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Losartan but not enalaprilat acutely reduces reperfusion ventricular tachyarrhythmias in hypertrophied rat hearts after low-flow ischaemia

Silvia Butz, Sergey Driamov, Andrea Remondino, Mohamed Bellahcene, Konstantin Beier, André Ziegler, Peter T. Buser and Christian E. Zaugg

Abstract

Based on clinical and experimental studies, angiotensin II receptor blockers and angiotensin converting enzyme inhibitors have been proposed to exert acute anti-arrhythmic effects in heart failure patients. Therefore, the goal of this study was to assess acute anti-arrhythmic effects of losartan and enalaprilat in hypertrophied rat hearts during low-flow ischaemia and reperfusion. In dose-finding experiments in non-hypertrophied isolated perfused hearts, we performed dose–response curves of losartan and enalaprilat studying monophasic action potential duration at 90% repolarisation ($MAPD_{90\%}$) and ventricular fibrillation (VF) threshold. Subsequently, we determined the effects of losartan and enalaprilat (in therapeutically relevant concentrations) on ventricular tachyarrhythmias induced by low-flow ischaemia/reperfusion in hearts demonstrating left ventricular (LV) hypertrophy 70 days after aortic banding. We found that neither drug significantly affected $MAPD_{90\%}$ (1 nM–1 mM) or VF threshold (1 μM losartan and 10 μM enalaprilat) in non-hypertrophied hearts. Similarly in hypertrophied hearts, neither drug significantly affected the incidence or the duration of ventricular tachyarrhythmias (ventricular tachycardia and VF) during low-flow ischaemia. However, 1 μM losartan significantly reduced the duration of ventricular tachyarrhythmias during reperfusion. In conclusion, neither losartan nor enalaprilat is acutely anti-arrhythmic in hypertrophied rat hearts during low-flow ischaemia. During reperfusion, however, losartan but not enalaprilat exerts acute anti-arrhythmic effects.

Experimental Cardiology Research Group, Department of Research, University Hospital of Basel, Switzerland

Silvia Butz, Sergey Driamov, Andrea Remondino, Mohamed Bellahcene, André Ziegler, Christian E. Zaugg

Institute of Anatomy, University of Basel, Switzerland

Konstantin Beier

Division of Cardiology, Department of Internal Medicine, University Hospitals Basel, Switzerland

Peter T. Buser

Correspondence: C. E. Zaugg, University Hospital of Basel, Dept. Research, Hebelstr. 20, ZLF 319, 4031 Basel, Switzerland. E-mail: Christian.Zaugg@unibas.ch

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Introduction

Sudden death is a major cause of mortality in patients with ventricular hypertrophy and heart failure (Stevenson et al 1993; Messerli 1999; Vakili et al 2001). In these patients, ventricular tachyarrhythmias, particularly ventricular fibrillation (VF), contribute importantly to sudden death (Stevenson et al 1995). These arrhythmias are largely due to electrophysiological abnormalities of the hypertrophied heart that include prolonged action potential duration, decreased resting membrane potential, slowed conduction velocity and heterogeneous recovery following depolarization (Aronson & Ming 1993; Stevenson et al 1995).

In this regard, it is important that the angiotensin II receptor type 1 (AT_1) blocker losartan has been proposed to be associated with a lower mortality than that found with the angiotensin converting enzyme (ACE) inhibitor captopril in elderly heart failure patients (Pitt et al 1997). This difference appeared to arise largely from a decrease of sudden death in losartan-treated patients, causing various investigators to attribute acute anti-arrhythmic effects to losartan (Thomas et al 1996; Lee et al 1997). However, the effect on sudden death was not confirmed by a recent trial that was adequately sized for mortality (Pitt et al 2000). Still, various experimental studies could demonstrate acute anti-arrhythmic effects of angiotensin II receptor blockers or ACE inhibitors. Accordingly, in human atrial tissue, losartan significantly reduced angiotensin I-induced noradrenaline (norepinephrine) release (Rump et al 1998). In an experimental study in guinea-pigs, losartan exerted anti-arrhythmic effects independent of AT_1 receptor blockade (Thomas et al 1996). In another study in spontaneously hypertensive rats, losartan

exerted anti-arrhythmic effects in the setting of myocardial infarction (Lee et al 1997). In contrast, in rat hearts during ischaemia/reperfusion, captopril was superior to losartan in reducing the incidence of irreversible VF (Ozer et al 2002). Based on the foregoing, it is controversial whether AT_1 blockers or ACE inhibitors exert anti-arrhythmic effects, particularly in hypertrophied hearts.

Therefore, the goal of this study was to assess acute anti-arrhythmic effects of losartan and enalaprilat in hypertrophied rat hearts during ischaemia/reperfusion. For dose-finding purposes, we determined the effects of these drugs on action potential duration in dose–response curves as well as the effects of these drugs on VF threshold in non-hypertrophied isolated perfused hearts. Subsequently, we determined the effect of theses drugs on the incidence and duration of ventricular tachycardia (VT) and of VF induced by low-flow ischaemia and reperfusion in hypertrophied hearts 70 days after aortic banding. Low-flow ischaemia was chosen because, from a clinical point of view, low-flow ischaemia is both relevant to hypertrophied hearts (due to reduced coronary reserve) and responsible for life-threatening ventricular arrhythmias (Furukawa et al 1991).

Table 1 Characterization of rat model of left ventricular hypertrophy.

	Aorta banded	Sham-operated
Body weight (g)	477 ± 41	488 ± 51
Heart wet weight (g)	2.04 ± 0.32	1.67 ± 0.21*
Wet heart weight/body weight ($g\,kg^{-1}$)	4.3 ± 0.5	3.4 ± 0.3*
LV mass (g)	0.98 ± 0.03	0.70 ± 0.11*
LV end-diastolic volume (μL)	753 ± 21	659 ± 76
LV end-systolic volume (μL)	316 ± 88	209 ± 35
LV stroke volume (μL)	338 ± 66	450 ± 42
LV wall thickness (mm)	2.4 ± 0.1	1.7 ± 0.3*
Ejection fraction	0.58 ± 0.10	0.68 ± 0.02

Values are mean $\pm$ s.d. 70 days after surgery (n = 28 and 18 hearts, respectively, for weights and n = 3 hearts each for magnetic resonance data). Left ventricular (LV) wall thickness was measured in diastole. LV mass was calculated from the end-diastolic myocardial volume multiplied by the myocardial specific gravity ($1.05\,g\,cm^{-3}$). The ejection fraction was calculated from the stroke volume/end-diastolic volume ratio. The stroke volume was calculated as end-diastolic minus end-systolic volumes. *$P < 0.05$ vs sham-operated rats.

Materials and Methods

Drugs

Losartan and enalaprilat were donated by Du-Pont-Merck (USA). Amiodarone (Cordarone Injection solution $50\,mg\,mL^{-1}$) was from Sanofi-Synthelabo (Switzerland) and lidocaine hydrochloride (Rapidocaine 1%) was from Sintetica (Switzerland). All other chemicals were from Fluka (Switzerland).

Animals

All experiments conformed to the rules of the Swiss Federal Act on Animal Protection (1998) and were approved by the veterinary department of Basel (Switzerland). Furthermore, all experiments were performed in male Sprague Dawley rats from Iffa credo (L'Arbresle, France).

Animal model of left ventricular hypertrophy

Left ventricular (LV) hypertrophy was induced by pressure overload consequent to abdominal aortic banding (coarctation) in rats. To this end, rats 182 ± 14 (mean $\pm$ s.d.) g were anaesthetized by inhalation of isoflurane (1.3%), N_2O ($90\,mL\,min^{-1}$) and O_2 ($30\,mL\,min^{-1}$). The abdominal aorta was isolated via lateral laparotomy and a tantalum clip (Horizon ligating clip; Weck closure systems, USA; inner diameter set to 0.4 mm) was applied to the aorta proximal to the branching of the A. renalis. Sham-operated rats were treated the same way but with no clip applied. The rats were then allowed free access to a normal diet and water for 70 days to develop LV hypertrophy. Subsequently, LV hypertrophy was confirmed by repeated in-vivo magnetic resonance imaging (in 3 rats each, Table 1) as described previously (Ziegler et al 2002). Additionally, LV hypertrophy was confirmed ex-vivo by determining the wet heart weight/body weight ratio (of all rats). Imaging data revealed clear signs of LV hypertrophy in rats after aortic banding, including increased LV mass and LV wall thickness (Table 1). However, ejection fraction (calculated from the stroke volume/end-diastolic volume ratio) was similar in both groups, indicating compensated LV hypertrophy in banded rats. Seventy days after surgery, hearts were isolated and perfused to assess the acute anti-arrhythmic effects of losartan and enalaprilat during low-flow ischaemia and reperfusion.

Isolated rat heart preparation

Rats were anaesthetized by intraperitoneal injection of $30\,mg\,kg^{-1}$ sodium pentobarbital (Nembutal; Abbott Laboratories, Chicago, IL). After midline sternotomy, hearts were excised rapidly and perfused within 30 s according to Langendorff as previously described (Zaugg et al 1996b). All hearts were perfused at a constant pressure of 80 mmHg with a filtered (pore size 0.65 μm) non-recirculating modified Krebs-Henseleit buffer containing (in mM) NaCl 117, KCl 4.3, $MgCl_2$ 1.2, $CaCl_2$ 2.0, $NaHCO_3$ 25, EDTA 0.5 and glucose 15 at pH 7.4. No albumin was added to the buffer to avoid losartan binding to albumin (Christ 1995). The buffer was saturated with 95% O_2/5% CO_2 ensuring a minimal pO_2 of 550 mmHg. A pair of electrodes (0.28 mm diameter, 2–3 mm contact length) was placed on the right appendage and apex to record a bipolar epicardial electrocardiogram (ECG). Ventricular stimulation was carried out using a pair of platinum electrodes (0.28 mm diameter) connected to a PowerLab 4/20 data acquisition system (AD Instruments, Castle Hill, Australia) controlled by a Macintosh computer running Chart software (AD Instruments, Castle Hill, Australia). The stimulation electrodes

were implanted in the right ventricular free wall. During all experiments, the hearts were immersed in perfusate maintained at exactly 37.0 °C (Zaugg et al 1996b), preventing bradycardia due to cardiac cooling (Curtis 1998). Furthermore, in all experiments, a 15-min stabilization period preceded any drug administration or pacing protocol.

Experimental protocols

Dose-finding experiments of losartan and enalaprilat (dose–response curves for action potential duration and VF threshold experiments) were performed in isolated non-hypertrophied hearts from non-operated rats weighing 353 ± 52 g. Acute anti-arrhythmic effects of these drugs during low-flow ischaemia and reperfusion, however, were assessed in isolated hypertrophied hearts 70 days after aortic banding surgery.

Dose–response curves of losartan and enalaprilat on action potential duration

To find suitable concentrations for subsequent experiments and to assess acute electrophysiologic effects of the drugs, we performed dose–response curves for losartan (1 nM–1 mM, n = 4) and enalaprilat (1 nM–1 mM, n = 4) studying monophasic action potential duration at 90% repolarisation ($MAPD_{90\%}$). The potassium-channel blocker amiodarone (10 μM), which has been shown to prolong action potential duration (Rochetaing et al 2001), was used as positive control. In these dose-finding experiments, monophasic action potentials were recorded using a contact electrode (Ag–AgCl electrode, model 225; Hugo Sachs Elektronik-Harvard Apparatus, March-Hugstetten, Germany) on the epicardium of the left ventricle. A digitized readout was recorded at 1 kHz sampling rate using a PowerLab 4/20 data acquisition system. The validity of monophasic action potential recording was confirmed by determining the effect of increasing heart rate on $MAPD_{90\%}$. Accordingly, $MAPD_{90\%}$ consistently decreased when the heart rate was increased from 100 to 300 beats per min (at 30 °C to allow for heart rates < 200 min^{-1}). For experiments determining the effects of losartan or enalaprilat on $MAPD_{90\%}$, the heart rate was held constant at 240 beats per min (at 37 °C). The dose–response curves were to select a concentration of losartan and enalaprilat for subsequent experiments (VF threshold and ischaemia/reperfusion experiments).

VF threshold

VF threshold determination was performed using a train-of-pulses method at increasing voltage to scan the vulnerable period of repolarisation (Zaugg et al 1996a). Specifically, the heart rate was held constant at 200 ms pulse interval using the stimulator mode of PowerLab 4/20 data acquisition system (1 ms monophasic square wave pulses at 4 V). After 30 regular pulses, a train-of-pulses (100 Hz, 250 ms duration) was generated at increasing voltage. The voltage was increased starting at 0.25 V in 0.25-V increments (until 1 V) and in 0.5-V increments (until 10 V) until VF occurred. After each train-of-pulses, pacing was stopped for 2.5 s to allow detection of VF. The VF threshold was defined as the mean voltage (in V) of at least three successive measurements, which were reproducible within limits of ± 15%. VF was detected as ECG waves of irregular morphology without corresponding effective left ventricular pressure for longer than 1 s (Zaugg et al 1996a). VF thresholds were determined before and after perfusion with losartan (1 μM, n = 6; for choice of concentration see Results), enalaprilat (10 μM, n = 5), or vehicle (control, n = 6). As a positive control, VF thresholds were determined before and after perfusion with the sodium-channel blocker lidocaine (3 μM). To reduce VF threshold variability, the pacemaker electrodes were coated and held in position by polyethylene tubes to ensure a constant implantation depth of 2 mm (uncoated electrodes) and consistent distance of 5 mm from each other (Zaugg et al 1996a). Thereby, the spatial separation and anatomical position of electrodes on the heart were kept consistent, as recommended for reproducible VF threshold determination (Van Tyn & MacLean 1961).

Arrhythmias induced by low-flow ischaemia and reperfusion

The hypertrophied hearts were randomly assigned to one of three groups: control (receiving only Krebs-Henseleit buffer (vehicle), n = 10); losartan (1 μM, n = 9) or enalaprilat (10 μM, n = 9). Hearts of sham-operated rats (n = 18) were perfused with the vehicle only. After a drug-free stabilization period of 20 min, losartan or enalaprilat were administered 15 min before low-flow ischaemia until the end of the experiment. Low-flow ischaemia lasted for 60 min and was induced by reducing perfusion pressure from 80 to 15 mmHg, reducing coronary flow by ≈ 90%. To prevent bradycardia and asystole during low-flow ischaemia, hearts were paced (as recommended by Curtis (1998)) at 300 beats per min via a pair of platinum pacemaker wires implanted in the right ventricular free wall and connected to a pulse generator (Grass SD 5; Grass Instruments, Quincy, MA). Pacing was stopped before normalizing the perfusion pressure to 80 mmHg and subsequent reperfusion lasted for 60 min.

Measurement of haemodynamic variables

Coronary flow was measured within the aortic cannula using an inline flowprobe (Transonic 2N) connected to a transit time flowmeter (Transonic TTFM-SA type 700; Hugo Sachs Elektronik-Harvard Apparatus, March-Hugstetten, Germany). LV pressure was measured by a fluid-filled polyethylene catheter inserted through the left atrial appendage into the LV cavity. The catheter was connected to a pressure transducer (MLT1050 Pressure transducer; AD Instruments, Castle Hill, Australia). A digitized readout of the LV pressure and the ECG was recorded at 400 Hz sampling rate throughout the experiment using a PowerLab 8e data acquisition system (AD Instruments, Castle Hill, Australia) connected to a Macintosh computer. LV developed pressure was defined as the difference between systolic and diastolic values of LV pressure.

Analysis of arrhythmias

Analysis of ventricular arrhythmias was based on the Lambeth Conventions (Walker et al 1988). Accordingly, arrhythmias were categorized as ventricular tachycardia (VT, run of four or more consecutive ventricular premature

beats with corresponding effective LV pressure) or ventricular fibrillation (VF, ECG waves of irregular morphology without corresponding effective LV pressure). Sustained and spontaneously reverted VF were not analysed separately, and VF persisting longer than 30 s was terminated by a bolus of 0.25 mg lidocaine hydrochloride injected into the perfusion cannula proximal to the aorta. After termination of VF, lidocaine was washed out within 5 min (Zaugg et al 1996a) and the corresponding heart was re-included in the arrhythmia analysis until the end of the experimental protocol. This way, we avoided potential study bias due to early exclusion of experiments after sustained VF.

Evaluation and statistical analysis

Normal distribution of numerical variables (magnetic resonance data, ratio wet heart weight/body weight, coronary flow, LV developed pressure, $MAPD_{90\%}$ and VF threshold) was confirmed by Shapiro-Wilk test. Consequently, these variables were expressed as mean ± s.d. and compared among groups by one-way analysis of variance. Dose–response curves for losartan and enalaprilat on $MAPD_{90\%}$ were analysed by repeated-measures analysis of variance. The incidence of VT and of VF were evaluated on the digitized ECG and pressure readouts and compared among groups by chi-squared analysis. To obtain a more sensitive measure of potential anti-arrhythmic effects, an overall duration of ventricular tachyarrhythmias was calculated and compared among groups. To this end, the duration of VT and of VF was pooled during low-flow ischaemia and during reperfusion for each experiment. Because of non-Gaussian distribution, the duration of ventricular tachyarrhythmias was expressed as median with interquartile range (distance between 25th and 75th percentile) and compared among groups by Kruskal-Wallis test followed by Dunn's test. One hypertrophied heart demonstrating non-sustained VT before low-flow ischaemia was excluded from analysis. Testing for far outliers was performed according to the method of Velleman & Hoaglin (1981), excluding one experiment in the sham-operated, and one in the losartan-treated hypertrophied hearts in the analysis of arrhythmias during ischaemia and reperfusion. Statistical computations were done using Prism software (version 3.0a; GraphPad, San Diego, CA). In an approximation of sample size determination for this study, 9 rats in each group had 90% power to detect a biologically meaningful difference of at least 20% in most variables assuming an s.d. of 15% and a 0.05 two-sided significance level. The study was, however, not meant and powered to demonstrate differences in the incidence of VF or VT. For all statistical analyses, the null hypothesis was rejected at the 95% level, considering a two-tailed $P < 0.05$ significant.

Results

Dose–response curves

In dose–response curves of losartan and enalaprilat on $MAPD_{90\%}$ in non-hypertrophied hearts, neither drug significantly affected $MAPD_{90\%}$ at a constant heart rate of 240 beats per minute (Figure 1). Similarly, $MAPD_{90\%}$ of control hearts (no drug treatment, n = 3) remained unaltered and stable throughout the duration of the experiment. In contrast, amiodarone (10^{-5} M), which was used as positive control, significantly prolonged the $MAPD_{90\%}$ from 41.9 ms to 52.2 ms (n = 3). Because none of the tested concentrations of losartan or enalaprilat affected $MAPD_{90\%}$, further experiments were carried out using concentrations that had previously been shown to be cardioprotective under similar conditions (Grover et al 1991; Zhu et al 1999). Therefore, 1 μM losartan (Zhu et al 1999) and 10 μM enalaprilat (Grover et al 1991) were used for VF threshold and ischaemia/reperfusion experiments. These concentrations correspond to the plasma concentrations achieved in man after oral administration of the drug in a therapeutic dosage (Yasar et al 2002; Najib et al 2003).

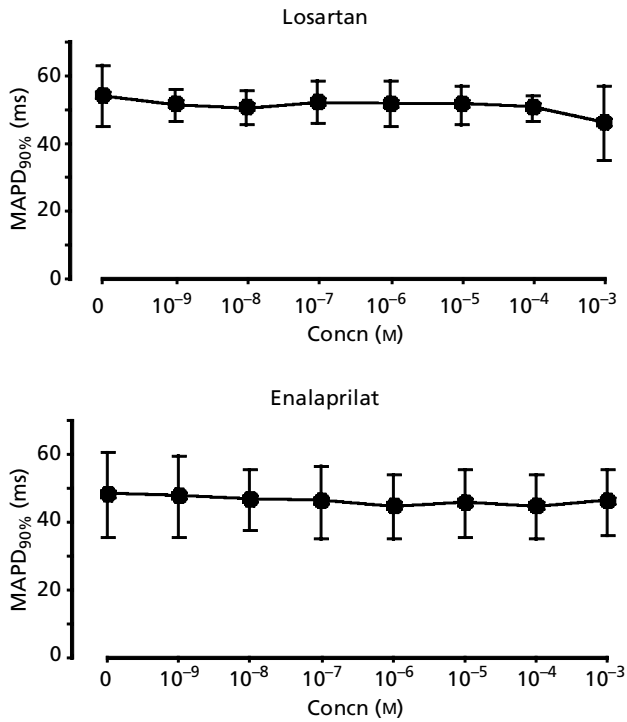

Figure 1 Dose–response curve of losartan and of enalaprilat on action potential duration at 90% repolarisation ($MAPD_{90\%}$) recorded at a constant heart rate of 240 beats per min in non-hypertrophied rat hearts. Values are mean ± s.d. of 4 hearts per drug. Note that neither drug significantly affected $MAPD_{90\%}$.

VF threshold

Similar to their effects on $MAPD_{90\%}$, neither 1 μM losartan nor 10 μM enalaprilat significantly affected VF threshold (Figure 2). In contrast, 3 μM lidocaine, which was used as positive control, increased VF threshold to > 10 V. As a pre-requirement for valid and reproducible measurements, no differences in VF threshold could be detected among groups before drug treatment.

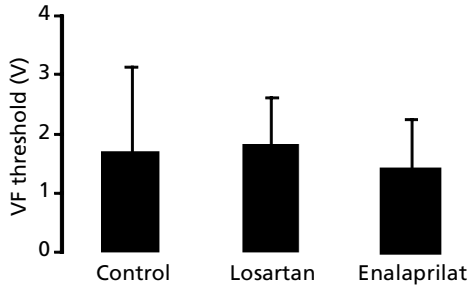

Figure 2 Effect of losartan (1 μM) and of enalaprilat (10 μM) on ventricular fibrillation (VF) threshold in non-hypertrophied rat hearts. Control hearts were perfused with the vehicle only. Values are mean ± s.d. of 5 or 6 hearts per group. Note that neither drug significantly affected VF threshold.

Arrhythmias induced by low-flow ischaemia and reperfusion

In contrast to their effects on $MAPD_{90\%}$ and on VF threshold, losartan and enalaprilat had differential effects on ventricular tachyarrhythmias induced by low-flow ischaemia and reperfusion in hypertrophied hearts (Table 2, Figure 3; characterization of ventricular hypertrophy in Table 1). Specifically, during low-flow ischaemia, neither drug significantly affected the incidence or median duration of VT or VF. During reperfusion, however, 1 μM losartan significantly reduced the median duration of tachyarrhythmias (from 51 s to 0 s; Figure 3). Enalaprilat appeared to reduce the duration of tachyarrhythmias during reperfusion too (4 s), but this reduction was not statistically significant ($P = 0.24$). Similarly, the duration of ventricular tachyarrhythmias appeared longer in hypertrophied hearts (51 s) than in non-hypertrophied hearts from sham-operated rats (4 s), but again this difference was not statistically significant ($P = 0.60$).

Haemodynamic variables were similar in all groups throughout the experiments (Table 3). Specifically at baseline, no significant differences in coronary flow and LV developed pressure could be detected among the groups. Inducing low-flow ischaemia by reducing the perfusion pressure led to a consistent reduction of coronary flow and LV developed pressure from 23.2 ± 4.2 mL min^{-1} to 2.5 ± 1.2 mL min^{-1} (= 11% residual coronary flow) and from 84.5 ± 15.5 mmHg to 13.1 ± 5.7 mmHg, respectively. Importantly, throughout low-flow ischaemia, coronary flow and LV developed pressure values did not significantly differ among the groups. Finally, at the end of the reperfusion, coronary flow and LV developed pressure were similar in all groups, recovering to almost baseline levels.

Table 2 Incidence of VT and VF in rat hearts during low-flow ischaemia and reperfusion.

	Hypertrophied hearts			Sham-operated
	Control	Losartan (1 μM)	Enalaprilat (10 μM)	
Ischaemia				
VT (%)	80	67	100	89
VF (%)	90	56	89	78
Reperfusion				
VT (%)	70	33	67	67
VF (%)	40	22	11	39
n (hearts)	10	9	9	18

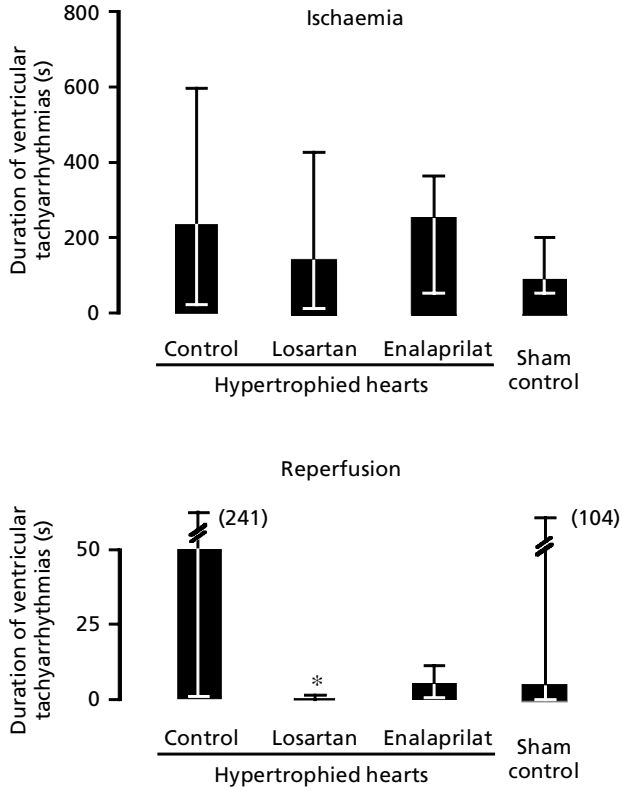

Figure 3 Effect of losartan (1 μM) and of enalaprilat (10 μM) on the duration of ventricular tachyarrhythmias (sum of VT and VF duration) during low-flow ischaemia (60 min) and reperfusion (60 min) in hypertrophied rat hearts. Control hypertrophied hearts and sham-operated (non-hypertrophied) hearts were perfused with the vehicle only. The data are shown as median and quartiles of 9, 9, 10 and 18 hearts. *$P < 0.05$ vs control. Note that losartan significantly reduced the duration of ventricular tachyarrhythmias during reperfusion.

Discussion

This study in hypertrophied rat hearts demonstrates that neither losartan nor enalaprilat is acutely anti-arrhythmic during low-flow ischaemia. During reperfusion, however, losartan, but not enalaprilat, exerts acute anti-arrhythmic effects. Specifically, during low-flow ischaemia, neither drug reduced the median duration of ventricular tachyarrhythmias. However, during reperfusion, losartan, but not enalaprilat, reduced the median duration of ventricular

Table 3 Haemodynamic variables.

	Hypertrophied hearts			Sham-operated
	Control	Losartan	Enalaprilat	
Coronary flow ($mL\,min^{-1}$)				
Baseline	21.3 ± 2.3	21.1 ± 4.4	24.8 ± 4.2	24.4 ± 4.3
Low-flow ischaemia	3.1 ± 1.4	2.7 ± 1.2	2.9 ± 1.2	2.1 ± 1.1
End reperfusion	18.6 ± 2.9	16.0 ± 5.0	18.6 ± 5.6	14.6 ± 3.2
LV developed pressure (mmHg)				
Baseline	90.3 ± 29.4	80.6 ± 12.2	80.2 ± 9.4	85.2 ± 5.5
Low-flow ischaemia	14.6 ± 6.3	15.7 ± 6.3	12.0 ± 5.5	11.5 ± 5.1
End reperfusion	85.9 ± 28.8	79.9 ± 10.8	69.2 ± 21.4	74.5 ± 17.8

Low-flow ischaemia values are averaged over the entire 60-min period. Values are mean ± s.d. of 10, 9, 9 and 18 hearts. Neither coronary flow nor left ventricular (LV) developed pressure differed among groups.

tachyarrhythmias indicating acute anti-arrhythmic effects. Importantly, these effects occurred in concentrations comparable with those in human plasma after oral administration of losartan in a therapeutic dosage (Yasar et al 2002; Najib et al 2003).

These different electrophysiological and anti-arrhythmic effects of losartan and enalaprilat partially agree with, and extend previous reports about, acute anti-arrhythmic effects of AT_1 blockers and ACE inhibitors. Similar to our findings, losartan ($10\,\mu M$) only exerted electrophysiologic and anti-arrhythmic effects in early reperfusion but not under normoxic conditions in guinea-pig ventricles (Thomas et al 1996). Similarly, in isolated rat hearts, an AT_1 blocker reduced the median duration of VF during reperfusion (Fleetwood et al 1991). Moreover, during reperfusion after left descending coronary artery occlusion, losartan ($50\,\mu g\,kg^{-1}\,min^{-1}$) beneficially affected both the VF threshold and the incidence of ventricular tachyarrhythmias in dogs (Matsuo et al 1997). Finally, losartan ($2\,mg\,kg^{-1}$) reduced the incidence of VT (but not VF) during reperfusion in rats in-vivo (Ozer et al 2002). These reports, as well as our findings, support the notion that angiotensin II is a mediator of reperfusion-induced tachyarrhythmias (Fleetwood et al 1991). During low-flow ischaemia, however, neither losartan nor enalaprilat significantly affected the incidence or duration of ventricular tachyarrhythmias in our experiments. In this regard, however, it should be considered that this study was not meant and powered to demonstrate differences in the incidence of VF or VT. Instead, we calculated the median duration of ventricular tachyarrhythmias to obtain a more sensitive measure of potential anti-arrhythmic effects. Nevertheless, similar to our results, neither losartan nor the ACE inhibitor captopril reduced the incidence of ischaemia-induced ventricular arrhythmias in dogs (Lynch et al 1999). In contrast, in the setting of myocardial infarction, losartan reduced the incidence of VF in spontaneously hypertensive rats (Lee et al 1997). Furthermore, in contrast to our $MAPD_{90\%}$ data, enalaprilat exerted electrophysiologic effects that caused action potential prolongation in a multisite optical mapping study in isolated guinea-pig hearts (Gilat et al 1998). Similar to our findings, this effect was not of sufficient magnitude to suppress the initiation of VF or re-entrant VT.

Interestingly, losartan demonstrated anti-arrhythmic effects in hypertrophied hearts during reperfusion without exerting electrophysiologic effects in non-hypertrophied hearts under normoxia. It may thus be speculated that losartan exerts little or no electrophysiologic and anti-arrhythmic effects in normal hearts during normoxia. During post-ischaemic reperfusion however, losartan might alter cardiac electrophysiology and therefore be acutely anti-arrhythmic. Our study shows for the first time that such an anti-arrhythmic effect is present in hypertrophied hearts after low-flow ischaemia. This could be of particular relevance to patients demonstrating LV hypertrophy that are at increased risk of life-threatening ventricular tachyarrhythmias induced by low-flow ischaemia. The latter, in turn, is a consequence of reduced coronary reserve in hypertrophied hearts. Moreover, losartan showed only anti-arrhythmic activity during reperfusion (but not during low-flow ischaemia). Therefore, it may be speculated that losartan affects automatic (pacemaker-induced) arrhythmias or triggered arrhythmias.

It is difficult, however, to speculate about the electrophysiologic effects of losartan on mechanisms initiating ventricular tachyarrhythmias in our experimental model. This is because global low-flow ischaemia presumably generated little flow of injury current and because of ventricular pacing (avoiding asystole during ischaemia (Curtis (1998)) that most likely created a site of ectopic automaticity. Still, losartan reduced the duration of ventricular tachyarrhythmias in our experiments and thus probably affected re-entry mechanisms that maintain ventricular tachyarrhythmias. Such acute anti-arrhythmic effects might be mediated by altering angiotensin II-related ventricular electrophysiologic properties of the ventricles, including increasing cardiac refractoriness (De Mello & Crespo 1999), reducing dispersion of action potential duration (De Mello 2001), and prevention of

Ca^{2+} overload in cardiomyocytes during reperfusion (Yahiro et al 2003). Additionally or alternatively, acute anti-arrhythmic effects of both losartan and enalaprilat may arise from free radical scavenger effects (Birincioglu et al 1997; Donmez et al 2002) or the accumulation of bradykinin. The latter possibility does not necessarily favour ACE inhibitors because losartan has also been shown to produce bradykinin-dependent cardioprotective effects in rat hearts during ischaemia and reperfusion (Zhu et al 1999). The view that ACE inhibitors and angiotensin do not equally well suppress the actions of angiotensin II, as well as additional effects of losartan on cardiac K^+ currents, may explain why losartan but not enalaprilat was acutely anti-arrhythmic in our experiments. In this regard, it may be important that losartan exerted anti-arrhythmic effects independent of AT_1 receptor blockade (Thomas et al 1996) and acutely modified cardiac delayed rectifier K^+ currents (hKv1.5, HERG, and Ks channels; but not Na^+ and Ca^{2+} currents) in guinea-pig ventricular myocytes, canine Purkinje fibres or ventricular myocytes (Timmermans et al 1993; Timmermans & Smith 1994; Caballero et al 2000). Consequently, losartan might acutely prolong action potential duration (Caballero et al 2000) and such a prolongation could render the heart less susceptible to re-entrant arrhythmias during early reperfusion when action potential is shortened (Thomas et al 1996).

Unfortunately, we could not record reliable action potentials during ischaemia or reperfusion because varying electrode contacts resulted in action potential artifacts. For this reason, we do not know whether the anti-arrhythmic effects of losartan were paralleled by electrophysiologic effects during ischaemia and reperfusion. Other authors measuring $APD_{90\%}$ in guinea-pig ventricles found a shortened $APD_{90\%}$ during simulated ischaemia and early reperfusion but no differences between preparations treated with losartan and vehicle (Thomas et al 1996). However, it should be considered that inter-species differences in cellular electrophysiology and action potential morphology limit the comparison of rat hearts with hearts of other species, including guinea-pigs and man. Crucial ionic currents in man do not contribute to the repolarisation in rats. Specifically, in rats, I_{to} is the most important repolarizing current (Cerbai et al 1994). In contrast, in man, I_{Kr} and I_{Ks}, the two components of the delayed rectifier potassium current, play a dominant role in the repolarisation of the action potential (Viswanathan et al 1999). Nevertheless, in this study, the rat heart served as a readily available model of LV hypertrophy during ischaemia/reperfusion and had previously been used for similar studies (Fleetwood et al 1991; Thomas et al 1996; Lee et al 1997; Matsuo et al 1997; Lynch et al 1999). Furthermore, in this study, we did not assess the effects of chronic administration of drugs as has been done previously (Zhu et al 2000). It is likely that beneficial effects of AT_1 blockers and ACE inhibitors in patients demonstrating LV hypertrophy or heart failure result from chronic effects such as regression of LV hypertrophy (Kohya et al 1995). Taken together with the findings of other authors (Fleetwood et al 1991; Thomas et al 1996; Lee et al 1997; Matsuo et al 1997; Lynch et al 1999), our results suggest that part of the beneficial effects of losartan may be due to acute actions of this drug. However, the molecular mechanism(s) of these actions, and of possible differences to ACE inhibitors, remain speculative at present.

Conclusion

This study in hypertrophied rat hearts demonstrates that neither losartan nor enalaprilat is acutely anti-arrhythmic during ischaemia. During reperfusion, however, losartan, but not enalaprilat, exerts acute anti-arrhythmic effects. Beneficial effects in heart failure patients might thus not only be due to chronic effects but also to acute effects of losartan.

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