

Deleterious Effects of Acute Treatment With a Peroxisome Proliferator–Activated Receptor- γ Activator in Myocardial Ischemia and Reperfusion in Pigs

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Thiazolidinediones exert electrophysiologic effects in noncardiac cells in vitro, but to date there have been no reports of effects on cardiac rhythm. We previously demonstrated that chronic pretreatment with a thiazolidinedione peroxisome proliferator-activated receptor (PPAR)- γ activator, troglitazone, improves recovery of left ventricular (LV) function and substrate metabolism after ischemia and reperfusion, without causing arrhythmias. In this study, we determined whether similar salutary effects are achieved with acute treatment with troglitazone. Anesthetized pigs underwent 90 min of regional LV ischemia and 90 min of reperfusion. Fifteen pigs were treated with troglitazone (10 mg/kg load, 5 mg \cdot kg⁻¹ \cdot h⁻¹ infusion i.v.) beginning 1 h before ischemia. Seven pigs received corresponding vehicle. Plasma troglitazone concentration (mean 5 μ g/ml) was similar to that achieved in clinical use of this agent. Before ischemia, acute troglitazone treatment had no effect on LV function, electrocardiogram, or substrate utilization. During ischemia or reperfusion, eight pigs in the troglitazone group died of ventricular fibrillation, compared with no pigs in the vehicle group ($P < 0.05$). Pigs that developed ventricular fibrillation had shorter QT intervals than survivors of either group. Among survivors, neither LV function nor substrate utilization differed between groups. Acute treatment with troglitazone increases susceptibility to ventricular fibrillation during myocardial ischemia and reperfusion. Whether thiazolidinediones have proarrhythmic potential in clinical use requires further investigation. *Diabetes* 52:1187–1194, 2003

Peroxisome proliferator-activated receptors (PPARs) are a family of nuclear receptors that regulate gene transcription, particularly those affecting energy substrate metabolism and inflammation (1,2). Thiazolidinedione drugs activate PPAR- γ

and are used clinically to treat patients with type 2 diabetes (3). Currently, several million patients are treated with these agents worldwide. Although the clinical use of thiazolidinediones in type 2 diabetes is based on effects of these agents in adipose tissue, liver, and skeletal muscle to improve glycemic control, PPAR- γ is also expressed in myocardium (4–6). Considering the prevalence of coronary heart disease among diabetic patients, surprisingly little is known about the effects of PPAR- γ activation in myocardium.

In addition to activation of nuclear PPAR- γ receptors, thiazolidinediones produce immediate (nontranscriptional) effects on ion channel conductances across the cell membrane of vascular smooth muscle cells (7–9). To date, however, cardiac electrophysiologic effects of thiazolidinediones have not been reported.

Our laboratory previously studied the effects of 8 weeks' pretreatment with the thiazolidinedione compound, troglitazone, in low-flow myocardial ischemia and reperfusion in nondiabetic pigs. We found that pretreated pigs had significantly greater recovery of myocardial mechanical function and carbohydrate metabolism after ischemia and reperfusion than untreated pigs (10). There were no sustained ventricular arrhythmias among treated pigs. These findings indicated a beneficial effect of chronic pretreatment with troglitazone in experimental myocardial ischemia and reperfusion. In clinical practice, however, there are inherent limitations to a strategy of chronic, anticipatory pretreatment for episodes of myocardial ischemia that may occur sporadically and unpredictably. If acute treatment with a PPAR- γ activator afforded similar benefits to chronic pretreatment, there might be clinical utility of acute treatment in situations where ischemia is known to occur, such as in acute coronary syndromes or cardiopulmonary bypass. Conversely, if salutary effects of PPAR- γ activation require prolonged drug exposure to alter the expression of target genes (11), acute treatment may be futile. Recent studies using rat models of total coronary occlusion and reperfusion demonstrated reduction of infarct size with either chronic oral pretreatment or acute intravenous treatment with the thiazolidinedione compound, rosiglitazone (12,13). The present study was undertaken to determine whether acute treatment with a thiazolidinedione improves recovery of myocardial me-

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FFA, free fatty acid; LAD, left anterior descending coronary artery; LV, left ventricular; PPAR, peroxisome proliferator-activated receptor.

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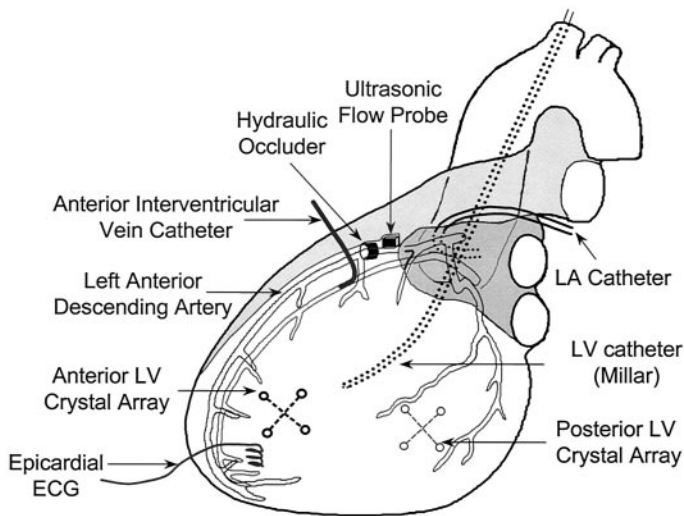


FIG. 1. Instrumentation of the heart.

chanical function and substrate metabolism after low-flow ischemia and reperfusion in pigs.

RESEARCH DESIGN AND METHODS

Surgical preparation and instrumentation. Altogether 22 domestic farm pigs weighing 28 ± 1 kg were studied. Techniques of anesthesia, surgical instrumentation, and physiologic measurements conformed to the *Guide for the Care and Use of Laboratory Animals* of the U.S. National Institutes of Health and were similar to those used in our previous study of chronic troglitazone pretreatment (10). After an overnight fast, pigs were sedated with ketamine HCl (25 mg/kg i.m.), anesthetized with α -chloralose (100 mg/kg i.v. induction, $30 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ i.v. maintenance), and mechanically ventilated with oxygen-enriched air. Arterial blood glucose was monitored every 15 min and an intravenous infusion of 10% glucose adjusted (14) to maintain arterial blood glucose at 4 mmol/l. Pigs were treated with propranolol (1 mg/kg i.v.) and atropine (0.2 mg/kg i.v.) to prevent activation of autonomic reflexes and with indomethacin (2 mg i.v.) to prevent hemodynamic response to the injection of fluorescent microspheres in dilute Tween vehicle.

The instrumentation of the heart is shown in Fig. 1. Micromanometer catheters (Millar, Houston, TX) were inserted in the aortic arch and left ventricle via the carotid arteries. A fluid-filled catheter was inserted in the left atrium. A hydraulic occluder (In Vivo Metrics, Healdsburg, CA) and transit-time ultrasonic flow probe (Transonic Systems, Ithaca, NY) were placed around the left anterior descending coronary artery (LAD) just distal to the first diagonal branch. A catheter was inserted in the anterior interventricular coronary vein to sample effluent blood from the ischemic region. Arrays of four ultrasonic crystals (two orthogonal pairs) were implanted in the anterior and posterior left ventricular (LV) subendocardium (ischemic and nonischemic regions, respectively). The crystals were connected to a digital sonomicrometer (Sonometrics, London, Canada) to measure regional LV wall area (a two-dimensional analog of segment length) and LV pressure versus wall area loops. A hydraulic occluder was placed around the inferior vena cava to vary cardiac preload. A bipolar pacing electrode was affixed to the left atrial appendage to maintain a heart rate slightly higher than the spontaneous rate. A screw-in unipolar electrode was affixed to the epicardial surface of the ischemic region to measure the regional myocardial electrogram.

Treatment groups. Altogether 15 pigs were treated with intravenous troglitazone. Crystalline troglitazone (Sankyo Pharmaceutical Research, Tokyo, Japan) was dissolved in an aqueous solution of 50% polyethylene glycol-400 and 0.15 mol/l NaHCO_3 to a concentration of 10 mg/ml (25 mmol/l). Beginning 1 h before ischemia, a loading dose of troglitazone (10 mg/kg i.v.) was given over 15 min, followed by a continuous infusion ($5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ i.v.) for the remaining duration of the experiment (total dose 30 mg/kg). This dose of troglitazone was chosen to achieve plasma concentrations similar to those achieved with chronic, oral troglitazone treatment in our previous experiments and to those achieved in previous clinical use of troglitazone. The intravenous route of administration was chosen to maintain constant plasma concentrations during the experiment. Seven pigs served as controls and received the corresponding volume of vehicle without troglitazone.

Ischemia/reperfusion protocol. Measurements of hemodynamics, regional LV function, myocardial blood flow, substrate uptake, and electrogram were

made under baseline conditions, after 60 min of preischemic treatment with troglitazone or its vehicle, at the end of 90 min ischemia, and at the end of 90 min of reperfusion. Regional ischemia was produced during continuous monitoring of LAD flow. The LAD occluder was constricted using a microsyringe (Gilmont Instruments) until LAD flow was reduced to 50% of baseline. Continuous fine manual adjustment of the microsyringe/occluder system ensured that LAD flow did not vary by >1 ml/min during the 90-min ischemic period. This severity and duration of ischemia results in myocardial stunning without infarction (15–17). Lidocaine (0.6 mg/kg i.v.) was given every 30 min during ischemia and before reperfusion. Selected 10-s intervals of data (electrocardiogram recorded from the epimyocardial electrode, hemodynamic and sonomicrometry data, and phasic coronary flow) were digitized at 200 Hz, analyzed using custom software, and stored in a personal computer. After conclusion of the in vivo protocol and euthanasia, myocardium was excised for measurements of regional blood flow, expression of PPAR- γ mRNA, and troglitazone concentration.

Assessment of LV function. Regional LV wall area was defined as the instantaneous product of the orthogonal crystal pair separations (i.e., the area subtended by four sonomicrometry crystals). LV pressure versus wall area loops were recorded in both ischemic and nonischemic regions during brief suspension of mechanical ventilation. Three measures of regional systolic function were calculated (18,19). 1) Fractional systolic wall area reduction, a 2-dimensional analog to segment shortening in one dimension or ejection fraction in three dimensions, was calculated as the difference between end-diastolic and end-systolic wall area, divided by end-diastolic wall area. This measure is sensitive to changes in both preload and afterload. 2) Regional external work was calculated as the area of LV pressure versus wall area loops under prevailing loading conditions. This measure is sensitive to changes in preload (end-diastolic wall area), which generally increases during the course of an experiment. 3) Therefore, a load-insensitive measure of regional systolic function, preload-adjusted regional external work was calculated as follows: Regional Frank-Starling relations were derived from recordings of LV pressure versus wall area loops were during brief occlusion of the inferior vena cava (10). Under each experimental condition, preload-adjusted regional external work was calculated from the Frank-Starling relation for that condition with end-diastolic wall area set to its baseline, steady-state value. This is illustrated in Fig. 2. Preload-adjusted regional work does not indicate actual work performed by the region under prevailing loading conditions but provides a basis for comparing intrinsic myocardial function among pigs or groups of pigs at comparable loading conditions. In some cases, the calculated value of preload-adjusted regional external work may be negative during ischemia or reperfusion, but this does not necessarily imply that negative work was performed under the prevailing loading conditions. Rather, it indicates that contractile dysfunction was so severe that positive work would not be performed if loading conditions were set back to baseline.

In each pig, steady-state and preload-adjusted external work are expressed as fractions of their baseline values. The reason for normalization is that the absolute value of external work depends on the distance between ultrasonic crystals at implantation (i.e., a source of experimental rather than physiologic variability among pigs) and is therefore not physiologically meaningful.

Regional lusitropic function was assessed by the maximum rate of regional wall area expansion in early diastole ($\text{dA}/\text{dt}_{\text{max}}$), also normalized to the baseline value in each pig. Global systolic and lusitropic function were assessed from maximum positive and negative derivatives of LV pressure with respect to time ($+\text{dP}/\text{dt}_{\text{max}}$ and $-\text{dP}/\text{dt}_{\text{max}}$).

Myocardial blood flow, substrate uptake, and oxygen consumption. Under each experimental condition, measurement of regional transmural myocardial blood flow was performed using fluorescent microspheres, as described previously (16). Myocardial extraction of glucose, lactate, free fatty acids (FFAs), and oxygen was determined from paired arterial and coronary venous blood samples. Glucose and lactate were measured with an autoanalyzer (YSI, Yellow Springs, OH). FFAs were measured using a modification of the method of Ko and Royer (20). Myocardial substrate or oxygen uptake in the anterior LV was calculated as the coronary arteriovenous concentration difference multiplied by the mean transmural blood flow. Plasma insulin was measured by radioimmunoassay (Diagnostic Products, Los Angeles, CA). Plasma and myocardial troglitazone concentrations were determined before ischemia, at the end of ischemia, and at the end of reperfusion using previously published methods (21).

Expression of PPAR- γ in porcine myocardium. Expression of PPAR- γ in porcine myocardium was demonstrated by ribonuclease protection assay using a published nucleotide sequence (6). A cDNA construct for porcine PPAR- γ was provided by Dr. Stefan Neuenschwander of the Swiss Federal Institute of Technology, Zürich. Myocardial homogenates were prepared from frozen specimens of subendocardium from the nonischemic region of each LV. Total RNA (20 μg) was hybridized with PPAR- γ 1 riboprobe (5'-end) at 42°C

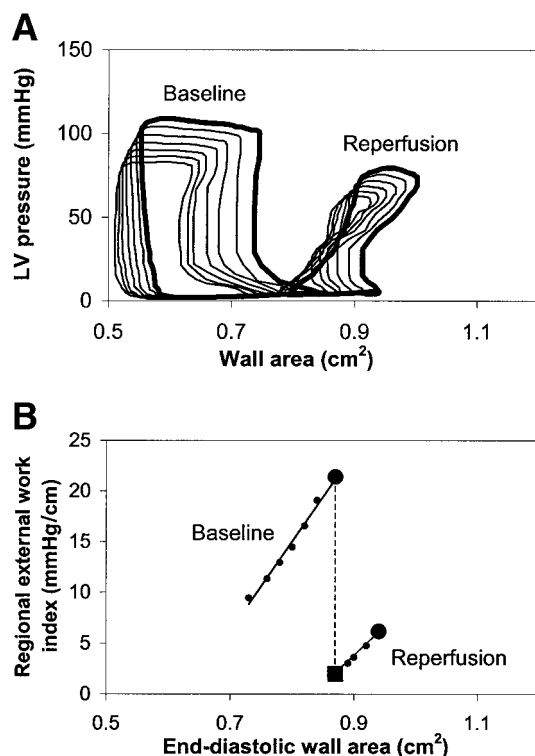


FIG. 2. Assessment of regional LV function after ischemia and reperfusion. **A:** LV pressure versus wall area loops. Bold loops were recorded under steady-state conditions (without occlusion of the vena cava). Fine loops were recorded consecutively during brief occlusion of the inferior vena cava to reduce preload. The area of each loop is a measure of regional external work in that cardiac cycle. End-diastolic wall area is the regional preload. **B:** Frank-Starling relations derived from the data in the top panel by plotting the area of each loop against its end-diastolic wall area. In the top panel, loops recorded after reperfusion is shifted rightward and reduced in area, indicating reduced regional external work, compared with loops recorded at baseline. In the bottom panels, Frank-Starling relations are shifted downward and to the right after reperfusion, indicating reduced external work at any given preload. Large filled circles indicate external work under steady-state conditions (without occlusion of the vena cava). Filled squares indicate preload-adjusted external work (see text). Preload-adjusted external work is a construct that allows assessment of regional LV function independent of changes in loading conditions between baseline and reperfusion. It is calculated using the Frank-Starling relation after reperfusion and the preload (end-diastolic wall area) that prevailed at baseline, as indicated by the dashed line.

overnight. Digestion was carried out using 1/100 ribonuclease A+T1. Protected fragment specific corresponding to PPAR- γ 1 and PPAR- γ 2 isoforms were identified. Expression of PPAR- γ was quantified by densitometry and normalized to the expression of a housekeeping gene, GAPDH.

Statistical analysis. Data are expressed as the mean \pm SE. Within groups, changes in a variable from baseline were assessed using one-way ANOVA for repeated measures, followed by Dunnett's procedure. Between groups, comparison of the response of a variable to ischemia and reperfusion was assessed using two-way ANOVA for repeated measures. The incidence of ventricular fibrillation was compared using the Fisher exact test. The significance level was defined as $P < 0.05$.

RESULTS

Expression of PPAR- γ . Ribonuclease protection assays demonstrated PPAR- γ -specific bands in myocardium from each heart examined. Fig. 3 shows representative results from two hearts. Both isoforms of PPAR- γ were expressed, with predominance of PPAR- γ 1. Acute treatment with troglitazone did not significantly alter expression of either isoform. Expression of PPAR- γ 1 (normalized to GAPDH) was 0.021 ± 0.008 and 0.048 ± 0.012 in vehicle

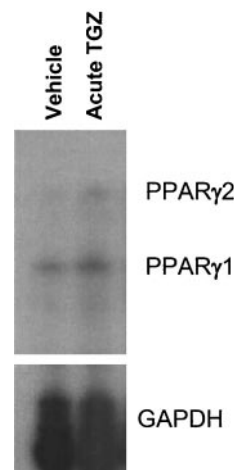


FIG. 3. Expression of PPAR- γ mRNA in porcine myocardium. Ribonuclease protection assay of LV myocardium from a pig in the vehicle group (left lane) and a pig from the acute troglitazone group (right lane). In both cases, protected fragments corresponding to PPAR- γ 1 and PPAR- γ 2 isoforms are observed, with predominant expression of PPAR- γ 1. In pooled group data, there was no significant effect of troglitazone treatment on expression of PPAR- γ .

and troglitazone groups, respectively ($P = 0.10$); expression of PPAR- γ 2 was 0.010 ± 0.003 and 0.014 ± 0.005 , respectively ($P = 0.52$).

Ventricular arrhythmia and epimyocardial electrogram. Ventricular fibrillation occurred in 8 of 15 pigs in the troglitazone group (during ischemia in 1 pig and during reperfusion in 7 pigs). In contrast, ventricular fibrillation occurred in none of seven pigs in the vehicle group ($P < 0.05$). The unexpected finding of increased arrhythmic death with acute intravenous troglitazone treatment contrasts with findings of our previous studies in which none of 12 pigs pretreated chronically with troglitazone ($75 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ orally for 8 weeks) and 3 of 20 untreated pigs suffered ventricular fibrillation during ischemia or reperfusion (Fig. 4).

There were no differences between vehicle and acute troglitazone groups in heart rate or QRS duration measured from epimyocardial electrograms. QT intervals measured in the ischemic zone are shown in Table 1. The QT interval in nonsurvivors of the troglitazone group was shorter than that in the survivors of both groups, both under baseline conditions (286 ± 19 vs. 326 ± 10 ms; $P = 0.08$) and during ischemia (279 ± 19 vs. 335 ± 10 ms; $P = 0.02$). These differences persisted when the QT was corrected for heart rate.

Plasma and myocardial troglitazone concentrations. Data in this and following sections of the results pertain to pigs that survived the ischemia/reperfusion protocol without ventricular fibrillation ($n = 7$ each in group). In the troglitazone group, plasma troglitazone concentration averaged $5.3 \mu\text{g/ml}$ ($6.4 \pm 0.7 \mu\text{g/ml}$ before ischemia, $5.1 \pm 0.4 \mu\text{g/ml}$ at the end of ischemia, and $4.4 \pm 0.8 \mu\text{g/ml}$ at the end of reperfusion). In our previous studies of pigs treated chronically with troglitazone ($75 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ orally), trough and peak plasma troglitazone concentrations were 1.6 ± 0.6 and $8.0 \pm 3.0 \mu\text{g/ml}$. Thus, plasma troglitazone concentrations achieved with acute, intravenous administration were intermediate between the trough and peak levels obtained with chronic oral administration. Myocar-

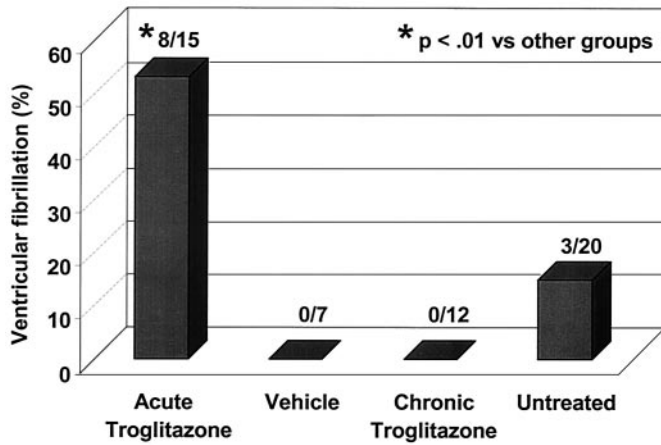


FIG. 4. Incidence of ventricular fibrillation during ischemia or reperfusion. Data from the present experiments (vehicle and acute troglitazone groups) are compared with historical data using the same experimental model in pigs that were pretreated chronically with troglitazone or received no treatment before ischemia and reperfusion. Compared with all other groups, acute troglitazone treatment resulted in a significant excess of ventricular fibrillation.

dial troglitazone concentration was $7.5 \pm 1.2 \mu\text{g/g}$ wet weight at the conclusion of the ischemia/reperfusion protocol in the present study. This compares with a myocardial troglitazone concentration of $2.3 \pm 1.1 \mu\text{g/g}$ wet weight in pigs that received chronic, oral treatment.

Myocardial blood flow. There were no significant differences between groups in LAD blood flow (measured by flow probe) or myocardial blood flow (measured by the microsphere method) under any experimental condition (Table 2). Neither acute treatment with troglitazone nor its vehicle had an effect on LAD or myocardial blood flow. During ischemia, the extent of blood flow reduction was similar in both groups and with reperfusion blood flow recovered to a similar degree in both groups.

Hemodynamics and regional left ventricular function. There were no significant differences between groups in any measured hemodynamic variable or index of regional LV systolic or diastolic function under any experimental condition (Table 3 and Fig. 5). During preischemic treatment, there were no effects of intravenous troglitazone or its vehicle on any measured variable. During ischemia, there was similarly severe impairment of regional LV systolic function in both groups, paralleled by reductions in measures of regional lusitropic function (dA/dt_{max}) and global contractile and lusitropic function ($LV + dP/dt_{\text{max}}$ and $-dP/dt_{\text{max}}$). With reperfusion, systolic function deteriorated further and to a similar extent in both groups. Compared with baseline, LV pressure versus wall area loops after reperfusion were reduced in size and shifted to the right, while regional Frank Starling relations were shifted downward and to the right (Fig. 2). Regional external work recovered to only 0.18 ± 0.06 times baseline in the vehicle group and to only 0.16 ± 0.07 times baseline in the troglitazone group (Fig. 5). Reperfusion also caused further deterioration in indexes of regional and global lusitropic function (dA/dt_{max} and $LV - dp/dt_{\text{max}}$, respectively). **Myocardial substrate metabolism.** Table 4 shows metabolic data for each group of pigs. At baseline, the groups were closely balanced. There were no significant differences among groups in plasma insulin concentration,

TABLE 1
QT interval

	Vehicle survivors ($n = 7$)			Troglitazone survivors ($n = 7$)			Troglitazone nonsurvivors ($n = 8$)					
	Baseline	Pre-ischemic treatment	Ischemia	Reperfusion	Baseline	Pre-ischemic treatment	Ischemia	Reperfusion	Baseline	Pre-ischemic treatment	Ischemia	Reperfusion
Heart rate (min^{-1})	125 ± 2	126 ± 2	126 ± 2	128 ± 3	124 ± 2	128 ± 2	128 ± 2	131 ± 3	127 ± 2	129 ± 2	137 ± 8	N/A
QT interval (ms)	321 ± 14	325 ± 13	338 ± 12	335 ± 14	330 ± 15	323 ± 14	333 ± 13	313 ± 15	$286 \pm 19^\dagger$	$288 \pm 19^\dagger$	$279 \pm 22^*$	N/A
Corrected QT interval (ms)	464 ± 18	470 ± 17	489 ± 17	487 ± 17	474 ± 17	470 ± 15	484 ± 23	461 ± 25	$416 \pm 30^\dagger$	$428 \pm 34^\dagger$	$420 \pm 41^*$	N/A

Data are means \pm SE. QT interval is corrected by dividing by the square root of the cycle length in seconds. * $P < 0.05$ vs. survivors of both vehicle and troglitazone groups; $^\dagger P < 0.10$ vs. survivors of both vehicle and troglitazone groups.

TABLE 2
LAD Flow and regional myocardial blood flow

	Vehicle	Troglitazone
LAD flow (ml/min)		
Baseline	22 ± 2	23 ± 2
Preischemic treatment	23 ± 1	25 ± 3
Ischemia	12 ± 1*	13 ± 1*
Reperfusion	23 ± 5	19 ± 2
Anterior LV (ischemic region) subendocardial blood flow (ml · g ⁻¹ · min ⁻¹)		
Baseline	1.01 ± 0.10	1.09 ± 0.15
Preischemic treatment	0.96 ± 0.03	1.17 ± 0.20
Ischemia	0.23 ± 0.05*	0.23 ± 0.05*
Reperfusion	0.81 ± 0.19	0.71 ± 0.07*
Anterior LV (ischemic region) mean transmural blood flow (ml · g ⁻¹ · min ⁻¹)		
Baseline	1.09 ± 0.08	1.11 ± 0.11
Preischemic treatment	1.11 ± 0.06	1.15 ± 0.14
Ischemia	0.43 ± 0.03*	0.43 ± 0.04*
Reperfusion	0.82 ± 0.13*	0.79 ± 0.05*
Posterior LV (non-ischemic region) mean transmural blood flow (ml · g ⁻¹ · min ⁻¹)		
Baseline	1.35 ± 0.11	1.37 ± 0.22
Preischemic treatment	1.27 ± 0.10	1.44 ± 0.21
Ischemia	1.15 ± 0.13	1.43 ± 0.26
Reperfusion	1.19 ± 0.15	1.28 ± 0.21

Data are means ± SE. *n* = 7 in each group. **P* < 0.05 vs. baseline in same group; there were no significant differences between groups.

arterial substrate concentrations, myocardial substrate uptake, or oxygen consumption. Acute treatment with troglitazone or its vehicle produced no discernible effects on circulating insulin or substrate concentrations, myocardial substrate uptake, or oxygen consumption. As expected, ischemia decreased myocardial oxygen consumption and increased transmural glucose extraction and lactate release, again without differences between groups. With reperfusion, myocardial lactate uptake and the ratio of lactate uptake to glucose uptake remained significantly depressed compared with baseline. These findings indicate a persistent impairment of carbohydrate oxidation despite restoration of oxygen delivery, one of the metabolic signatures of reperfused myocardium (22). Notably, our previous studies showed that chronic troglitazone pretreatment corrected this abnormality of substrate utilization after reperfusion (10), but there was no evidence of a similar effect of acute troglitazone treatment in the present study.

DISCUSSION

This study shows that acute treatment with the thiazolidinedione compound, troglitazone, does not preserve contractile function or energy metabolism during myocardial ischemia and reperfusion and, in fact, markedly increases the likelihood of ventricular fibrillation in an anesthetized porcine model. These results are in direct contrast to the beneficial effects on contractile function and energy metabolism and the absence of pro-arrhythmic effects observed after chronic pretreatment with troglita-

zone in the same model (10). The present findings also contrast with protective effects of either acute or chronic rosiglitazone treatment in myocardial ischemia/reperfusion in rats. (12,13) What mechanism(s) underlie the disparate findings of these studies?

Neither the absence of protective effects nor the increased risk of ventricular fibrillation with acute troglitazone treatment is explained by plasma or myocardial troglitazone concentrations of the drug, since these were similar to concentrations achieved in our previous experiments with chronic troglitazone treatment. It is important to note that myocardial troglitazone concentration in the present experiments (mean 7.5 μg/g) was measured immediately after intravenous treatment, while myocardial troglitazone concentration in experiments with chronic oral treatment (mean 2.3 μg/g) was a trough level measured at the end of a 24-h dosing interval. Considering that we measured a fivefold difference between peak and trough plasma concentrations with chronic oral treatment and that distribution of troglitazone between plasma and myocardium is rapid and complete (23), it is likely that peak myocardial troglitazone concentration during chronic treatment was similar to the level in the present experiments.

While plasma and tissue concentrations of troglitazone do not explain the disparate effects of acute and chronic treatment, duration of exposure may be a pivotal factor. The demonstration of PPAR-γ expression in porcine myocardium fulfills a necessary condition for the specific action of a PPAR-γ activator to alter expression of PPAR-γ-responsive genes. Evidence of transcriptional effects of chronic troglitazone treatment include our finding of increased content of glucose transport proteins in myocardium of treated pigs (24). Transcriptional regulation of genes that are protective in myocardial ischemia and reperfusion may explain the benefit of chronic pretreatment with troglitazone. In contrast, acute treatment may not afford sufficient time to achieve protective transcriptional effects of PPAR-γ activation. On the other hand, proarrhythmic effects of troglitazone in myocardial ischemia/reperfusion were observed after acute administration of the drug but not after chronic pretreatment, as discussed below.

The present studies are the first report of proarrhythmic effects of thiazolidinedione compounds. While this is a sentinel observation in vivo, it may not be surprising given electrophysiologic effects of these compounds that have been demonstrated in vitro. Similar to ligands of other nuclear receptors, such as steroid hormones (25), thiazolidinediones may exert immediate, nontranscriptional effects at the plasma membrane. In vascular smooth muscle and other excitable cells, immediate inhibition of several ion channels has been demonstrated in response to exposure to thiazolidinediones in general and troglitazone in particular, at concentrations similar to those measured in the myocardium in the present experiments. Inhibition of voltage-gated calcium and potassium channels, calcium-activated potassium channels, and ATP-sensitive potassium channels has been demonstrated (7–9,26–28). Although analogous electrophysiologic studies have not been performed in cardiac myocytes, effects on one or more of these ion channels may have contributed to the

TABLE 3
Systemic hemodynamics and regional LV function

	Baseline		Pre-ischemic treatment		Ischemia		Reperfusion	
	Vehicle	Troglitazone	Vehicle	Troglitazone	Vehicle	Troglitazone	Vehicle	Troglitazone
Systemic hemodynamics								
Heart rate (min^{-1})	125 \pm 2	125 \pm 3	126 \pm 2	128 \pm 2	126 \pm 2	128 \pm 2	128 \pm 3	131 \pm 2
Mean aortic pressure (mmHg)	89 \pm 4	88 \pm 4	87 \pm 7	90 \pm 4	78 \pm 5	89 \pm 5	78 \pm 6	74 \pm 5*
Mean left atrial pressure (mmHg)	6 \pm 3	6 \pm 2	6 \pm 2	7 \pm 2	5 \pm 1	6 \pm 2	6 \pm 1	7 \pm 1
LV systolic pressure (mmHg)	104 \pm 4	102 \pm 3	102 \pm 6	104 \pm 5	93 \pm 4	99 \pm 7	90 \pm 5*	86 \pm 6*
LV end diastolic pressure (mmHg)	6 \pm 1	5 \pm 1	5 \pm 1	8 \pm 2	8 \pm 1	13 \pm 3*	10 \pm 2*	12 \pm 2*
LV +dP/dt _{max} (mmHg/s)	1,762 \pm 176	1,463 \pm 150	1,636 \pm 134	1,389 \pm 153	1,210 \pm 55*	1,169 \pm 129*	1,117 \pm 70*	925 \pm 85*
fraction of baseline			0.95 \pm 0.03	0.94 \pm 0.03	0.72 \pm 0.06*	0.80 \pm 0.03*	0.65 \pm 0.04*	0.65 \pm 0.05*
LV -dP/dt _{max} (mmHg/s)	-2,200 \pm 92	-1,979 \pm 215	-2,086 \pm 157	-1,966 \pm 168	-1,460 \pm 82*	-1,469 \pm 118*	-1,256 \pm 77*	-1,208 \pm 130*
fraction of baseline			0.95 \pm 0.06	1.02 \pm 0.04	0.67 \pm 0.04*	0.78 \pm 0.07*	0.58 \pm 0.04*	0.65 \pm 0.08*
Regional LV function								
Regional systolic function								
Fractional systolic wall area reduction	0.28 \pm 0.04	0.27 \pm 0.03	0.28 \pm 0.04	0.27 \pm 0.04	0.03 \pm 0.02*	0.01 \pm 0.02*	0.02 \pm 0.02*	0.01 \pm 0.01*
External work (fraction of baseline)			0.98 \pm 0.07	1.06 \pm 0.07	0.32 \pm 0.04*	0.32 \pm 0.07*	0.18 \pm 0.06*	0.16 \pm 0.07*
Preload-adjusted external work (fraction of baseline)			0.98 \pm 0.08	0.92 \pm 0.08	0.08 \pm 0.11*	-0.06 \pm 0.15*	-0.01 \pm 0.13*	-0.27 \pm 0.17*
Regional diastolic function								
Maximum dA/dt (fraction of baseline)			1.07 \pm 0.08	1.10 \pm 0.08	0.84 \pm 0.07*	0.70 \pm 0.11*	0.64 \pm 0.07*	0.51 \pm 0.08*

Data are means \pm SE. dP/dt, first derivative of LV pressure with respect to time; dA/dt, first derivative of regional wall area with respect to time. All measurements in anterior (ischemic) region of left ventricle. $n = 7$ in each group; * $P < 0.05$ vs. baseline in same group; there were no significant differences between groups. External work is measured at the prevailing regional preload (end-diastolic wall area) under each condition. Preload-adjusted regional external work is a construct to assess intrinsic cardiac function independent of prevailing loading conditions. In each pig under each experimental condition, preload-adjusted regional external work is determined from the regional Frank-Starling relation for that condition and regional preload that prevailed under baseline conditions.

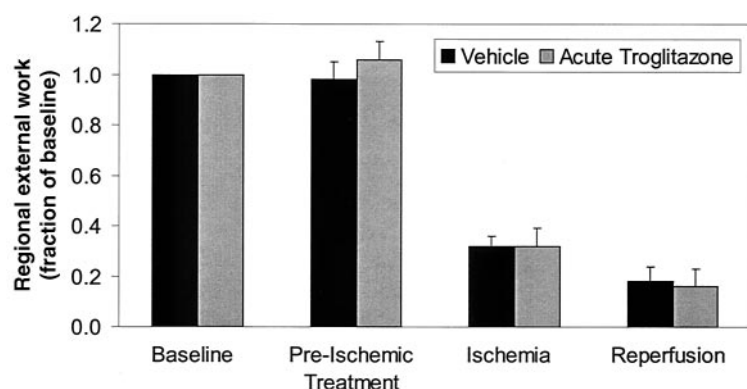


FIG. 5. Regional external work after ischemia and reperfusion. Data are shown for the pigs that survived the protocol of 90 min regional ischemia and 90 min reperfusion ($n = 7$ in each group, mean \pm SE). There was significant reduction of external work during ischemia and reperfusion, but no significant differences in the responses of the two groups.

proarrhythmic effects of acute troglitazone treatment in the present study. In contrast, we observed no proarrhythmic effects in ischemia/reperfusion after chronic pretreatment with troglitazone. This dichotomy is unexplained; however, precedent exists for compensatory upregulation of ion channels in response to chronic exposure to inhibitory ligands (29,30).

The pro-arrhythmic response to acute troglitazone treatment was an unexpected finding of the present investigation, therefore limited electrophysiologic data were collected. Nonetheless, we observed that pigs that died of ventricular fibrillation had a shorter QT interval at baseline and during ischemia than pigs that survived ischemia and reperfusion. Dynamic shortening of the QT interval occurs clinically with hypercalcemia, tachycardia, catecholamines, or acetylcholine but is not associated with increased arrhythmic risk under these circumstances. However, a persistent abnormal shortening of the QT interval has been associated with arrhythmia and sudden death in otherwise healthy subjects; the mechanism underlying this association is undefined (31). It is also possible that pigs with a shorter QT interval at baseline may have greater potential for transmural QT dispersion during ischemia and reperfusion, thereby increasing susceptibility to develop ventricular arrhythmias. However, the epicardial electrogram may have been insensitive to detect potential alterations in ion channel conductances, particularly if the

electrophysiologic effects of troglitazone were localized to the more ischemic subendocardium. More detailed cellular and in vivo electrophysiologic studies will be necessary to determine the mechanism of proarrhythmia with acute troglitazone treatment and to confirm the possible association with shorter QT intervals.

Are the observations of the present study relevant to the clinical use of thiazolidinediones? Although the dose of troglitazone used in the present study (30 mg/kg) is higher than the maximum daily dose of troglitazone that was approved for clinical use (600 mg/day or ~ 5 –10 mg/kg), the plasma concentration of troglitazone in the present study (averaging 5.3 $\mu\text{g/ml}$) is similar to peak plasma concentrations in human subjects after an oral dose of 600 mg (averaging 2.8 $\mu\text{g/ml}$) (32). It remains to be determined whether the risk of arrhythmia in an individual subject bears a direct relation to plasma or myocardial concentration of troglitazone.

Although troglitazone is no longer in clinical use, the proarrhythmic effects observed in this study, in conjunction with evidence from in vitro electrophysiologic studies (7–9,26–28), raise the possibility that other thiazolidinediones may be proarrhythmic at clinically relevant plasma concentrations. If an increased risk of arrhythmia exists only if an ischemic event occurs during the initial period of treatment with a thiazolidinedione drug, it would not be surprising that clinical databases have failed to identify

TABLE 4
Regional LV substrate metabolism

	Baseline		Pre-ischemic treatment		Ischemia		Reperfusion	
	Vehicle	Troglitazone	Vehicle	Troglitazone	Vehicle	Troglitazone	Vehicle	Troglitazone
Plasma insulin ($\mu\text{U/ml}$)	3 ± 1	2 ± 1	4 ± 1	4 ± 1	6 ± 1	3 ± 1	$8 \pm 1^*$	5 ± 2
Arterial substrate concentration ($\mu\text{mol/ml}$ blood)								
Glucose	4.4 ± 0.4	4.2 ± 0.1	4.2 ± 0.1	4.3 ± 0.1	3.9 ± 0.1	4.0 ± 0.2	3.9 ± 0.1	4.1 ± 0.2
Lactate	0.9 ± 0.1	1.0 ± 0.2	0.9 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
FFAs	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
Myocardial substrate uptake ($\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$)								
Glucose	0.22 ± 0.16	0.20 ± 0.04	0.24 ± 0.09	0.33 ± 0.07	0.32 ± 0.04	0.21 ± 0.03	0.23 ± 0.04	0.24 ± 0.07
Lactate	0.57 ± 0.07	0.62 ± 0.13	0.57 ± 0.06	0.66 ± 0.15	$-0.08 \pm 0.03^*$	$-0.07 \pm 0.05^*$	$0.19 \pm 0.04^*$	$0.17 \pm 0.04^*$
FFAs	0.03 ± 0.02	$0.01 \pm .03$	0.05 ± 0.03	0.01 ± 0.02	0.02 ± 0.02	0.03 ± 0.01	0.03 ± 0.03	0.03 ± 0.01
Myocardial oxygen consumption ($\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$)	4.3 ± 0.5	4.3 ± 0.6	4.6 ± 0.2	4.6 ± 0.5	$1.8 \pm 0.1^*$	$1.6 \pm 0.2^*$	$1.8 \pm 0.2^*$	$1.8 \pm 0.4^*$

*Data are means \pm SE. All measurements made in anterior (ischemic) region of left ventricle. $n = 7$ in each group. * $P < 0.05$ vs. baseline in same group. There were no significant differences between groups.

such an effect to date. In another example, it took many years of widespread clinical use before the proarrhythmic potential of antihistamines was appreciated.

We believe that the findings of this study, in conjunction with mounting electrophysiologic data in noncardiac cells in vitro, provide enough of a "smoking gun" to warrant further research into the potential cardiac electrophysiologic effects of thiazolidinedione drugs in vivo. These issues bear on the important and unanswered question of whether PPAR- γ activators are helpful or harmful to patients with ischemic heart disease.

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