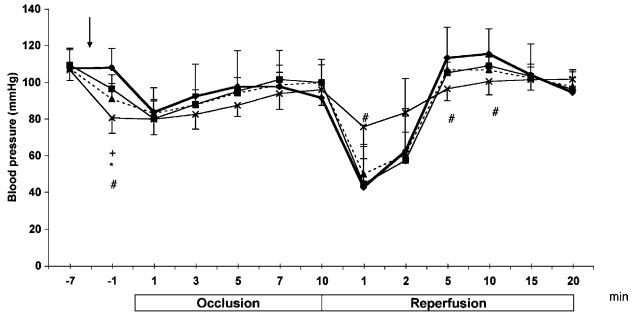

Fig. 2. Effect of ME10092 on blood pressure in rats subjected to 10 min coronary occlusion and 20 min reperfusion. The compound was administered in doses 1 (■), 3 (▲) or 10 (×) mg/kg i.v. 5 min before the occlusion of the left coronary artery (indicated by the arrow). Controls (♦) received the i.v. injection of the equal volume of saline. Significant differences from the control at $p<0.05$ indicated by +, * and # for, respectively 1, 3 and 10 mg/kg ME10092.

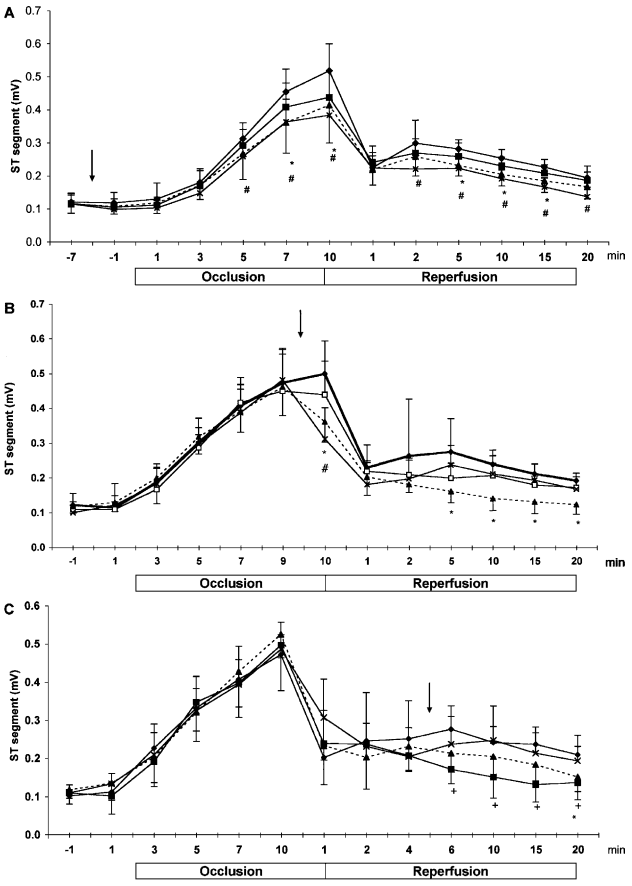

Fig. 3. Effect of ME10092 on the potential of the ECG ST-segment in rats subjected to 10 min coronary occlusion and 20 min reperfusion. The compound was administered in doses1 (■), 3 (▲) or 10 (×) mg/kg i.v. either 5 min before the occlusion of the left coronary artery (A), 1 min before the reperfusion (B) or 5 min after the onset of the reperfusion (C) (indicated by the arrows). Controls (♦) received the i.v. injection of the equal volume of saline. Significant differences from the control at $p<0.05$ indicated by +, * and # for, respectively, 1, 3 and 10 mg/kg ME10092.

ME10092 given, this resulted in that, toward the end of the experiments, the ST-potential was only about 0.05 mV above the ST-potential level seen in the animals before the induction of the ischemia (Fig. 3A).

When ME10092 was administered 1 min before the start of reperfusion, the ST-potential seemed to drop immediately, and the second phase of ST-potential elevation during reperfusion became attenuated; the effect being significant at 10 mg/kg of ME10092 during the 5th–20th min following the reperfusion. The attenuation of ST-potential elevation resulted also in this case to that the ST-potential at the end of the experiment was only slightly elevated compared to the rats' normal ST-potential (Fig. 3B). Also when ME10092 was administered 5 min after the start of the reperfusion, the ST-potential became reduced; the effect being significant at the 6th–20th min of the reperfusion for 10 mg/kg ME10092, and at the 20th min for 3 mg/kg of ME10092 (Fig. 3C).

3.4. Evaluation of the dose and time effect relations of ME10092 on cardiac arrhythmias and mortality during short-term ischemia–reperfusion

The 10-min coronary artery occlusion, with the following 20 min reperfusion, caused disturbances of the heart rhythm. The patterns of these rhythm disturbances are illustrated in Fig. 4 for individual animals. Thus, toward the end of the coronary occlusion, occasions of ventricular premature beats and episodes of ventricular tachycardias were seen in the control animals (Fig. 4A). Following the reperfusion, many episodes of ventricular fibrillations, tachycardias and premature beats were then induced, and many of the control animals died due to irreversible ventricular fibrillations (Fig. 4A).

The effect of ME10092 on ventricular ectopic activity was evaluated after its i.v. administration at doses 1–10 mg/kg, either 5 min before the occlusion, or 1 min before releasing the coronary artery clamp. The effects of 3 mg/kg of ME10092 administered 1 min before the reperfusion are illustrated on individual rats in Fig. 4B. As can be seen, ME10092 caused a clear reduction of the ectopic heart activity, compared to that seen in the control animals.

The effects of ME10092 on ventricular ectopic activity and mortality are summarized in Fig. 5. Thus, clear and significant reductions of mortality were seen when ME10092 was administered 5 min before the occlusion, as well as when it was administered 1 min before the reperfusion. Thus, about 50% of the control animals died during the reperfusion, whereas among the 3 and 10 mg/kg ME10092-treated animals, only 1 out of a total of 25 animals died when ME10092 was administered 5 min before the occlusion (see Fig. 5A), and only 1 out of a total of 12 animals died when ME10092 was administered 1 min before the reperfusion (see Fig. 5B). Dose-dependent and significant reductions were also seen on ventricular fibrillations and

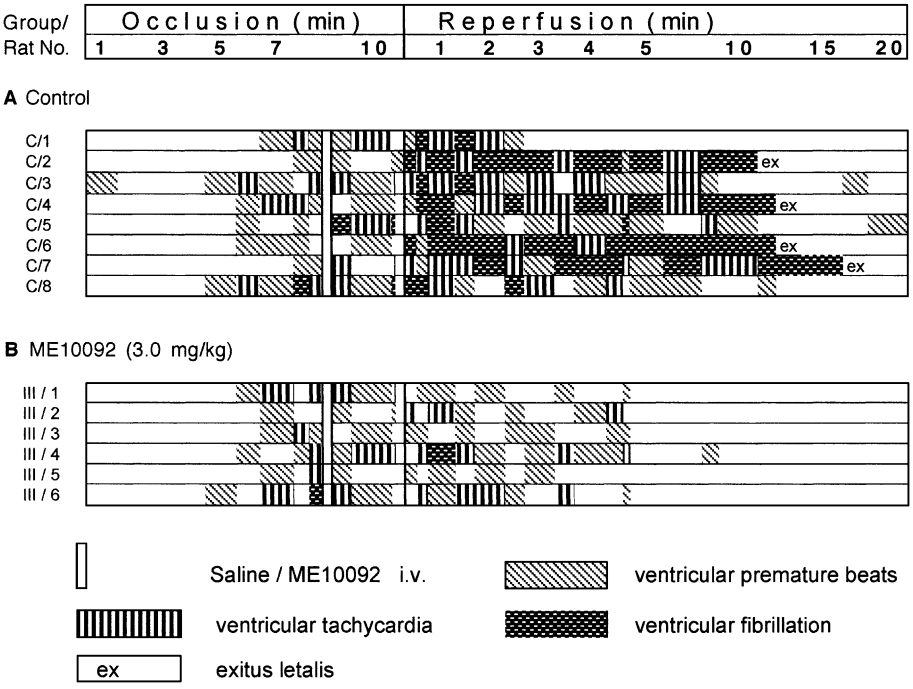

Fig. 4. Occurrence of ventricular ectopic activity and exitus letalis in rats subjected to 10 min coronary occlusion and 20 min reperfusion. Time periods where ventricular premature betas, ventricular tachycardia, ventricular fibrillation and exitus letalis are indicated as shown in the figure. The upper panel shows results for control animals receiving injections of saline 1 min before the onset of reperfusion. The lower panel indicates results for rat receiving ME10092 1 min before the onset of reperfusion.

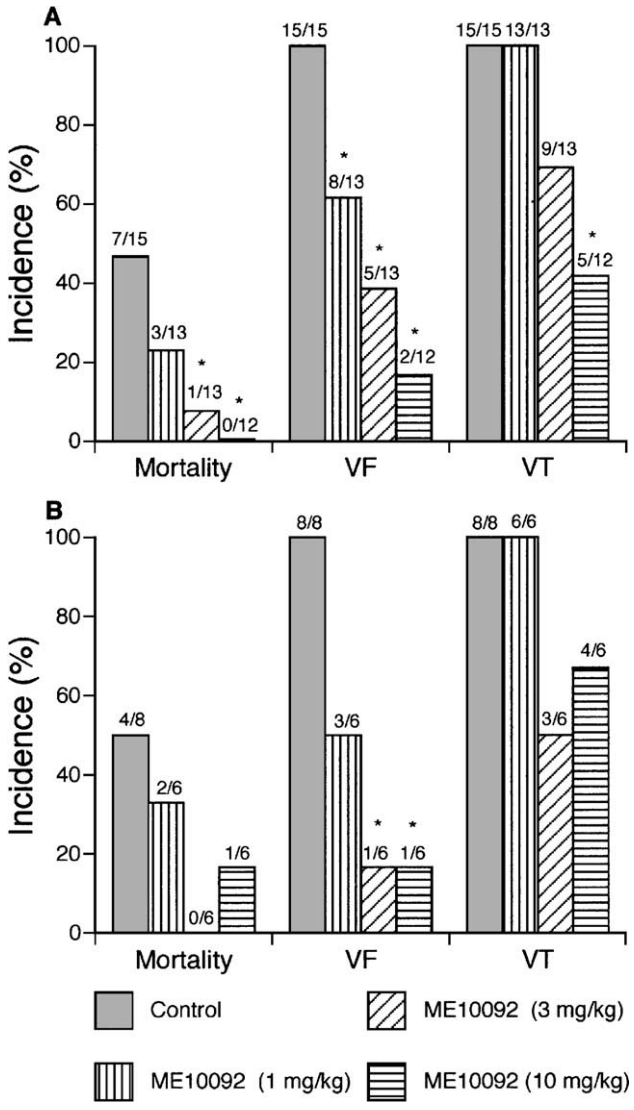

Fig. 5. Effect of ME10092 on the incidence of ectopic activity and mortality in rats subjected to 10 min coronary occlusion and 20 min reperfusion. Shown in panel A are results for rats receiving ME10092 5 min before the occlusion and in B for rats receiving ME10092 1 min before the reperfusion. ME10092 was given intravenously in doses 1, 3 or 10 mg/kg as indicated. Control rats received injection of saline. * indicates $p < 0.05$ vs. control. VF = ventricular fibrillation, VT = ventricular tachycardia.

ventricular tachycardias, both when ME10092 was administered 5 min before the occlusion and 1 min before the reperfusion (Fig. 5A,B).

3.5. Influence of ME10092 on the morphology of rat hearts during long-term ischemia–reperfusion

In these tests, the animals were subjected to 60 min coronary occlusion followed by a 120-min reperfusion. At the end of the experiment, the hearts were isolated and investigated morphologically (see methods for details). ME10092 was administered at a dose of 3 mg/kg 5 min before the occlusion followed by a second injection of 1.5 mg/kg 5 min before the reperfusion. As a control, served animals received injections of saline 5 min before occlusion and 5 min before the reperfusion.

ANOVA showed that body weights and heart and left ventricle weights were essentially the same for the two treatment groups (Table 1). Also, the weight of the ischemic zones caused by the coronary artery occlusion was practically the same (Table 1). However, as seen from the table, the weight of the necrotic tissue within the ischemic zone was markedly reduced by the ME10092 treatment. In Fig. 6, the myocardial salvaging effect of ME10092 is illustrated by normalising to the necrotic zone relative to the size of either the ischemic zone or the left ventricular weight. The marked effect of ME10092 was ascertained at a statistical significance of $p < 0.01$.

3.6. Demonstration of the metabolic transformation of PR5 to ME10092 in vivo

In a previous study (Veveris et al., 1999), we reported the effects of PR5 using the same or closely similar experimental models as in the present study. PR5 is a close structural analogue to ME10092, PR5 being the hydroxyguanidine analogue of the ME10092 guanidine. As the effects of ME10092 found herein were practically identical with those of PR5, we suspected that the PR5 might actually be a prodrug forming ME10092, the pharmacological actions of PR5 thus being caused by ME10092.

We accordingly collected rat serum 30 or 60 min after the oral administration of 30 mg/kg PR5 or ME10092 and analysed it by high-pressure liquid chromatography (HPLC). For the rats given PR5, the PR5 was not detectable in the serum of any of the samples analysed. However, all these samples contained ME10092; the ME10092 concentrations being, respectively, 0.053 ± 0.029 μg/ml ($n = 12$) and 0.074 ± 0.015 μg/ml ($n = 6$) 30 and 60 min after the administration of PR5. For the rats given ME10092, 0.23 ± 0.04

Table 1
Body weight and weight of hearts, left ventricle, ischemic zone and necrotic zone in rats subjected to 60 min left coronary artery occlusion followed by 120 min of reperfusion

Group	Body weight (g)	Heart weight (mg)	Left ventricle (mg)	Ischemic zone (mg)	Necrotic zone (mg)
Control	373.4 ± 10.0	1133.6 ± 48.5	808.4 ± 24.6	429.4 ± 17.7	321.9 ± 23.9
ME10092	370.2 ± 18.6	1164.0 ± 66.7	802.0 ± 56.6	423.6 ± 25.8	195.6 ± 44.4[a]

Shown are data for control rats injected with saline and for rats receiving ME10092 3 mg/kg 5 min before the occlusion and 1.5 mg/kg 5 min before the reperfusion.

[a] Indicates $p < 0.05$.

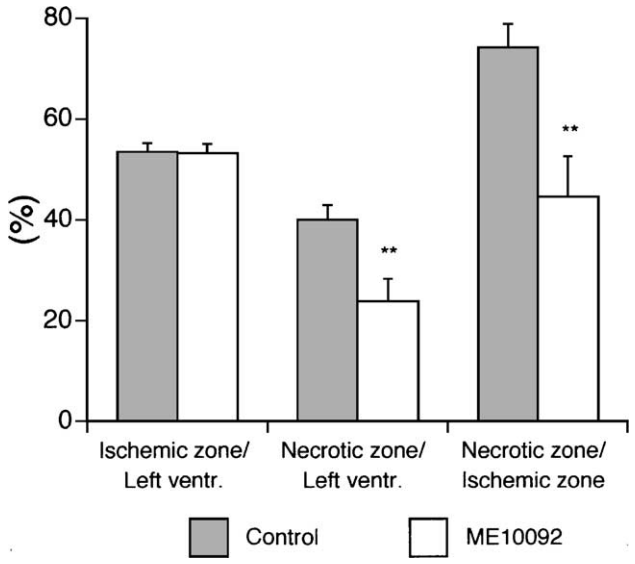

Fig. 6. Effect of ME10092 on the rat heart ischemic zone/left ventricular index, necrotic zone/left ventricular index and necrotic zone/ischemic zone index. Animals were subjected to 60 min left artery occlusion followed by 120 min reperfusion. Black bars represent controls injected with saline and white bars rats injected with ME10092, 3 mg/kg 5 min before the occlusion and 1.5 mg/kg 5 min before the reperfusion. ** indicates $p < 0.01$ vs. the controls.

μg/ml ($n = 4$) and 0.20 ± 0.05 μg/ml ($n = 4$) of ME10092 were detected in the sera 30 and 60 min after the administration of the ME10092. No traces of PR5 were detectable in any of the chromatograms of the animals that had been administered ME10092.

4. Discussion

We have shown here that the guanidine compound ME10092 possesses a protective effect on the heart during ischemia and reperfusion. The most pertinent of its effect is an about 40% reduction in the infarction size seen after 1 h ischemia followed by 2 h of reperfusion. This effect of ME10092 is manifested by a consistent reduction of the ST-segment potential during the ischemia, as well as during the reperfusion of the hearts. Thus, the effect of ME10092 on the ST-segment is seen already during the anoxic phase (i.e. when the compound is given before the coronary occlusion). Moreover, a clear effect is seen also during the reperfusion phase, both when ME10092 is administered before anoxia, when it is administered immediately before reperfusion and as well as even when it is administered some time after the start of the reperfusion. The almost complete restoration of the ST-segment to its normal potential by the highest dose of ME10092 is quite remarkable and may be taken as a sign of a cardioprotective effect, as the magnitude of the ST-segment rise is a measure of the degree of electrical malfunction of parts of the heart muscle. The ability of the compound to significantly prevent the secondary rise in the ST-segment during the reperfusion is also quite interesting. The reperfusion of the previously ischemic rat myocardium is known to cause reperfusion arrhythmia (Maxwell and Lip, 1997). The main factors causing the phenomena are oxygen-derived radicals, disturbed metabolism and ionic homeostasis during the ischemia period (Black, 2000; Piper et al., 1998; Maxwell and Lip, 1997). This in turn leads to a progression of the myocardial damage induced during the anoxic phase (Maxwell and Lip, 1997; Wang and Pincky, 2000). The reduction of rhythm disturbances by ME10092 may thus be an indirect effect caused by preventing or reducing the tissue damage during the anoxia and reperfusion. Of course, a direct effect of ME10092 on the electrical activity can at the present moment not be rigorously excluded, but taken together, all the data seem to indicate that ME10092 has a remarkable protective effect on the heart during ischemia and reperfusion.

The ability of ME10092 to prevent the marked drop in mean arterial pressure seen during the reperfusion phase might also be explained on the basis of a protective effect on the myocardium. In part, this effect might be mediated by the marked prevention of the reperfusion-induced arrhythmias induced by ME10092, but a direct effect on the sarcolemma is a possibility as well. ME10092 also appears to have a direct action on the heart as it causes a dose-dependent but transient reduction in the heart rate. ME10092 per se seems to cause a slight and transient reduction in mean arterial blood pressure but the relation of this effect to the effect on the myocardium is not clear.

The molecular mechanism of action of ME10092 is at present not very well understood. In a recent study, we reported on the in vivo pharmacological properties of PR5, which is the hydroxyguanidine analogue of ME10092. In the same (or similar models) as the present ones, PR5 caused essentially the same protection of the heart (Veveris et al., 1999) as we have reported here for ME10092. Thus, PR5 reduces the initial ST-segment potential rise seen during occlusion of the left coronary artery of rats' hearts, as well as the secondary rise seen during reperfusion and it reduces the size of the myocardial infarction seen after long-term ischemia–reperfusion. Moreover, PR5 also reduces the reperfusion-induced bursts of cardiac arrhythmia as well as it powerfully reduces the signs of myocardial insufficiency during the reoxygenation phase, as manifested by the inhibition of the rapid blood pressure drop seen upon reopening the occluded left coronary artery. On top of that, we showed that administration of PR5 induces a reduction of the necrotic zone in the rat hearts subjected to long-term ischemia–reperfusion; the magnitude of this effect being similar to the one induced by ME10092 in the present study after the long-term ischemia–reperfusion. In addition to these effects, we also observed in our previous study that PR5 causes the same transient reduction in heart rate and blood pressure in the normal rat, as does ME10092.

The pharmacological effects of PR5 and ME10092 during heart ischemia–reperfusion are thus closely similar. We show in the present study that the administration of PR5 to

rats gives rise to detectable levels of ME10092 in the blood, while the PR5 per se was not detectable at all. It appears therefore highly likely that our previously observed cardioprotective actions of PR5 are in fact mediated by the ME10092 metabolite; hence PR5 serving as a pro-drug to ME10092. The almost indistinguishable effects of ME10092 (present study) and PR5 (Veveris et al., 1999) in heart ischemia–reperfusion models speak strongly in favour of this hypothesis.

In another study, we have shown that the hydroxyguanidine PR5 may become metabolically reduced by the enzyme xanthine oxidase yielding the corresponding guanidine, which is in fact ME10092 (Dambrova et al., 2000; note that in our previous study, ME10092 was termed PR9). In this process, PR5 acts as an alternative electron acceptor during the oxidation of xanthine preventing the formation of superoxide from molecular oxygen. Our data thus showed that PR5 is capable of sustaining the anaerobic oxidation NADH by the xanthine oxidase by an action at the molybdenum centre of the xanthine oxidase (Dambrova et al., 2000). However, we have also found that the compound of the present investigation, ME10092, can prevent the formation of superoxide during the aerobically sustained oxidation of xanthine by xanthine oxidase (Dambrova et al., 2000). It is conceivable therefore that similarly to PR5, ME10092 may bind to the molybdenum centre of the xanthine oxidase, but then it acts as a xanthine oxidase inhibitor rather than as an electron acceptor. Another important observation of the present study was that we found no evidence for the metabolic transformation of ME10092 into PR5 after the administration of ME10092 to the rats. This observation supports the idea that the primary pharmacological effect of ME10092 is mediated by the ME10092 itself, and not by an indirect effect caused by the generation of PR5 from ME10092.

Whether or not a xanthine oxidase-mediated mechanism is responsible for the cardioprotective effects of ME10092 is elusive. The apparent affinity of ME10092 for the xanthine oxidase enzyme appears to be about 10-fold lower than the xanthine oxidase inhibitor allopurinol (Dambrova et al., unpublished data). It is possible that some of the effects of the ME10092 guanidine are mediated via an action on the xanthine oxidase enzyme. However, even the role of xanthine oxidase inhibition as a mechanism for cardiac protection during ischemia–reperfusion is questionable. For example, allopurinol was reported ineffective in experimental heart infarction in the dog (Reimer and Jennings, 1985). Moreover, although in a clinical study allopurinol seemed to improve cardiac performance following coronary bypass, as assessed by postoperative cardiac output and left ventricular stroke work (Castelli et al., 1995), a recent study suggest allopurinol, as well as the related compound oxypurinol, to be potent myofilament Ca^{2+} sensitisers, thereby mediating a positive inotropic effect in the heart (Pérez et al., 1998). Thus, even the eventual beneficial effect of allopurinol when observed during heart ischemia may be nonrelated to its xanthine oxidase inhibitory effect.

The mechanisms underlying the observed quite remarkable beneficial effects of ME10092, and its pro-drug PR5, on the heart during ischemia–reperfusion should thus deserve further investigations. In such investigations, it may be of interest to investigate their eventual effects on α_2-adrenoceptors seen by other fairly structurally related guanidines (Gehr et al., 1986) as well as possible effects on Na^+/H^+ exchange mechanisms shown by some other guanidine derivatives (Hoque and Karmazyn, 1997). In such studies, the possibility of a distinctly new type of mechanism of action of ME10092 should not be overruled.

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