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Effects of trimetazidine on myocardial preconditioning in anesthetized rats

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Abstract

Trimetazidine is a widely used anti-ischemic agent, but its effect on myocardial preconditioning in anesthetized animals has not been investigated. The aim of this study was to examine the effects of trimetazidine on ischemic preconditioning and carbachol preconditioning in anesthetized rats. Ischemic preconditioning, induced by 5-min coronary artery occlusion and 5-min reperfusion, decreased the incidence of ventricular tachycardia and abolished the occurrence of ventricular fibrillation during 30-min ischemia. Trimetazidine (10 mg/kg, i.v.) alone attenuated these parameters of arrhythmia. Carbachol infusion induced preconditioning with a marked depression of mean arterial blood pressure, heart rate and ventricular tachycardia. The marked reductions in parameters of arrhythmia induced by ischemic preconditioning and carbachol preconditioning were preserved in the presence of trimetazidine. Arrhythmia scores and myocardial infarct size were significantly reduced with ischemic preconditioning or carbachol preconditioning and were not inhibited by trimetazidine. These results show that trimetazidine protects the heart against ischemia-induced arrhythmias, reduces myocardial infarct size, preserves the effects of ischemic preconditioning and pharmacological preconditioning, and is able to mimic ischemic preconditioning in anesthetized rats.

Keywords: Arrhythmias; Carbachol; Infarct size; Preconditioning; Trimetazidine

1. Introduction

Trimetazidine is an anti-ischemic agent devoid of hemodynamic effects. Trimetazidine selectively inhibits the activity of the final enzyme of the fatty acid β-oxidation pathway, long-chain 3-ketoacyl-coenzyme A thiolase (3-KAT) (Kantor et al., 2000). This leads to a switch in energy substrate preference with a partial inhibition of fatty acid β-oxidation together with an increase in glucose oxidation. Consequently, trimetazidine reduces intracellular acidosis and electrolyte abnormalities (Renaud, 1988; El Banani et al., 2000) by optimizing the oxygen demand of mitochondria (Fantini et al., 1994) and by preventing the decrease in intracellular ATP levels (Lavanchy et al., 1987). Findings from in vitro and ex vivo studies of myocardial ischemia demonstrated that trimetazidine also inhibits the production of free radicals (Maupoil et al., 1990), protects against oxygen-free radical-induced toxicity (Maupoil et al., 1990), inhibits neutrophil infiltration after ischemia and reperfusion (Williams et al., 1993), counteracts calcium overload (El Banani et al., 2000), and reduces the area of necrosis (Drake-Holland et al., 1993; Noble et al., 1995). Trimetazidine protects myocyte structure (Ruiz-Meana et al., 1996) and function (Fantini et al., 1994, 1997), and increases cell resistance to hypoxic stress (Demaison et al., 1995). In isolated rat hearts undergoing ischemia and reperfusion, trimetazidine delays the occurrence of ischemic contracture (Boucher et al., 1994), improves recovery of post-ischemic left ventricular dysfunction (Aussedat et al., 1993), and accelerates the recovery of mitochondrial oxidative phosphorylation and phosphocreatine resynthesis (Allibardi et

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al., 1998). These anti-ischemic effects of trimetazidine could be useful for the treatment of ischemic heart disease, alone (Detry et al., 1994), or in combination with other drugs (McClellan and Plosker, 1999; Szwed et al., 2001). Clinical studies have shown the therapeutic efficacy of trimetazidine in reducing the frequency of anginal attacks and increasing the exercise capacity of patients with angina pectoris (Detry et al., 1994; McClellan and Plosker, 1999).

It has been shown that short periods of ischemia with intermittent reperfusion protect tissue from a subsequent prolonged ischemic insult, defined as ischemic preconditioning (Murry et al., 1986). Although the exact mechanism responsible for preconditioning has not been fully elucidated, a growing body of evidence suggests that endogenous myocardial protective substances such as adenosine, bradykinin, opioids, or reactive oxygen and nitrogen species may play a pivotal role (Yellon and Downey, 2003). Investigations have been directed to finding therapeutic approaches to mimic ischemic preconditioning with pharmacological agents. We tested the hypothesis that trimetazidine is a potential pharmacological agent to mimic cardiac preconditioning. Although trimetazidine significantly reduced the protection afforded by adenosine or ischemic preconditioning in rat isolated hearts (Minners et al., 2000), in another study, administration of trimetazidine induced an increase in adenosine plasma levels in patients, a response which may represent "pharmacological preconditioning" (Blardi et al., 2002). Therefore, the definitive mechanism of action of trimetazidine on ischemic preconditioning has not been determined. The aim of the present study was to investigate the effects of trimetazidine on myocardial preconditioning in anesthetized rats. In addition, results obtained with trimetazidine were compared with those obtained in hearts treated with carbachol.

2. Materials and methods

2.1. Animals and surgical preparation

Male Wistar rats, weighing 220–320 g, were used in this study. Animals were kept in colony rooms with 12-h light/dark cycles at a room temperature of 21±1 °C, and supplied with standard laboratory diet and tap water ad libitum. The investigation conformed with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The study was approved by the local Ethics Committee.

Rats were anesthetized with thiopental sodium (120 mg/kg, i.p., Pental Sodyum, I.E. Ulagay, Istanbul, Turkey) and anesthesia was maintained with supplementary injections (~10 mg/kg, i.v.) of thiopental sodium as required. The rats were intubated and ventilated with room air by means of a small animal ventilator (SAR-830, IITC Life Science, California, USA) with a stroke volume of 15 ml/kg and a rate of 70 strokes/min. A standard limb lead II electrocardiogram (ECG) was continuously monitored and recorded on a computer throughout the experiment, using a computerized data acquisition system (MP30, BIOPAC Systems, California, USA). Body temperature was measured via a rectal probe and maintained at 37±1 °C with a lamp. The left carotid artery was cannulated with a polyethylene PE-50 catheter and connected to a pressure transducer to monitor mean arterial blood pressure. Body temperature and arterial blood pressure were also continuously monitored and recorded throughout the experiment with the same data acquisition system. The left jugular vein was cannulated for the administration of drugs. An infusion pump (74900 series, Cole-Parmer, Illinois, USA) was used for i.v. drug infusion. Rats were given heparin i.v. (200 IU/kg), and then the chest was opened by a left thoracotomy performed between the fourth and the fifth ribs approximately 3 mm from the sternum, the pericardium was incised, and the heart was gently exteriorized by pressure on the abdomen. A loose ligature, 6/0 braided silk suture attached to a 10-mm micro-point reverse cutting needle, was placed around the left anterior descending coronary artery, close to its origin. The heart was immediately replaced in the chest cavity with the ligature ends exteriorized. Both ends of the ligature were then passed through a short piece of polyethylene tube (1 mm i.d. and 15 mm long) to form a snare. Any animal in which this procedure produced dysrhythmia or a sustained fall in mean arterial pressure to less than 60 mm Hg was discarded from the study at this point. Following a stabilization period of 15 min, the snare around the left anterior descending coronary artery was tightened and held in place with a small clip to induce transient regional myocardial ischemia for 30 min. Reperfusion was initiated by releasing the ligature and removing the tube. Successful occlusion was confirmed by a 20–30% reduction in arterial blood pressure compared to the pre-ischemic values. Successful reperfusion was confirmed by the return of arterial blood pressure to pre-ischemic values.

2.2. Measured parameters

For all the groups, heart rate was measured from the electrocardiogram recordings and the incidence of arrhythmias was registered, in accordance with the Lambeth Conventions (Walker et al., 1988), as ventricular tachycardia, ventricular fibrillation, and ventricular ectopic beat. Ventricular ectopic beat is defined as a discrete and identifiable premature QRS complex. Ventricular tachycardia was diagnosed as four or more consecutive ventricular ectopic beats. Ventricular fibrillation was diagnosed when the ECG recording showed chaotic activity with an amplitude less than that of the normal ECG. Complex forms (e.g., bigeminy) were included in the ventricular ectopic beat count and were not analyzed separately. Ventricular fibrillation may be sustained or may revert spontaneously to a normal sinus rhythm in the rat.

Irreversible ventricular fibrillation was defined as ventricular fibrillation which did not reverse within 5 min of onset. The onset and duration of arrhythmia were also measured. The arrhythmia score for these experiments was calculated by using the previously published scale (Demiryurek et al., 2002). The following values were used:

0. 0–50 ventricular ectopic beats with no ventricular tachycardia or ventricular fibrillation over the 30-min ischemia period,
1. 50–500 ventricular ectopic beats only,
2. more than 500 ventricular ectopic beats, or one episode of spontaneously reversible ventricular tachycardia or ventricular fibrillation,
3. spontaneously reversible ventricular tachycardia and/or ventricular fibrillation for 2–30 episodes,
4. spontaneously reversible ventricular tachycardia and/or ventricular fibrillation for more than 30 episodes,
5. occurrence of irreversible ventricular fibrillation.

2.3. Cardiac area at risk and infarct size determination

At the end of experiments, the left anterior descending coronary artery was occluded again at the same site as previously, and 3 ml of a 2% solution of Evans blue dye was infused into the jugular vein catheter to distinguish between perfused and non-perfused (area at risk) sections of the heart (Murry et al., 1986). The Evans blue solution stains the perfused myocardium, while the occluded vascular bed remains uncoloured. Then the heart was excised. Both atria and the roots of the great vessels were removed. The entire ventricle was cut, from the apex to the base, into slices of 3–4 mm, the right ventricular wall was removed, and the area at risk (pink) was separated from the non-ischemic (blue) area. The area at risk was cut into small pieces and incubated with a 1% solution of 2,3,5-triphenyltetrazolium chloride (TTC, in 20 mM phosphate buffer, pH 7.4) stain for 20-min at 37 °C, to visualize the infarct area. The area at risk of infarction was colored brick red, due to the formation of a precipitate that results from the reaction of TTC with dehydrogenase enzymes. The loss of these enzymes from the infarcted myocardium prevents the formation of the precipitate and the infarcted area within the region at risk remains pale yellow (i.e. necrotic area). Pieces were separated according to staining and weighed to determine the infarct size as a percentage of the weight of the area at risk. Area at risk is expressed as a percentage of the left ventricle.

2.4. Experimental protocols

After completion of the surgical procedures, all hearts were allowed to stabilize for 15 min prior to the experimental protocol. These protocols are diagrammatically represented in Fig. 1. In the first group of experiments (protocol 1, control, n=27), hearts were subjected to 30 min of left anterior descending coronary artery occlusion after a 30-min stabilization period. In the second group of experiments (protocol 2, Effects of trimetazidine, n=10), hearts were subjected to trimetazidine at doses of 10 mg/kg, i.v. bolus (Komori et al., 1985; Iskit and Guc, 1996), given 20 min prior to 30-min occlusion. In the third group of experiments (protocol 3, ischemic preconditioning, n=24), hearts were preconditioned against 30-min ischemia by 5-min occlusion and 5-min reperfusion. In protocol 4 (trimetazidine+ischemic preconditioning, n=10), hearts

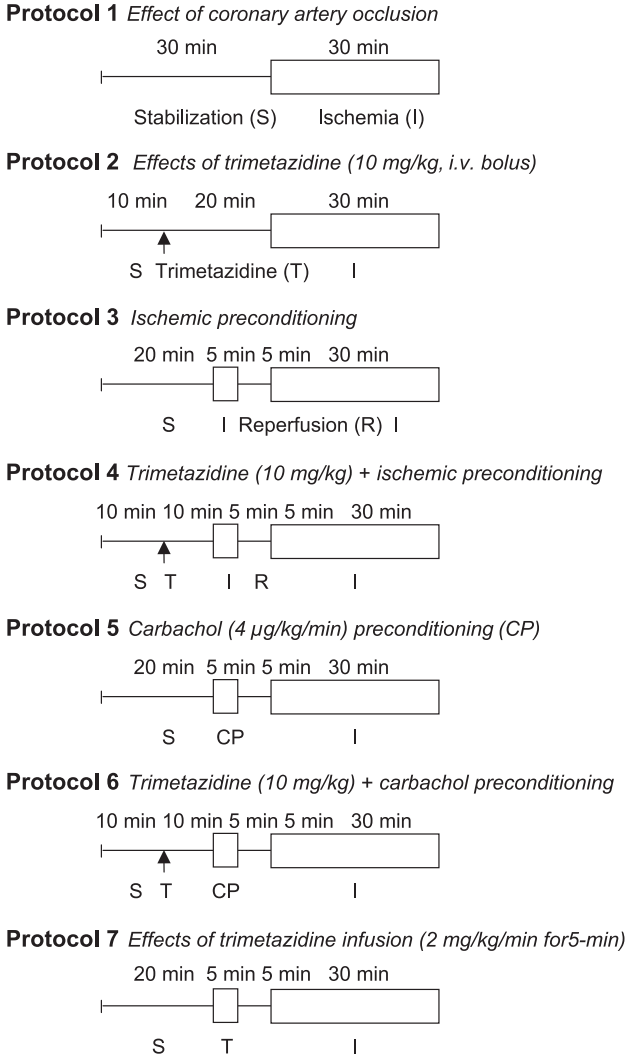

Fig. 1. Experimental protocol for the study. Rat hearts were subjected to 30-min coronary artery occlusion (protocol 1). Hearts were subjected to trimetazidine at the dose of 10 mg/kg 20 min prior to the 30-min occlusion period (protocol 2). Hearts were preconditioned against 30-min ischemia by 5-min occlusion and 5-min reperfusion (protocol 3). Hearts were preconditioned with 5-min ischemia as in protocol 2, but received a bolus of trimetazidine (10 mg/kg) 10 min prior to preconditioning ischemia (protocol 4). Hearts were preconditioned against 30-min ischemia by 5-min carbachol infusion (4 µg/kg/min) (protocol 5). Hearts were preconditioned with 5-min carbachol infusion as in protocol 5, but received a bolus of trimetazidine (10 mg/kg) 10 min prior to carbachol infusion (protocol 6). Hearts were subjected to i.v. infusion of trimetazidine (2 mg/kg/min for 5-min) prior to the 30-min occlusion period (protocol 7).

were preconditioned with 5-min ischemia as in protocol 2, but received an i.v. bolus of trimetazidine (10 mg/kg) 10 min prior to preconditioning ischemia. In the fifth group of experiments (protocol 5, carbachol preconditioning (n=9), hearts were preconditioned against 30-min ischemia by 5-min carbachol infusion (4 μg/kg/min, i.v.). This dose of carbachol has been shown to induce pharmacological preconditioning in rat hearts (Yamaguchi et al., 1997). In the sixth group of experiments (protocol 6, trimetazidine+-carbachol preconditioning, n=10), hearts were preconditioned with 5-min carbachol infusion as in protocol 5, but received an i.v. bolus of trimetazidine (10 mg/kg) 10 min prior to carbachol infusion. For the last series of experiments (protocol 7), the effects of i.v. infusion of trimetazidine on ischemic preconditioning were investigated. In this group of experiments (effects of trimetazidine infusion, n=10), hearts were subjected to 5-min i.v. infusion of trimetazidine (2 mg/kg/min for 5-min) followed by washout and then 30-min occlusion.

2.5. Biochemical analysis

Blood samples were collected at the end of the experiment. The samples were promptly centrifuged at 5000 rpm, 4 °C, for 15 min, and the plasma was removed and stored at −40 °C until assayed.

2.5.1. Measurement of lactate

For lactate measurement, blood samples were collected in tubes containing sodium fluoride/potassium oxalate. Quantitative measurement of lactate concentration in plasma was performed with the Vitros LAC Kit (Ortho-Clinical Diagnostics, Rochester, USA). The lower detection limit of the assay is 0.5 mM.

2.5.2. Assays for cardiac troponin T

Plasma cardiac troponin T levels were determined with the Elecsys Troponin T Kit (Roche Diagnostics, Mannheim, Germany), an automated two-site immunoassay using monoclonal antibodies specific for the cardiac troponin T isoenzyme, using an Elecsys 2010 immunoanalyzer. The lower detection limit of the assay is 0.01 ng/ml.

2.5.3. Measurements of creatine kinase-MB (CK-MB) activity

Plasma CK-MB activity was measured with a CK-MB Kit (Roche Diagnostics), using an autoanalyzer (Roche Hitachi Modular DP Systems, Mannheim, Germany). The lower detection limit of the assay is 5 U/l.

2.5.4. Malondialdehyde measurements

Malondialdehyde was measured in plasma as previously described (Ohkawa et al., 1979). Malondialdehyde generation was evaluated by the assay of thiobarbituric acid-reacting substances (TBARS). In particular, the addition of a solution of 0.2 ml of sodium dodecyl sulfate (8.1%), 1.5 ml of 20% acetic acid solution (pH=3.5), 1.5 ml of 0.8% thiobarbituric acid (pH=3.5), 0.1 ml 90 mM butylated hydroxytoluene (to prevent the formation of TBARS in vitro) (Jentzsch et al., 1996) and 0.6 ml of distilled water produced a chromogenic product which was extracted in n-butanol and pyridine. The organic layer was removed and the malondialdehyde content was read at 532 nm in a spectrophotometer and the results expressed as nmol/ml. The amount of TBARS was calculated as malondialdehyde equivalents using 1,1,3,3-tetramethoxypropane as standard.

2.6. Materials

Trimetazidine dihydrochloride was generously supplied by Servier Pharmaceuticals, France. Evans blue, carbachol, and 2,3,5-triphenyltetrazolium chloride were obtained from Sigma (St. Louis, MO, USA). All the other materials used were of analytical grade and all stock solutions were prepared in non-pyrogenic saline (0.9% NaCl; Eczacý-basý-Baxter, Istanbul, Turkey) immediately before use.

2.7. Statistical analysis

All data are expressed as means±S.E.M. or the percentage incidence. Statistical comparison of more than two groups was performed by a one-way analysis of variance followed by Student–Newman–Keuls multiple comparisons test. A Fisher's exact test was used to detect significant differences in the incidence of ventricular tachycardia, ventricular fibrillation and irreversible ventricular fibrillation between groups. The Mann–Whitney U-test was used to detect significant differences between arrhythmia scores. In all tests, P values less than 0.05 were considered to be statistically significant.

3. Results

3.1. Hemodynamics

Tables 1 and 2 summarize mean arterial blood pressure and heart rate in all groups, respectively. Occlusion of the left anterior descending coronary artery produced a marked decrease in blood pressure, and reperfusion partly restored these values in the control group. Trimetazidine itself had no marked effect on mean arterial blood pressure and heart rate. Although preconditioning ischemia and carbachol infusion produced marked decreases in blood pressure, no significant changes were observed in the presence of trimetazidine. No significant differences were observed in heart rate between groups except in the carbachol-treated groups (Table 2).

3.2. Effects on ischemia-induced arrhythmias

Ischemic preconditioning produced marked antiarrhythmic effects in anesthetized rats, as shown in Table 3.

Table 1
Mean arterial blood pressure (mm Hg) during coronary occlusion in anesthetized rats

	n	Baseline	i.v. bolus trimetazidine		Preconditioning or drug infusion		Reperfusion or no infusion		Occlusion	
			1 min	20 min	1 min	5 min	1 min	5 min	1 min	30 min
Control	27	138±5	–	–	–	–	–	–	108±5[a]	94±5[a]
Trimetazidine (10 mg/kg)	10	151±6	154±7	142±9	–	–	–	–	122±9	105±14[a]
Ischemic preconditioning	24	134±4	–	–	108±3[a]	101±4[a]	118±3[a]	124±3	103±4[a]	97±4[a]
Trimetazidine (10 mg/kg)+ ischemic preconditioning	10	157±5	158±5	153±5[b]	138±5	134±7	132±6	138±6	127±6[a]	111±10[a]
Carbachol preconditioning	9	145±5	–	–	113±8[a]	103±7[a]	109±8[a]	113±5[a]	98±6[a]	87±6[a]
Trimetazidine (10 mg/kg)+ carbachol preconditioning	10	144±4	146±3	142±4[b]	143±4	128±4	131±4	132±3	123±4[a]	106±6[a]
Trimetazidine infusion (2 mg/kg/min for 5 min)	10	139±6	–	–	137±6	135±6	134±6	134±6	121±4	103±8[a]

[a] $P<0.05$ when compared to baseline values.
[b] 10 min after i.v. bolus trimetazidine.

Preconditioning the hearts with 5-min ischemia suppressed arrhythmias during the 30-min occlusion period. The total number of ventricular ectopic beats (36±10, $n=24$, $P<0.05$) was markedly lower than in the controls (622±143, $n=27$). The incidence of ventricular tachycardia was markedly reduced (from 100%, $n=27$, to 25%, $n=24$, $P<0.05$) and no ventricular fibrillation was observed in the ischemic preconditioning group. Carbachol infusion appeared to decrease the severity of arrhythmias, but these differences were not significant. The total number of ventricular ectopic beats and the incidence of ventricular tachycardia and ventricular fibrillation were attenuated with trimetazidine, but these changes were not significant. The ischemic preconditioning-induced reduction in arrhythmia parameters seemed to be potentiated in the presence of trimetazidine (Table 3). In order to examine whether or not trimetazidine itself was able to mimic the effects of preconditioning, trimetazidine was infused for 5-min prior to 30-min ischemia. Trimetazidine infusion suppressed the number of total ventricular ectopic beats (to 171±105, $n=9$), decreased the incidence of ventricular tachycardia (to 30%, $n=3$) and abolished the incidence of ventricular fibrillation. Ventricular fibrillation was not observed in the trimetazidine (10 mg/kg)+carbachol preconditioning group.

The effects of ischemic preconditioning, carbachol preconditioning and trimetazidine on the time of onset of first arrhythmia, duration of ventricular tachycardia and ventricular fibrillation and arrhythmia score are shown in Table 4. The occurrence of the first arrhythmias was markedly delayed in the trimetazidine+ischemic preconditioning and trimetazidine+carbachol preconditioning groups, whereas there were no significant differences in the other groups. Although there was no marked change in the duration of ventricular tachycardia, ventricular fibrillation duration was markedly depressed in the ischemic preconditioning, carbachol preconditioning and trimetazidine-treated groups. The arrhythmia scores decreased markedly. The most marked reductions were observed in the ischemic preconditioning and trimetazidine+ischemic preconditioning groups. The carbachol preconditioning and trimetazidine-treated groups also showed attenuation of the arrhythmia scores, but the effects of trimetazidine were more marked than those of carbachol (Table 4).

Table 2
Heart rate (beats/min) during coronary occlusion and reperfusion in anesthetized rats

	n	Baseline	i.v. bolus trimetazidine		Preconditioning or drug infusion		Reperfusion or no infusion		Occlusion	
			1 min	20 min	1 min	5 min	1 min	5 min	1 min	30 min
Control	27	387±9	–	–	–	–	–	–	377±12	374±15
Trimetazidine (10 mg/kg)	10	357±12	349±18	354±10	–	–	–	–	348±11	338±23
Ischemic preconditioning	24	382±9	–	–	371±7	364±13	377±9	379±7	388±8	365±15
Trimetazidine (10 mg/kg)+ ischemic preconditioning	10	351±12	340±20	353±18[b]	355±22	354±12	377±22	359±11	357±11	362±20
Carbachol preconditioning	9	396±10	–	–	368±11	206±18[a]	218±22[a]	299±13[a]	283±23[a]	303±29[a]
Trimetazidine (10 mg/kg)+ carbachol preconditioning	10	353±8	313±11	348±12[b]	351±13	213±17[a]	225±16[a]	279±16[a]	266±14[a]	236±30[a]
Trimetazidine infusion (2 mg/kg/min for 5 min)	10	367±10	–	–	368±11	371±11	360±12	373±12	372±12	362±22

Values are given as means±S.E.M.
[a] $P<0.05$ significantly different when compared to baseline values.
[b] 10 min after i.v. bolus trimetazidine.

Table 3
Effects of ischemic preconditioning and trimetazidine on the severity of arrhythmia induced by 30-min coronary artery occlusion in anesthetized rats

	n	Total ventricular ectopic beats	%Ventricular tachycardia	%Total ventricular fibrillation	%Irreversible ventricular fibrillation
Control	27	622.4±143.3 (27)	100.0 (27)	40.7 (11)	11.1 (3)
Trimetazidine (10 mg/kg)	10	282.5±160.5 (10)	50.0 (5)	10.0 (1)	0 (0)
Ischemic preconditioning	24	35.5±10.3[a] (19)	25.0[a] (6)	0[a] (0)	0 (0)
Trimetazidine (10 mg/kg)+ ischemic preconditioning	10	17.6±4.5[a] (10)	20.0[a] (2)	0[a] (0)	0 (0)
Carbachol preconditioning	9	288.9±160.9 (9)	66.7 (6)	11.1 (1)	0 (0)
Trimetazidine (10 mg/kg)+ carbachol preconditioning	10	252.2±65.0 (10)	60.0 (6)	0[a] (0)	0 (0)
Trimetazidine infusion (2 mg/kg/min for 5 min)	10	171.2±104.9 (9)	30.0 (3)	0[a] (0)	0 (0)

Numbers in parentheses are the number of hearts that exhibited that particular type of arrhythmia.
[a] $P<0.05$ compared to control group.

3.3. Biochemical analysis

Lactate and malondialdehyde levels were markedly reduced in the ischemic preconditioning group (Fig. 2A–B). Lactate and malondialdehyde levels were also decreased in the trimetazidine+ischemic preconditioning group, but this reduction was not significant. There were no marked changes in lactate and malondialdehyde levels in the other groups. There was a tendency towards a reduction in troponin T levels (Fig. 3A), but these changes did not reach significance. CK-MB levels were not significantly altered in the preconditioning or the trimetazidine-treated groups (Fig. 3B).

3.4. Area at risk and infarct size measurements

No significant differences were noted in the left ventricular area at risk between the groups (Fig. 4). The necrotic area was markedly reduced in the ischemic preconditioning, carbachol preconditioning and trimetazidine-treated groups. Trimetazidine appeared to potentiate the cardioprotective effects of ischemic preconditioning or carbachol preconditioning. Trimetazidine infusion seemed to generate slightly smaller infarct than carbachol infusion.

4. Discussion

The results of the present study show that trimetazidine has an antiarrhythmic effect against coronary artery occlusion-induced arrhythmias and protects hearts from ischemic injury in anesthetized rats. Trimetazidine did not alter the mean arterial blood pressure or heart rate. To our knowledge, this is the first experimental evidence showing that trimetazidine on its own can mimic preconditioning and does not inhibit ischemic preconditioning or carbachol preconditioning in anesthetized rats. In addition to arrhythmias and infarct size, trimetazidine appeared to reduce biochemical parameters in our experiments.

There are only limited numbers of published studies showing the effects of the anti-ischemic drug trimetazidine, a known mitochondrial “protector”, on preconditioning. One study showed trimetazidine to limit the effect of ischemic preconditioning and to completely reverse the drug-induced reduction in infarct size in Langendorff-perfused isolated rat hearts (Minners et al., 2000). In another study, significant increases in plasma adenosine levels after administration of trimetazidine in single oral doses were observed in patients with angina pectoris (Blardi et al., 2002). We observed that

Table 4
Effects of ischemic preconditioning, carbachol preconditioning, and trimetazidine on the time of onset of first arrhythmias, durations of ventricular tachycardia and ventricular fibrillation, and on arrhythmia scores in anesthetized rats

	n	Time of onset of first arrhythmias (s)	Duration of ventricular tachycardia (s)	Duration of ventricular fibrillation (s)	Arrhythmia scores
Control	27	49.2±16.1	72.7±18.7	20.7±4.3	3.3±0.1
Trimetazidine (10 mg/kg)	10	237.0±55.9	50.8±42.9	4[a]	1.7±0.4[a]
Ischemic preconditioning	24	135.6±36.8	7.8±4.3	0[a]	0.6±0.2[a]
Trimetazidine (10 mg/kg)+ ischemic preconditioning	10	511.9±117.4[a,b]	3.0±1.0	0[a]	0.5±0.3[a]
Carbachol preconditioning	9	32.4±12.7	35.4±20.4	37[a]	2.1±0.4[a]
Trimetazidine (10 mg/kg)+ carbachol preconditioning	10	286.8±65.2[a,c]	26.8±5.5	0[a]	2.2±0.3[a]
Trimetazidine infusion (2 mg/kg/min for 5 min)	10	240.6±70.2	49.0±43.0	0[a]	1.1±0.4[a]

[a] $P<0.05$ compared to control group.
[b] $P<0.05$ significantly different when compared to ischemic preconditioning group.
[c] $P<0.05$ compared to carbachol preconditioning.

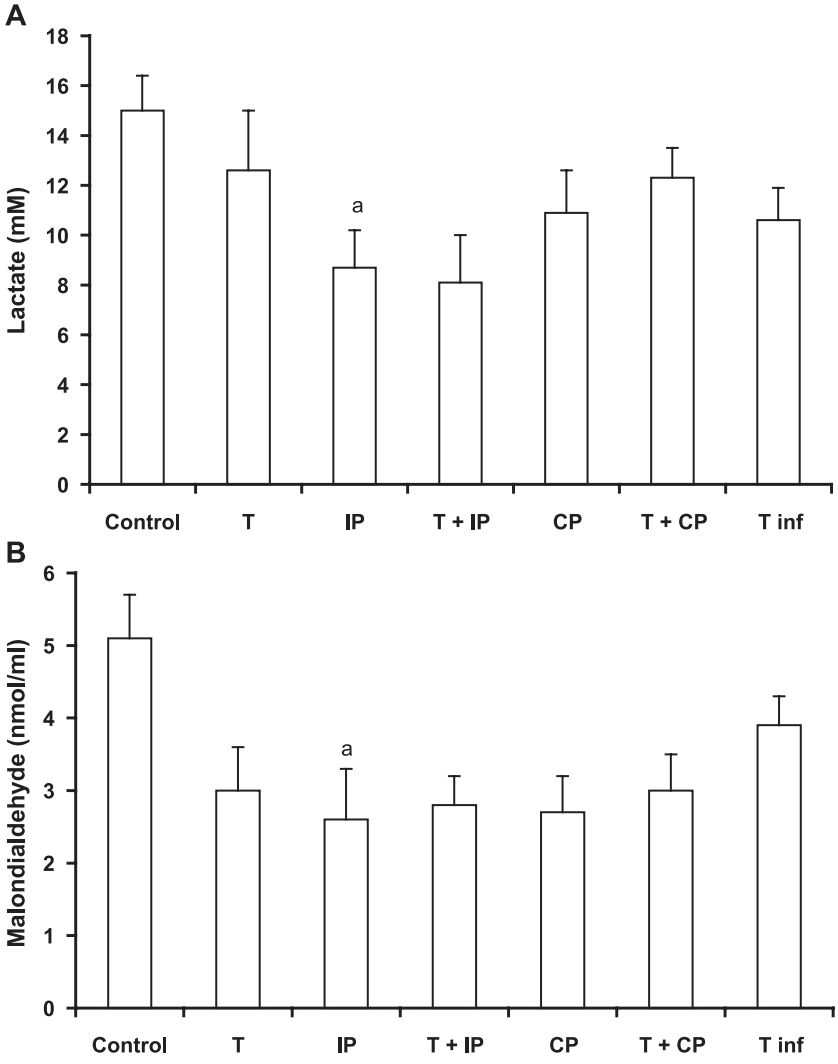

Fig. 2. Effects of trimetazidine on plasma lactate (A) and malondialdehyde (B) levels in anesthetized rats. All values are the means±S.E.M. [a]$P<0.05$ versus control (n=27). IP, ischemic preconditioning (n=24); T, trimetazidine (n=10); T+IP, trimetazidine (10 mg/kg) plus ischemic preconditioning group (n=10); CP, carbachol preconditioning (n=9); T+CP; trimetazidine (10 mg/kg) plus carbachol preconditioning (n=10), T inf, trimetazidine infusion (2 mg/kg/min for 5-min) (n=10).

trimetazidine functions as a preconditioning-mimetic agent and did not diminish the cardioprotective effects of ischemic preconditioning or carbachol preconditioning in anesthetized rats. These results are not in agreement with the data presented by Minners et al. (2000), who showed that short-term administration of 2,4-dinitrophenol, cyclosporin A, and adenosine pretreatment reduced infarct size to a similar to ischemic preconditioning, and that trimetazidine limited the effect of ischemic preconditioning and completely reversed the 2,4-dinitrophenol, cyclosporin A, and the adenosine-mediated reduction in infarct size in isolated rat hearts. The reasons for this discrepancy are not known, but may involve the differences in techniques used, i.e. isolated hearts vs. anesthetized animals. However, our results may support the data presented by Blardi et al. (2002), who reported that the cardioprotective effects of trimetazidine may be mediated, at least in part, by adenosine release. Our results are also in agreement with the studies of Drake-Holland et al. (1993) and Noble et al. (1995), who found that trimetazidine decreased myocardial infarct size in rabbits. Additionally, our results may support studies of rat isolated hearts showing that acute treatment with trimetazidine improves contractile function during ischemia (Lavanchy et al., 1987).

No changes in blood pressure or heart rate were recorded in the present study, which may support the previous observation that this drug has no hemodynamic effect (McClellan and Plosker, 1999). As there were no effects of trimetazidine on coronary flow, contractility, or heart rate in isolated heart studies, our results are also in line with these in vitro findings (Boucher et al., 1994; McClellan and Plosker, 1999; El Banani et al., 2000).

The present study is also first to describe the antiarrhythmic effects of trimetazidine during preconditioning. The antiarrhythmic effects of ischemic preconditioning or carbachol preconditioning were preserved in the presence of trimetazidine. During ischemia, increased lactate and proton

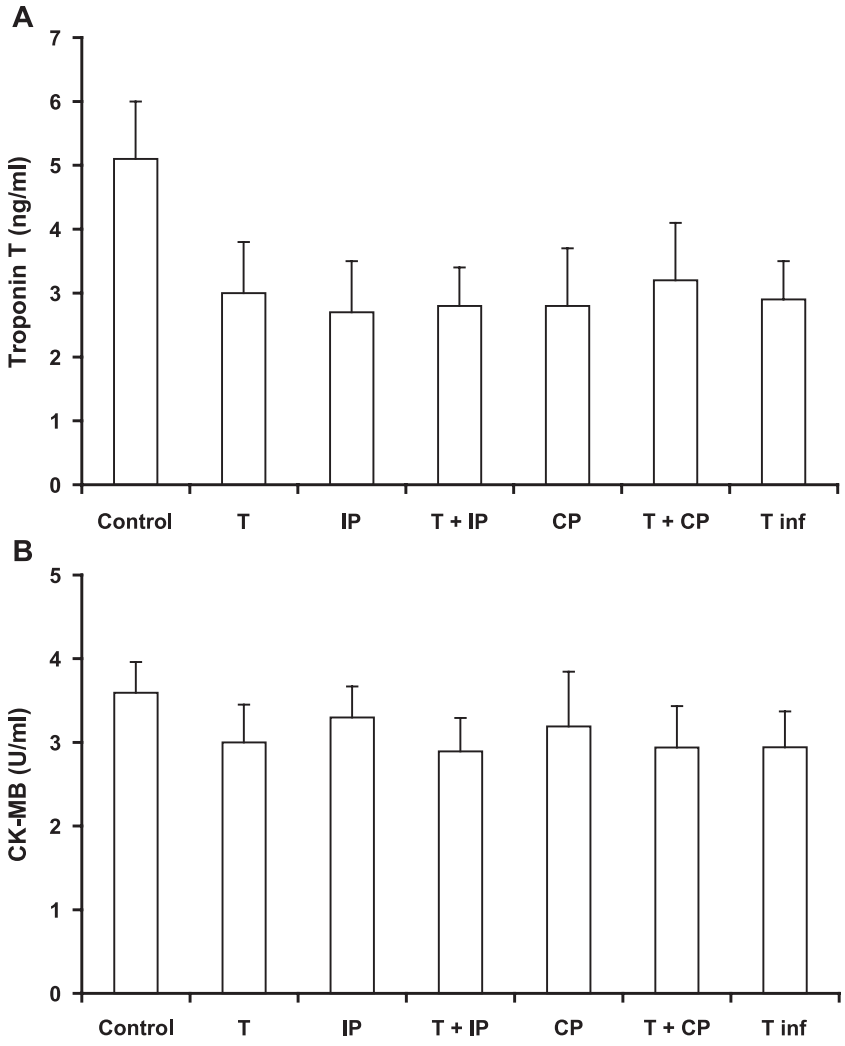

Fig. 3. Effects of trimetazidine on cardiac troponin T levels (A), and plasma creatine kinase MB (CK-MB) activity (B) in anesthetized rats. All values are the means±S.E.M., n=9–27. Abbreviations are as in the legend of Fig. 2.

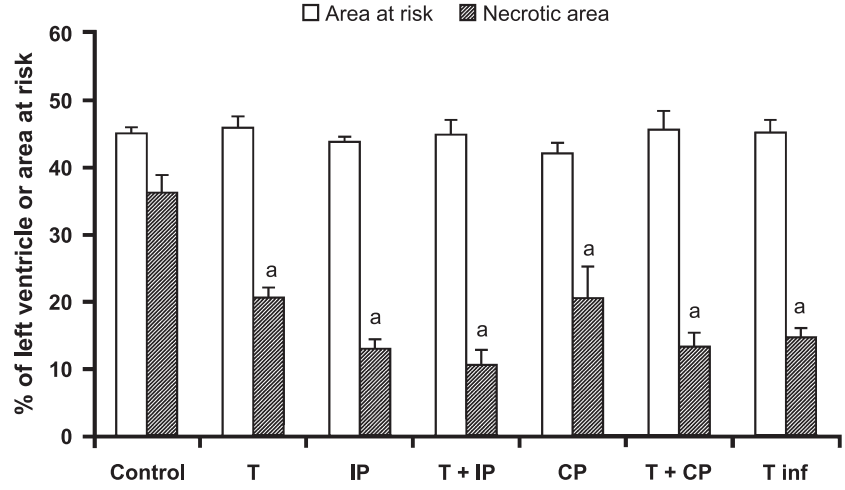

Fig. 4. Effects of trimetazidine on area at risk and infarct size. Area at risk indexed to total left ventricle (area at risk/total left ventricle×100) and necrotic area indexed to area at risk (necrotic area/area at risk×100) in percentage of wet weight. All values are the means±S.E.M., n=9–27. [a]P<0.05 versus control group. Abbreviations are as in the legend of Fig. 2.

content (and hence a fall in intracellular pH) is associated with a reduction in the contractile function of myocardial segments. Cardiac efficiency is decreased because ATP is required not only to support contractile function, but also to restore cellular ionic homeostasis. Furthermore, the accumulation of long-chain fatty acid intermediates during β-oxidation has previously been shown to reduce the ventricular arrhythmia threshold during ischemia (Murnaghan, 1981). Suppression of circulating levels of plasma non-esterified fatty acids, and thus myocardial fatty acid uptake and oxidation, has been shown to reduce ischemic damage and ventricular dysrhythmias during acute myocardial infarction or exercise-induced angina (Rowe et al., 1975; Oliver M.F., 1994). Experimentally, trimetazidine has been shown to decrease ST segment elevation during coronary artery occlusion of rabbit hearts (d'Alche et al., 1991), and is cardioprotective in in vitro models of ischemia (Libersa et al., 1990). Our results may support the finding that trimetazidine is effective against halothane-adrenaline arrhythmias in dogs (Komori et al., 1985).

Despite its cardioprotective properties, the actual mechanisms by which trimetazidine acts have not been completely elucidated. Since the effects of trimetazidine occur in the absence of detectable changes in systemic and coronary hemodynamics, the in vivo effects of trimetazidine on the ischemic myocardium are likely to depend upon direct cytoprotection at the cellular level. Trimetazidine has been reported to exert cardioprotective effects by limiting the fall in the intracellular adenosine triphosphate (ATP) content (Lavanchy et al., 1987; Aussedat et al., 1993), and by preventing intracellular sodium and calcium accumulation and acidosis (Renaud, 1988; El Banani et al., 2000). Others have suggested a membrane stabilizing effect (Fantini et al., 1997) or inhibition of neutrophil accumulation (Williams et al., 1993), and antioxidant activity (Guarnieri and Muscari, 1990; Maupoil et al., 1990) has been reported, but the exact mechanism underlying the beneficial effect of trimetazidine is still largely unknown. It has been proposed that these effects derive from inhibition of the mitochondrial long-chain 3-KAT, and thus inhibition of β-oxidation and re-coupling of glucose oxidation (Kantor et al., 2000).

The mitochondrial permeability transition pore (PTP) functions as a Ca^{2+}-, voltage-, pH-, redox-gated, cyclosporin A-sensitive channel, which is probably located at the contact site between the inner and outer mitochondrial membranes (Susin et al., 1998). Mitochondrial PTP opening is favored by high phosphate concentrations, ATP and ADP depletion, Ca^{2+} overload and oxidative stress, conditions prevailing during ischemia–reperfusion. Cyclosporin A, which is the most potent inhibitor of PTP (Broekemeier et al., 1989), protects cells from ischemia–reperfusion injury. Mitochondrial PTP contributes to cellular injury during ischemia and reperfusion. Inhibition of PTP constitutes a relevant pharmacological target to protect a cell from death. It has been recently proposed that PTP is the end-effector of ischemic preconditioning and that ischemic preconditioning may protect the myocardium by inhibiting PTP opening (Hausenloy et al., 2002; Yellon and Downey, 2003). The cardioprotective mechanism of trimetazidine could be related to direct inhibition of the mitochondrial PTP. The existence of low-affinity [^{3}H]trimetazidine binding sites involved in the regulation of the PTP has recently been reported (Morin et al., 1998; van Balen et al., 2002). Trimetazidine acts on mitochondrial function in at least two different ways, as a mitochondrial Ca^{2+} releaser when the mitochondrial PTP is closed, and by inducing its closure when the pore is open (Morin et al., 1998). The inhibition of mitochondrial PTP opening by trimetazidine may result from the displacement of Ca^{2+} (Morin et al., 2000; van Balen et al., 2002). These results suggest that the mechanism by which trimetazidine protects mitochondria against the deleterious effects of ischemia–reperfusion may involve inhibition of PTP opening.

Under conditions of decreased ischemia-induced intracellular acidification by trimetazidine, the activity of Na^+/H^+ exchange (and possibly of other Na^+-dependent intracellular pH regulating mechanisms) would be attenuated. This may also contribute to preconditioning, since it is known that inhibition of Na^+/H^+exchange produces cardiac preconditioning (Nakai et al., 2002). Several data demonstrate that trimetazidine exerts its action at the ventricular myocyte level by inducing a decrease in the intracellular accumulation of Ca^{2+} and Na^+, and by protecting cardiac cells against the accumulation of H^+ (Renaud, 1988; El Banani et al., 2000).

There was a tendency toward a reduction in troponin T levels in the present study, but these changes did not reach statistical significance. This may be related to the fact that our experimental period was not long enough to detect marked changes, since troponin T levels start to increase a few hours after the onset of myocardial damage and remain increased for several days (Mair et al., 1992). (Fabiani et al., 1992) measured CK-MB, myoglobin, malondialdehyde levels, and hemodynamic variables to detect the cardioprotective effect of trimetazidine in patients who had coronary artery bypass grafting. They found that pretreatment with trimetazidine had a cardioprotective effect but did not improve postoperative hemodynamics. Tunerir et al., (1999) have found significantly lower cardiac troponin T levels in a trimetazidine pretreatment group than in a placebo group during coronary artery bypass grafting. CK-MB levels were detectable in the present study but were not affected by preconditioning or trimetazidine administration, implying that longer experimental periods are needed to detect marked changes.

The effects of trimetazidine on the inhibition of neutrophil infiltration into myocardial tissue after ischemia and reperfusion have been examined in anesthetized rabbits. The number of neutrophils in the area at risk was significantly reduced by i.v. 2.5 mg/kg trimetazidine given 10 min before coronary artery occlusion (Williams et al.,

1993). Pretreatment with trimetazidine at the dose of 3 mg/kg was effective in reducing myocardial infarct size in rabbits (Drake-Holland et al., 1993; Noble et al., 1995), and 10 mg/kg trimetazidine was effective against halothane-adrenaline arrhythmia in dogs (Komori et al., 1985). Trimetazidine at the dose of 10 mg/kg was also tested in an anesthetized rat model of coronary artery occlusion–reperfusion-induced arrhythmias (Iskit and Guc, 1996). We used a high single i.v. dose of trimetazidine (10 mg/kg) in our experiments and found it to be effective in reducing arrhythmias and infarct size.

Trimetazidine may possess transition metal chelating properties. This ability in combination with its lipid barrier-penetrating capacity may allow the binding and inactivation of redox active transition metals associated with lipids in both low-density lipoprotein and cellular membranes (Tselepis et al., 2001). Although trimetazidine is not known to have a direct effect on neutrophils, the number of infiltrating neutrophils was significantly lower in trimetazidine-treated rabbits in a myocardial ischemia–reperfusion model (Williams et al., 1993). Additionally, in a rat intestinal ischemia–reperfusion injury model, decreased myeloperoxidase activity after trimetazidine therapy has been reported (Tetik et al., 1999). These effects of trimetazidine may explain the reduction in malondialdehyde levels in our experiments.

It has been reported that trimetazidine increases in the Ca^{2+} level and enhances ATP synthesis in isolated rat heart mitochondria (Guarnieri et al., 1997), and this increase in ATP levels would tend to keep the mitoK_{ATP} channel closed (Schultz et al., 1997; Minners et al., 2000). However, trimetazidine may have additional effects to mimic preconditioning, such as inhibition of mitochondrial PTP (Morin et al., 1998), release of adenosine (Blardi et al., 2002), increase in the activity of mitochondrial respiratory chain complex I (Monteiro et al., 2003), or possibly inhibition of Na^+/H^+ exchange. Trimetazidine treatment has a favorable effect on heart rate variability parameters, which probably can be explained by an increase in vagal activity. Trimetazidine treatment has been shown to alter the sympatho-vagal balance in favor of vagal activity (Ulgen et al., 2001). Birand et al. (1997) reported that trimetazidine treatment decreased sympathetic activity and increased vagal activity in patients who had undergone a percutaneus transluminal coronary angioplasty procedure. This effect of trimetazidine may also contribute to preconditioning under in vivo conditions.

In conclusion, these results suggest that trimetazidine has the ability to mimic preconditioning in anesthetized rats. Additionally, the results of this study support the concept that by shifting energy substrate preference away from fatty acid metabolism and toward glucose metabolism, trimetazidine, a 3-KAT inhibitor, may be an effective approach to induce or preserve preconditioning. The results of the present study provide new insights into the mechanism of trimetazidine.

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