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The Effects of Oral Pretreatment with Zofenopril, an Angiotensin-Converting Enzyme Inhibitor, on Early Reperfusion and Subsequent Electrophysiologic Stability in the Pig

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Summary. The effects of oral zofenopril pretreatment were investigated in a chronic closed-chest pig model of ischemia and reperfusion. Pigs (25–35 kg) were pretreated orally with zofenopril (15 mg/day) on the 2 days prior to ischemia, which was evoked by the inflation of a catheter balloon in the left anterior descending coronary artery over 45 minutes. The catheter was then removed and the myocardium was reperfused. After 2 weeks, infarct properties were assessed by signal averaging of the body surface electrocardiogram and the inducibility of malignant ventricular tachyarrhythmias was tested with a programmed electrical stimulation protocol.

A significant increase in the pressure-rate product (43 ± 11%, mean ± SEM), indicating the oxygen demand of the heart, was prevented by zofenopril (19 ± 8%, $p < 0.05$). Zofenopril reduced the peak efflux of adrenaline (1302 ± 213 vs. 3201 ± 760 pg/ml; $p < 0.05$), noradrenaline (402 ± 54 vs. 902 ± 282 pg/ml; $p < 0.05$), and of the adenosine catabolites inosine and hypoxanthine (56 ± 4 vs. 78 ± 9, pg/ml; $p < 0.05$) in the coronary venous effluent. The efflux of the cytoplasmatic enzyme creatine phosphokinase was not significantly reduced after zofenopril ($p = 0.08$). No difference in plasma renin levels between the groups were found. After 2 weeks, late potentials were found only in the surviving animals from the untreated group, i.e., the voltage vector magnitude was more reduced, and a prolongation of the QRS duration and of the terminal low-amplitude part of the high-frequency QRS were found. Moreover, zofenopril had caused a significant reduction of the inducibility of sustained ventricular tachyarrhythmias.

These findings support the view that catecholamine and purine efflux, and an adverse increase in the oxygen demand during early reperfusion are reduced by zofenopril pretreatment, leading to a higher electrophysiologic stability of the subsequently developed infarct after 2 weeks.

Key Words. converting enzyme inhibition, myocardial infarction, reperfusion, pig, signal averaging, zofenopril

Early reperfusion of ischemic myocardium by thrombolytic agents prevents a further extension of myocardial infarction [1–3] but can also be harmful by producing additional damage and severe ventricular arrhythmias [4–7]. The need for additional protective measures during early reperfusion therapy was recently emphasized by several authors [4,8,9].

Among other drugs, angiotensin-converting enzyme inhibitors may be suitable for this purpose, because they yield a reduction of this "paradoxical" damage. In previous studies, biochemical markers of tissue injury and reperfusion arrhythmias in the isolated rat heart were reduced after treatment with ACE inhibitors [10–12]. In vivo, converting-enzyme inhibitors salvage myocardial tissue during ischemia and early reperfusion in the rat [13], the pig [14,15], and the dog [10,16].

In these earlier studies, the converting-enzyme inhibitor was always administered shortly before [10–13] or during ischemia [14–16]. Because many (hypertensive) patients receive converting-enzyme inhibitor therapy nowadays, the chance that a patient with an acute myocardial infarction treated with reperfusion (thrombolytic) therapy is already taking converting-enzyme inhibitors is not insignificant. For this reason and because of the mechanisms involved, we investigated the effects of pretreatment with a converting-enzyme inhibitor on ischemia and reperfusion. In the present study, zofenopril (a recently developed potent captopril analogic) was administered over the 2 days before the ischemic event. Zofenopril is a potent captopril analogue that has a high bioavailability after

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oral administration and is rapidly deesterified in vivo to zofenoprilate (SQ 26333), a free sulfhydryl group containing a metabolite that is responsible for the pharmacologic actions of the drug [17]. Its pharmacokinetic properties are such that the drug still inhibited angiotensin-I pressor responses 24 hours after its administration to rats.

Moreover, the development of the infarction and the result after 2 weeks were evaluated using signal averaging of body surface electrocardiograms. Finally, the ultimate outcome was assessed after 2 weeks by studying the inducibility of malignant ventricular tachycardias associated with infarction using programmed electrical stimulation of endocardial sites in the periinfarct region. Drug effects on the inducibility of ventricular tachyarrhythmias can be studied reliably in patients and in animal models if the protocol used is sufficiently standardized [18].

Methods

Surgical procedure

The methods employed in this study have been described in detail before [15]. Briefly, male Yorkshire swine (body weight, 25–35 kg) were pretreated with 120 mg azaperone (Stresnil®; Janssen Pharmaceutica Beerse, Belgium) intramuscularly. After half an hour, 150 mg metomidate (Hypnodil®, Janssen) was injected in an ear vein. A cuffed endotracheal tube was introduced and the animals were ventilated with a mixture of O_2/N_2O. Anesthesia was maintained with an intravenous infusion of azaperone (2 mg/kg/min) and metomidate (8 mg/kg/min) through a double-lumen catheter in an inferior caval vein. Ventilation parameters were adjusted to keep arterial pO_2 concentrations between 16 and 20 kPa. Body temperature was kept at 36–38°C with a thermal mattress. Heparin was administered at an initial dose of 5000 IU, followed by 2500 IU/hr.

A polyethylene catheter was introduced under fluoroscopic guidance via the right external jugular vein into the coronary sinus to collect coronary venous blood draining the ischemic region. Acute coronary occlusion was produced with a balloon catheter, as used for percutaneous transluminal coronary angioplasty. An 8F Judkins guiding catheter was introduced via the left carotid artery, and the tip was positioned at the ostium of the left coronary artery, as verified by fluoroscopy. Through its lumen a 2F PTCA catheter (DJ-20-37 Grüntzig Dilaca, Schneider Medintag AG, Zürich) was introduced into the anterior descending branch of the left coronary artery and advanced beyond the first diagonal branch. The position of the balloon was carefully checked in order to occlude comparable perfusion areas.

Protocol

The animals received either no treatment (n = 9) or oral zofenopril 15 mg/day on 2 days before the experiment (n = 10). The last dose was given 1 day before the experiment. After an equilibration period of 30 minutes, during which hemodynamic parameters were allowed to stabilize, a catheter was introduced and ischemia was induced by inflating the balloon. The ischemic period lasted 45 minutes. The experiments were terminated 4 hours after reperfusion. In this study, a dose of 0.5 mg/kg body weight was chosen in order not to affect the resting blood pressure of the pigs. Therefore, the hemodynamic parameters before ischemia in both groups were comparable.

When ventricular fibrillation occurred, direct current cardioversion was attempted. After removal of the catheters, the pigs received postoperative care and were returned to their cages. After 2 weeks, the surviving animals were subjected to a programmed electrical stimulation protocol in order to study the inducibility of ventricular tachyarrhythmias. Finally, the animals were sacrificed. The hearts were removed and the left ventricle was dissected free and cut into slices of 1-cm thickness. The slices were washed and stained for dehydrogenase activity with the nitroblue tetrazolium method described by Nachlas [19]. The slices were photographed and the infarct size measured planimetrically, and the data were expressed as a percentage of the left ventricle.

Hemodynamic and biochemical parameters

Throughout the experiments arterial blood pressure (mmHg) was measured via a catheter in the left femoral artery. Heart rate and arrhythmias were monitored continuously by electrocardiography from conventional limb leads. A triple-lumen Swan-Ganz thermodilution catheter was inserted via an external jugular vein to measure right atrial pressure, cardiac output (l/min), and stroke volume (ml; calculated from cardiac output and heart rate). Systemic vascular resistance (dynes.s/cm^5) was calculated from the mean arterial pressure, the right atrial pressure, and the cardiac output.

Adrenaline and noradrenaline, and the purines inosine and hypoxanthine, were measured in the coronary venous effluent using a sensitive HPLC assay [20] at the end of the control period, at the end of the ischemic period, and at 1, 5, and 30 minutes of reperfusion. The efflux of adenosine catabolites from myo-

Table 1. *Cardiovascular parameters in the untreated and zofenopril pretreated groups at the beginning of the control period (t = 0), at the end of ischemia (t = 75), and at the end of the reperfusion period (t = 330)*

	Control t = 0		End of ischemia t = 75		End of reperfusion t = 330	
	Untr	Zof	Untr	Zof	Untr	Zof
HR	86 ± 6	83 ± 4	109 ± 6[b]	96 ± 8	140 ± 9[c]	110 ± 9[b]
BP	76 ± 3	78 ± 7	70 ± 4	72 ± 5	64 ± 4	64 ± 3
RAP	9 ± 0	8 ± 1	9 ± 0	7 ± 1	7 ± 0	6 ± 1
CO	2.7 ± 0.2	2.1 ± 0.2	2.9 ± 0.4	2.0 ± 0.2	2.6 ± 0.2	1.9 ± 0.1[a]
SV	32 ± 3	30 ± 6	27 ± 4	21 ± 2	19 ± 2	17 ± 2
RPP	322 ± 30	336 ± 46	366 ± 26	353 ± 45	450 ± 45[c]	372 ± 39
Art res	2040 ± 190	2720 ± 270[a]	1870 ± 220	2620 ± 210	1860 ± 210	2400 ± 200

Differences from untreated group: [a]$p < 0.05$.
Differences from initial value: [b]$p < 0.05$, [c]$p < 0.01$.
HR = heart rate (min^{-1}); BP = mean blood pressure (mmHg); RAP = right atrial pressure (mmHg); CO = cardiac output (l/min); SV = stroke volume (ml); RPP = rate pressure product ($.10^2$ mmHg/min/kg body weight); art res = arterial resistance (dynes.sec/cm^5); zof = zofenopril pretreated; untr = untreated.

cardial tissue reflects the loss of high-energy phosphorylated nucleotides from myocardial tissue and therefore represents a biochemical marker for the extent of ischemia [20]. Catecholamine efflux during reperfusion was assayed because catecholamines may contribute to the severity of reperfusion damage [15].

In order to assess irreversible myocardial damage, blood samples were collected during ischemia and reperfusion for monitoring creatine phosphokinase levels at the end of the control and of the ischemic period, and at 5, 30, 120, 180, and 240 minutes after reperfusion. Creatine phosphokinase (CPK) activity was determined with a spectrophotometric assay (Merck, Merckotest no. 14327).

Plasma renin activity (PRA) was measured by means of a radioimmunoassay (Du Pont, Rianen kit no. NEA-022,026) in arterial plasma samples at the end of the control and at the end of the ischemic period, and at 5, 10, 15, and 30 minutes of reperfusion. The values were expressed as nanograms per milliliter of angiotensin I per hour.

Signal averaging

Three body surface electrocardiograms, from leads in an orthogonal arrangement (X, Y, and Z) were recorded, averaged, vector summed, and filtered at 50 Hz. Recordings were made after equilibration, at the end of the ischemic period, at the end of the reperfusion period, and after 2 weeks in the surviving animals. Each recording contained 100–200 beats. From this filtered signal the following parameters were calculated: a) the QRS duration (ms), b) the root mean square of the QRS vector (V-total, μV), c) the root mean square of the terminal 30 ms (V-30, μV), and d) the duration of the terminal part below 30 μV (D-30, ms). For this purpose, a slightly modified computer algorithm developed by Simson was used [21].

Programmed electrical stimulation

After 2 weeks, the pigs were reanesthetized and the cardiac output was measured with a thermodilution catheter. Subsequently, signal averaging electrocardiography was performed and the inducibility of ventricular tachyarrhythmias was evaluated by a programmed electrical stimulation (PES) protocol. Details of the procedure used are described in a previous paper [18].

Statistical analysis

Differences between group means were tested with Student's t test. Fisher's exact probability test was used to evaluate differences in mortality and inducibility of ventricular tachyarrhythmias. Linear regression analysis was used to assess correlations between some parameters. Differences were considered to be significant if p was < 0.05. Data were represented as mean values ± SEM.

Results

Acute phase

Hemodynamic parameters and heart rhythm. In the untreated group, the heart rate increased during ischemia (from 86 to 109) and even more during reperfusion (from 86 to 140; Table 1). In the zofenopril-treated group, however, the heart rate during ischemia did not increase significantly (from 83 to 96, i.e., 15 ± 7% vs. 29 ± 9% in the untreated group; ns), and during

Table 2. Incidence of ventricular fibrillation and cardioversion in the acute phase

	Untreated	Zofenopril
Number of animals	9	10
Ventricular fibrillation (during ischemia)	3	3
Cardioversion unsuccessful	1	2
Survivors after acute phase	8	8
Survivors after 2 weeks	5	5
VT inducible	5	1[a]

Survival and inducibility of VT after 2 weeks. The number of animals in the untreated and zofenopril pretreated groups is indicated. Differences between the two groups were tested using Fisher's exact probability test.
[a] $p < 0.05$.

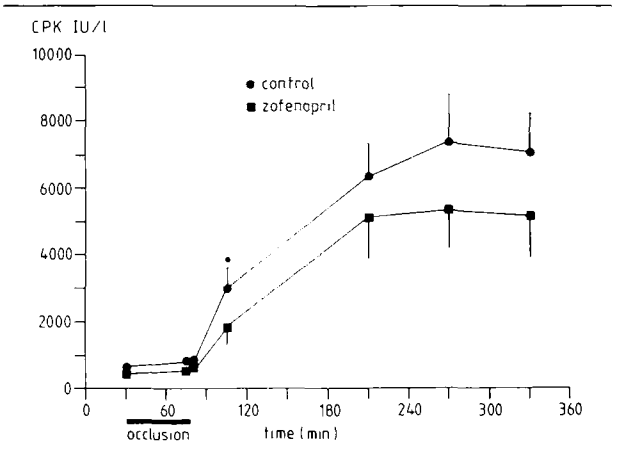

*Fig. 1. Time course of creatine phosphokinase levels in arterial blood, before, during, and after ischemia. The curve of the untreated group (n = 8) tends to be higher than the curve of the zofenopril-treated animals (n = 8), without reaching significance: p = 0.08. (mean ± SEM; * p < 0.05).*

reperfusion only moderately and to a lesser extent than in the untreated group (from 83 to 110, i.e., 33 ± 8% vs. 66 ± 12% in the untreated group; $p < 0.05$; Table 1). Concomitantly, the rate-pressure product in the untreated group increased (from 32,200 to 45,000), whereas in the zofenopril group no increase was found (from 33,600 to 37,200, i.e., 19 ± 8% vs. 43 ± 10% in the untreated group; $p < 0.05$).

The cardiac output after pretreatment with zofenopril was initially not significantly different from the untreated group (Table 1). The cardiac output did not change significantly in either of the groups at the end of the reperfusion period compared to control values, but at the end of the reperfusion period the cardiac output was significantly lower in the zofenopril group (Table 1). No changes in blood pressure were observed. The calculated arterial resistance was initially higher after pretreatment. The arterial resistance did not decrease during the experiment (Table 1).

No sinus nodal or AV-nodal conduction disturbances were found during the entire experiment in any of the animals. During the control period, no ventricular arrhythmias were present, but during ischemia ventricular extrasystoles occurred frequently. Moreover, 6 out of 19 animals developed ventricular fibrillation during ischemia, usually at 5–10 minutes or at 20–30 minutes of ischemia. Direct current cardioversion was attempted in all cases, but three animals died acutely (Table 2).

Upon reperfusion in the zofenopril group, one animal fibrillated, but the arrhythmia could be converted to sinus rhythm. Upon reperfusion an accelerated idioventricular rhythm developed in all pigs, indicating the restoration of myocardial perfusion [22]. Neither acute mortality nor the incidence of ventricular fibrillation during ischemia were affected by zofenopril (Table 2).

Biochemical assays. Initially, no differences in creatine phosphokinase plasma levels were observed (Figure 1). Only at t = 105 minutes, i.e., during the beginning of the reperfusion period, were the CPK levels higher in the untreated group. However, when the time course was evaluated as a whole, the CPK levels in the untreated group tended to be higher than in the zofenopril group, but this was not statistically significant ($p = 0.080$; Figure 1).

The efflux of purines (hypoxanthine and inosine), which are markers for reversible tissue damage, was lower in the zofenopril group (Table 3). Furthermore, pretreatment with zofenopril resulted in a reduction of the efflux of noradrenaline (Table 3; Figure 2B) and adrenaline (Table 3; Figure 2C). The transient increase in noradrenaline efflux observed after reperfusion in the untreated group was less pronounced in the pretreated group. The increase in adrenaline levels during ischemia occurred only in the untreated group (Figure 2C). Moreover, the transient increase after reperfusion was markedly reduced.

Zofenopril pretreatment did not affect plasma renin activity significantly when evaluated at the beginning of the protocol ($p = 0.076$). Furthermore, no differences between the groups were observed during ischemia and reperfusion (Figure 2D). Plasma renin activity rose twofold in both groups during ischemia and after reperfusion, in the untreated group from 4.9 ±

Table 3. *Efflux of noradrenaline and adrenaline, total purines (purines), and plasma renin activity (PRA)*

	Untreated	Zofenopril
Purines		
AUC (pg.min/ml)	16600 ± 5200	12100 ± 2200
Peak (pg/ml)	78 ± 9	56 ± 4[a]
Noradrenaline		
AUC (pg.min/ml)	141800 ± 54100	86000 ± 41700[a]
Peak (pg/ml)	900 ± 280	400 ± 50[a]
Adrenaline		
AUC (pg.min/ml)	464900 ± 182400	217100 ± 115800[b]
Peak (pg/ml)	3200 ± 760	1300 ± 210[a]
PRA		
AUC (ng.min/ml.hr)	3190 ± 2220	3610 ± 1780
Peak (ng/ml.hr)	12.2 ± 3.8	19.6 ± 5.9

Between-group differences: [a]$p < 0.05$; [b]$p < 0.01$.
The areas under the curve (AUC) and the maximal values (peak) during reperfusion are depicted.

1.5 to 13.6 ± 3.7 ($p < 0.01$), and in the pretreated group from 7.9 ± 2.3 to 14.9 ± 3.9 ($p < 0.05$; ng/ml angiotensin I per hour).

Chronic phase

During the 2 weeks after reperfusion, 3 out of 8 animals died in both the untreated and the zofenopril-pretreated groups. All these animals died within the first 24 hours after the infarction. At the start of the experiments after 2 weeks, cardiac output did not differ between the surviving animals of the groups (untreated 3.5 ± 0.5 vs. zofenopril 2.5 ± 0.2 l/min; ns).

Signal averaging. No bundle branch block was found after 2 weeks in any of the animals at the beginning of the experiment. The duration of the filtered QRS complex was not affected in either group before, during, or after ischemia (Table 4). After 2 weeks, however, the averaged QRS duration was prolonged in both groups. The root mean square of the QRS vector amplitude (V total) decreased markedly during ischemia and reperfusion. After 2 weeks, the V total in the

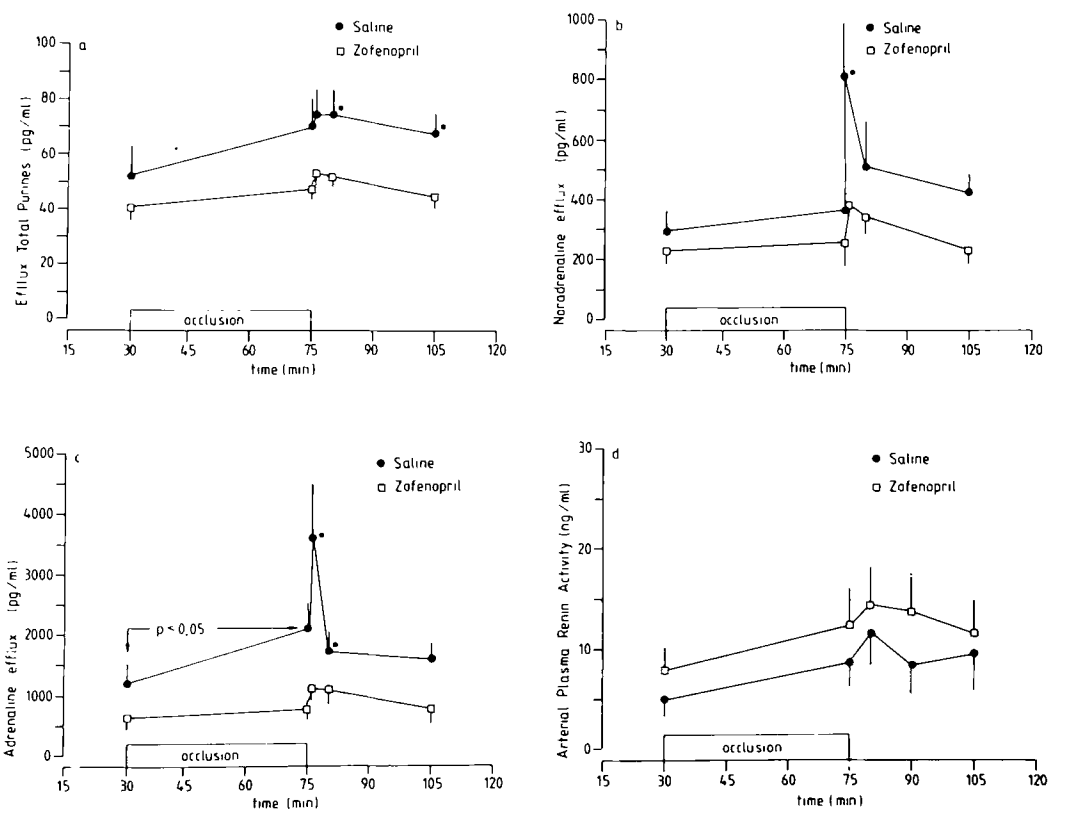

Fig. 2. *Time course of the efflux of (A) total purines, (B) noradrenaline, (C) adrenaline and (D) arterial plasma renin activity during ischemia (t = 30–45) and reperfusion (at t = 75). Significant differences between control (n = 8) and zofenopril-treated animals (n = 8) are indicated: * $p < 0.05$. (mean ± SEM).*

Table 4. Time domain analysis after signal averaging, vector summation, and filtering at 50 Hz of 100–200 beats

	Control	End of ischemia	End of reperfusion	After 2 weeks
QRS				
Untreated	63 ± 2	64 ± 5	60 ± 4	74 ± 8
Zofenopril	60 ± 2	62 ± 5	59 ± 2	66 ± 3
Vtot				
Untreated	211 ± 18	108 ± 11[d]	98 ± 27[c]	94 ± 20[d]
Zofenopril	195 ± 26	114 ± 13[b]	101 ± 16[b]	142 ± 3[a]
D30				
Untreated	20 ± 1	19 ± 4	21 ± 2	30 ± 4[b]
Zofenopril	20 ± 2	22 ± 6	21 ± 1	20 ± 4
V30				
Untreated	56 ± 31	55 ± 21	23 ± 4	16 ± 4
Zofenopril	46 ± 16	64 ± 23	52 ± 17[a]	29 ± 7[a]

Differences from untreated group: [a]$p < 0.05$.
Differences from initial vlaue: [b]$p < 0.05$; [c]$p < 0.01$; [d]$p < 0.001$.
QRS duration (QRS, ms), QRS vector voltage magnitude (Vtot, μV), duration of the terminal part of the QRS complex below 30 μV (D30, ms), and the energy content of the last 30 ms (V30, μV).

zofenopril group had partly recovered, whereas in the untreated group it remained decreased. This resulted in a significant difference between the groups after 2 weeks (Table 4).

A significant inverse correlation between the maximal CPK levels measured in the acute phase and the V total value after 2 weeks was found in the animals from both groups (rho = −0.849; $p < 0.01$) (Figure 3). The duration of the terminal part of the QRS complex below 30 μV (D-30) showed no alterations during the acute experiment. However, after 2 weeks the D-30 was elevated from 20 ± 1 ms to 30 ± 4 ms ($p < 0.05$) in the untreated group in contrast to the zofenopril group (20 ± 2 ms to 20 ± 4 ms) (Table 4). The V-30 (the root mean square of the terminal 30 ms of the QRS-complex) decreased during ischemia and after reperfusion in both groups. After the 2-week period it was further decreased in both groups. However, the decrease in the untreated group was greater than in the zofenopril group (Table 4; $p < 0.05$).

Taken together, a combination of a prolonged QRS duration, an increased D-30, and a reduced V-30 was found in the untreated group. Evidently, in the untreated group late potentials had developed, whereas such electrocardiographic abnormalities were prevented by zofenopril.

Programmed electrical stimulation. In the five untreated survivors a ventricular tachycardia (Figure 4) was inducible, which was monomorphic and sustained in four cases, whereas in the zofenopril-treated group in only one animal was tachycardia inducible after programmed electrical stimulation (Table 2). Therefore, a significantly lower susceptibility to malignant ventricular tachyarrhythmias was found after zofenopril ($p < 0.05$).

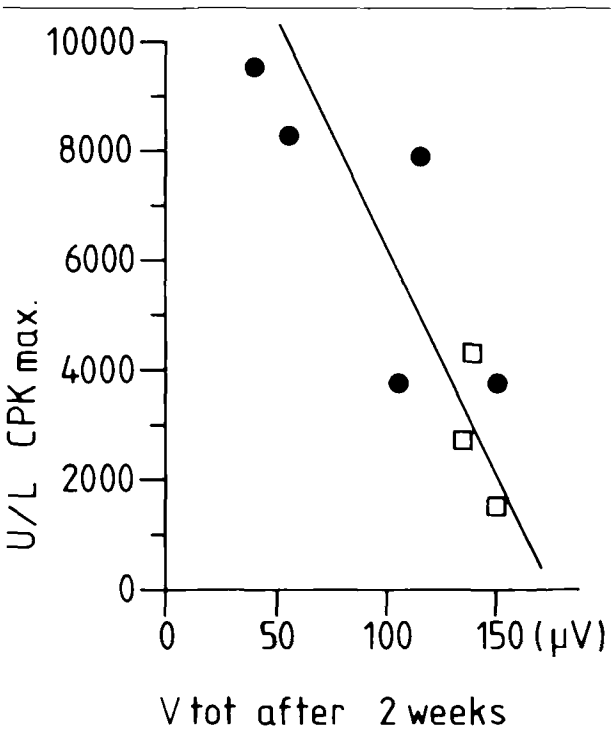

Fig. 3. Linear regression analysis of the maximal CPK levels (CPKmax) in the acute phase versus the QRS vector voltage magnitude (V tot) after 2 weeks. Filled circles represent control animals, the open squares the zofenopril-treated animals. rho = −0.849 and $p < 0.01$ ($n = 8$).

The anatomic infarct size ranged from 15% to 37% of the left ventricular tissue mass (26 ± 3%, mean ± SEM); due to technical failures the number of data was too small (n = 6) to draw conclusions about group differences. However, the overall data indicate that the variability of the infarct size was small. Furthermore, all infarcts were localized in the left ventricular apex and free wall, extending into the intraventricular septum in the larger infarcts only.

Discussion

This study shows that zofenopril ameliorates the deleterious effects of ischemia and reperfusion in the pig. The beneficial effect of zofenopril pretreatment was reflected during ischemia and reperfusion by various biochemical parameters. Firstly, zofenopril caused a smaller efflux of purines and catecholamines. Increased purine levels are known to represent the breakdown of high-energy phosphate nucleotides

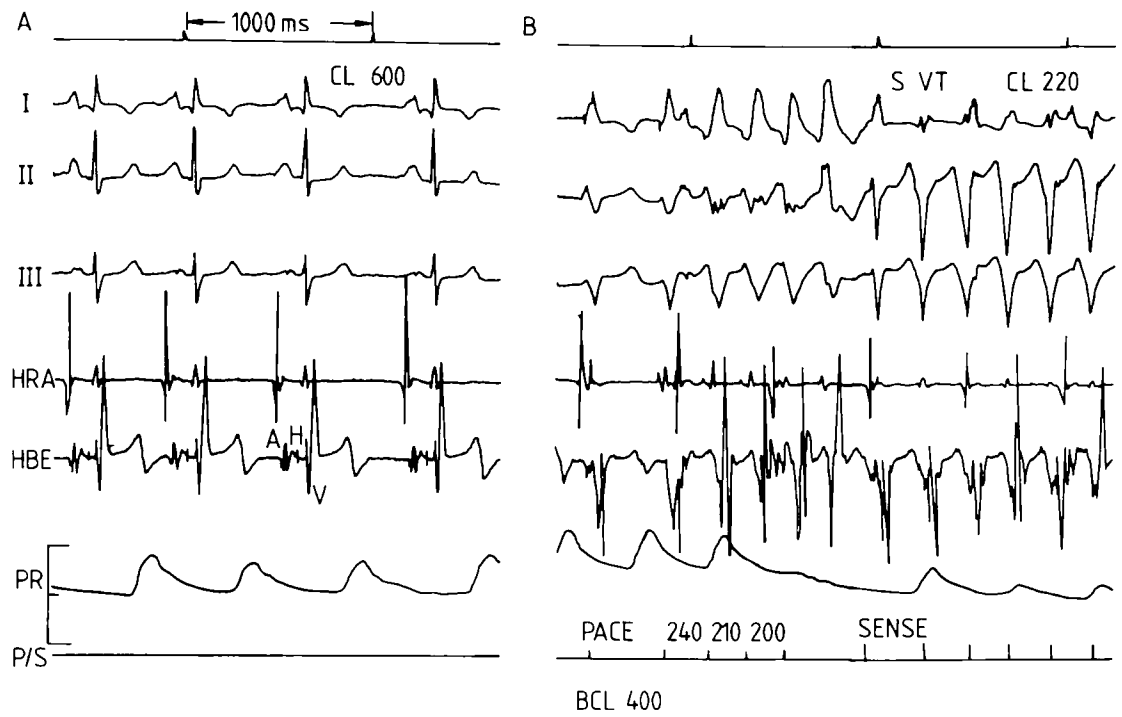

Fig. 4. *A typical recording of the induction of sustained monomorphic ventricular tachycardia by electrical stimulation of the right ventricular apex in an untreated animal, 2 weeks after ischemia and reperfusion. A: Sinus rhythm, B: Sustained ventricular tachycardia. CL = cycle length; BCL = basic cycle length; S VT = sustained ventricular tachycardia; A = atrial deflection; H = His-bundle deflection; V = ventricular deflection; HRA = high right atrium; HBE = His-bundle electrocardiogram; PR = arterial pressure. I, II, and III represent conventional Einthoven electrocardiographic leads. Pacing or sensing is indicated in the bottom tracing.*

caused by ischemia and can be used as a biochemical marker for the extent of ischemia [23]. Secondly, a reduction of irreversible myocardial tissue loss (CPK levels) was found, although this difference was marginally significant. In a previous study in this model, captopril dose-dependently reduced the CPK efflux after 60 minutes of coronary artery occlusion, when given in the acute ischemic phase [15].

In the chronic phase, electrophysiologic parameters were evaluated. Signal averaging of the ECG showed that in all untreated animals late potentials had developed, whereas in the zofenopril-treated animals no late potentials were present and signal-averaging parameters were (partly) recovered. The combination of a longer QRS duration, a decreased V-30, and a longer D-30, i.e., the presence of late potentials, has been shown to be the body surface representation of delayed conduction in partially surviving fibers in the infarct [24]. Therefore, zofenopril induces a modulation of infarct properties related to tachyarrhythmias.

Furthermore, an inverse correlation between maximal CPK levels and the V total after 2 weeks corroborates the results found by Keren et al. [25] in cardiac transplant patients in which V total is considered to reflect the amount of actively depolarizing myocardium. Therefore, apart from a noninvasive method to monitor rejection after heart transplantation, signal averaging might also be of value in evaluating surviving functional myocardial tissue after myocardial infarction. After 2 weeks, V total in the untreated group was further decreased (when compared with the values early after reperfusion) but V total had partially recovered in the zofenopril group. This observation indicates a salvage of reversibly damaged myocardial tissue, which is consistent with the lower purine overflow.

Finally, a programmed electrical stimulation protocol was performed to challenge the electrophysiologic stability of the infarcted hearts after 2 weeks. Electrophysiologic stability in the pretreated group was significantly better than in the untreated group. Previously, we found a similar greater electrophysiologic stability following prolonged oral treatment with captopril in the subsequent, chronic phase [14]. Moreover, it is well known that late potentials are a sen-

sitive and specific marker for sustained ventricular tachycardia in patients who are at risk after myocardial infarction [26,27]. Our results in the pig are in agreement with these observations.

Surprisingly, before ischemia and reperfusion, the arterial resistance in the zofenopril group was higher than in the untreated group. Probably the low cardiac output found in the zofenopril group was due to low circulating catecholamines in these healthy animals, since before ischemia the cardiac output in the pretreated group showed a positive correlation with adrenaline levels (rho = 0.85; $p < 0.05$; n = 9). In the zofenopril-pretreated group, a reduced sympathetic tone related to the low catecholamine levels might have developed. The low catecholamine levels in the zofenopril group may have prevented arterial vasodilation, i.e., lower peripheral $beta_2$ receptor stimulation might have led to a higher systemic vascular resistance.

It is well known that effects on heart rate are not found after ACE inhibition, not even after dosages that cause severe hypotension. During reperfusion, the heart rate in the untreated group rose considerably and zofenopril appeared to blunt this response to ischemia. This could be due to an attenuation of noradrenergic neurotransmission, as has been described for converting-enzyme inhibitors [28].

The present results indicate that converting-enzyme inhibition with a low dose of zofenopril prior to the acute phase of the infarction ameliorates the electrophysiologic instability in the chronic phase. Apparently, protection against the injurious component of ischemia and reperfusion is crucial. Whether the myocardial renin-angiotensin system plays a primary role in these effects of zofenopril is an intriguing question, but this cannot be answered from our data.

The mechanism of the protective effects of zofenopril, mainly apparent in the chronic phase of infarction, is probably multifactorial. Firstly, zofenopril can improve coronary perfusion through various mechanisms. Apart from the reduced synthesis of angiotensin II, the inhibition of bradykinin breakdown by the converting-enzyme inhibitor might play a role. Endogenous bradykinin is known to be released from the ischemic myocardium [29–31]. Bradykinin stimulates prostacyclin release from the vascular endothelium [32]. Furthermore, it is well known that prostacyclin can protect the myocardium against ischemic damage [33], which has also been shown in this model [34]. Therefore, it might be an essential mediator in the effects of zofenopril. Secondly, another important aspect might be the lower increase in heart rate and the absence of an increase in the rate-pressure product, and hence the prevention of an increased O_2 demand. Moreover, whatever the mechanism, the prevention of malignant tachyarrhythmias and a reduction of catecholamine efflux represent an important beneficial influence. In humans, this latter observation was corroborated by Kingma et al. [35], who, in a study in which acute myocardial infarction patients received reperfusion therapy with streptokinase and captopril as a concomitant intervention, observed a reduced catecholamine efflux.

In conclusion, the converting-enzyme inhibitor zofenopril salvages myocardial tissue by preventing a deleterious increase in oxygen consumption during ischemia and early reperfusion. Furthermore, a long-term beneficial effect of zofenopril on the anatomic substrate for ventricular tachycardia is evident from the signal-averaging data. Therefore, the development of electrophysiologic instability of the infarctions is prevented, as evidenced by a reduced inducibility of ventricular tachycardia. This effect is probably related to modulation of deleterious neurohumoral changes during ischemia and acute reperfusion. Therefore, the promising potential of zofenopril deserves further investigation in patients receiving thrombolytic therapy in the acute phase of myocardial infarction.

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