Effects of TA-3090, a New Calcium Channel Blocker, on Myocardial Substrate Utilization in Ischemic and Nonischemic Isolated Working Fatty Acid–Perfused Rat Hearts

Norman J. Davies, Jeffrey J. McVeigh, and Gary D. Lopaschuk

Experimental studies have shown that calcium channel blockade has a protective effect on the ischemic myocardium. Although these agents may act by decreasing intracellular Ca\(^{2+}\) accumulation during reperfusion or to reduce oxygen requirements by decreasing myocardial work load, recent evidence suggests that calcium blockers may also favorably alter energy substrate metabolism in ischemic and reperfused myocardium. In this study, TA-3090, a new calcium channel blocker with minimal effect on myocardial work load, was used to study the effect of calcium channel blockade on both myocardial substrate utilization and reperfusion recovery of ischemic hearts. Isolated working rat hearts were perfused at an 11.5 mm Hg preload and an 80 mm Hg afterload with Krebs-Henseleit buffer containing 11 mM glucose, 1.2 mM palmitate, and 500 microunits/ml insulin. In aerobically perfused spontaneously beating hearts, a 0.5 \(\mu\)M dose of TA-3090 had a mild depressant effect on heart rate but no effect on peak systolic pressure development. In paced hearts (250 beats/min), 0.5 \(\mu\)M TA-3090 had no effect on either peak systolic pressure development or contractility. Fatty acid and glucose oxidation was determined by measuring \(^{14}\)CO\(_2\) production in hearts perfused with either \(^{14}\)C-palmitate or \(^{14}\)C-glucose, respectively, whereas glycolysis was determined by measuring \(^3\)H\(_2\)O production from \(^{1}\)H-glucose. Under aerobic conditions, fatty acid oxidation was not altered by TA-3090, but a significant decrease in glucose oxidation and glycolytic rates was observed. If hearts were subjected to a 30-minute period of no-flow ischemia, the addition of 0.5 \(\mu\)M TA-3090 to the perfusate before ischemia significantly improved reperfusion recovery of mechanical function. The protective effects of TA-3090 were not observed if TA-3090 was added at the time of reperfusion and were not related to a depression of function before ischemia. TA-3090, added before ischemia, significantly reduced glycogen and ATP depletion during no-flow ischemia and also significantly decreased glycolytic rates in hearts subjected to low-flow ischemia (coronary flow=0.5 ml/min). Combined, our data suggest that the beneficial effects of calcium channel blockade on the ischemic myocardium are not related solely to a decrease in myocardial work load or metabolic demand before ischemia, but rather may in part be related to a decrease in myocardial energy demand during ischemia itself, resulting in preservation of ATP and a decrease in glycolysis. The decrease in glycolytic rates during ischemia may also result in a reduction of glycolytic product accumulation during ischemia. (Circulation Research 1991;68:807–817)

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The calcium channel antagonists have acquired a central role in the treatment of chronic ischemic heart disease. Their role in the treatment of acute myocardial ischemia and infarction, however, remains less clear. A large number of experimental investigations have shown a beneficial effect of calcium channel blockade in reduction of acute ischemic myocardial injury. Calcium channel blockers have been shown to preserve tissue morphology, reduce lactate dehydrogenase release, improve mechanical recovery, reduce ST elevation, and reduce infarct size when present during ischemia.\(^1\) This may occur because of improvement in collateral coronary flow or a decrease in calcium overload.\(^1\) In addition, however, calcium channel blockers also preserve ATP levels in the ischemic myocardium.\(^2\-4\)
At least two mechanisms may explain this effect on ATP. Reductions in myocardial work load, resulting in reduced ATP depletion, have been suggested as the primary mechanism by which calcium blockers exert their beneficial effects. For example, Neubauer and Ingwall demonstrated that ATP preservation during hypoxia in isolated intact hearts was fully explained by the negative inotropic action of verapamil. Bourdillon and Poole-Wilson showed that the cardioprotective effects of verapamil in ischemic isolated rabbit septa were also due to reduced work load. Recently, evidence has appeared supporting the contention that calcium channel blockade has a salutary effect that is not explained by work load effects. For example, Jenkins et al., using pacing and intravenous dobutamine infusion, demonstrated a work load–independent beneficial effect of calcium channel blockade on myocardial stunning after transient regional ischemia. Prevention of mitochondrial calcium overload during reperfusion, with a resultant increase in ATP synthesis, could explain enhanced reperfusion recovery after calcium blockade, but the evidence dissociating this phenomenon from work load effects is limited.

We have previously shown that metabolic substrate utilization is a key determinant of functional recovery after transient myocardial ischemia and reperfusion. Fatty acids, which depress glucose utilization, are the primary myocardial energy substrate in aerobic and reperfused myocardium but are nonetheless detrimental to reperfusion recovery when present at high concentrations. During ischemia itself, however, Neely and Grotjohann have suggested that glycolytic product accumulation contributes to tissue injury during no-flow conditions, implying that reperfusion recovery might be enhanced by inhibition of glycolytic flux during ischemia. Recent studies have suggested that calcium channel blockers may also favorably affect myocardial energy substrate utilization during ischemia. Watts and coworkers, using globally ischemic glucose-perfused Langendorff hearts, found that diltiazem reduced lactate accumulation to a degree that was not explained by its depressant effect on contractile performance, while De Jong and Huizer demonstrated that calcium channel blockade with nisoldipine reduces glycolysis in ischemic hearts. These studies, however, were performed in the absence of fatty acids, a condition that increases glycolytic flux.

The purpose of this study was to determine the effects of calcium channel blockade on myocardial energy substrate utilization and postischemic reperfusion recovery in hearts perfused in the presence of high concentrations of fatty acids (1.2 mM palmitate). These perfusion conditions are physiologically relevant, since serum concentrations of fatty acids similar to this are commonly seen after a myocardial infarction. To achieve this objective, we used TA-3090, a new benzothiazepine calcium antagonist that blocks both voltage-sensitive and receptor-operated calcium channels. TA-3090 is ideally suited for these studies, since previous work has demonstrated its relative lack of negative inotropism when compared with other calcium antagonists. By using isolated working rat hearts, the effects of TA-3090 on mechanical function, glucose and palmitate oxidation, and glycolytic flux were studied under aerobic conditions. The effect of TA-3090 on reperfusion recovery of hearts subjected to global no-flow ischemia and on glycolytic rates in hearts subjected to low-flow ischemia was also examined. Our data demonstrate that, at concentrations that do not reduce heart rate or myocardial contractility, TA-3090 decreases glycolysis during low-flow ischemia and has a significant beneficial effect on ATP levels and mechanical reperfusion recovery when present during no-flow ischemia. TA-3090 had a protective effect on ischemic myocardium that is not explained by changes in myocardial work before ischemia. Rather, our data suggest that the beneficial effects of calcium channel blockers may in part be due to their ability to reduce glycolysis in the ischemic myocardium and therefore the potential for glycolytic product accumulation during ischemia.

Materials and Methods

Heart Perusions

Hearts from sodium pentobarbital–anesthetized male Sprague-Dawley rats were excised, and the aorta and left atrium were cannulated as previously described. In hearts used to assess coronary flow and oxygen consumption, the pulmonary artery was also cannulated. Hearts were then perfused as working hearts (11.5 mm Hg preload, 80 mm Hg afterload) with Krebs-Henseleit buffer containing 11 mM glucose, 1.2 mM palmitate prebound to 3% bovine serum albumin, 2.5 mM free calcium, and 500 microunits/ml insulin. In hearts used to assess contractility, a cannula was inserted into the left ventricle through the apex of the heart to monitor left ventricular developed pressure. Heart rate, peak aortic systolic pressure, and left ventricular developed pressure were measured using pressure transducers (P21, Gould Inc., Cleveland, Ohio) connected to a physiograph (Grass Instrument Co., Quincy, Mass., or Gould). For measurement of myocardial contractility, a cannula was inserted through the apex into the cavity of the left ventricle and left ventricular developed pressure and its peak first derivatives (+ve and –ve dP/dt) were determined. Myocardial O2 consumption was determined using O2 microelectrodes (YSI, Yellow Springs, Ohio) to determine O2 levels in perfusate entering the left atrium and the cannulated pulmonary artery. Aortic flow was measured at 5-minute intervals by collecting perfusate off the top of the afterload column. Because pulmonary artery flow and dripout from the heart are derived only from coronary sinus flow in this model, coronary artery flow was taken to equal the sum of these two measurements. Myocardial O2 consumption was determined and expressed as μmol O2 consumed·min⁻¹·g dry
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wt\(^{-1}\) \cdot \text{or \(\mu\text{mol O}_2\) consumed \cdot min\(^{-1}\) \cdot (heart rate \times peak systolic pressure)}\(^{-1}\).

Hearts in which the initial rate–pressure product was less than 15.0 \times 10^5 \text{beats \cdot mm Hg/min} were not used in this study.

Assessment of Myocardial Function Under Aerobic Conditions

To assess the effect of TA-3090 (a generous gift of Nordic Laboratories, Laval, Quebec, Canada) on heart rate and peak systolic pressure (PSP) development, hearts were first perfused in working mode as described above for 15 minutes of baseline work. This 15-minute period allowed ample time for equilibration of heart function. TA-3090 was then added to the perfusate at progressively increasing concentrations, from 0.2 to 20 \(\mu\text{M}\), with 5-minute periods between each change in TA-3090 concentration provided to ensure adequate circulation of the drug throughout the perfusate between measurements of heart rate and PSP.

To assess the effects of TA-3090 on contractility, hearts were attached to the perfusion apparatus in the working mode as described above. After stabilization of heart rate and PSP, the hearts were paced at 250 beats/min. A cannula was inserted through the apex of the heart into the left ventricular chamber. A baseline afterload curve was constructed using afterloads of 40, 50, 60, 70, 80, 90, and 100 mm Hg. At each level, left ventricular developed pressure and PSP were determined. The first derivatives of the left ventricular developed pressure (+ve and −ve dP/dt) were calculated to determine the rates of contraction and relaxation, respectively. TA-3090 was then added to the perfusate at a concentration of 0.5 \(\mu\text{M}\). Aerobic perfusion was continued for 2 minutes to allow the drug to circulate throughout the perfusate, and the afterload curve was repeated. TA-3090 was then added to the perfusate to give a final concentration of 2.0 \(\mu\text{M}\), perfusion was continued for 2 minutes, and the afterload curve was again repeated.

Palmitate Oxidation, Glucose Oxidation, and Glycolysis Studies

To determine rates of palmitate oxidation, rates of \(^{14}\text{CO}_2\) production were measured in spontaneously beating hearts perfused with 11 \(\mu\text{M}\) glucose, 1.2 \(\mu\text{M}\) \(^{1-14}\text{C}\) palmitate, and 500 microunits/ml insulin. Steady-state \(^{14}\text{CO}_2\) production was measured between 20 and 60 minutes of perfusion as described previously.\(^9\),\(^10\)

Hearts were perfused in a closed recirculating system using an oxygenator with a large surface area in constant contact with 95% \(\text{O}_2\)-5% \(\text{CO}_2\). The gas mixture entered the perfusion apparatus at the top of the closed oxygenation chamber, exited the chamber through an exhaust tube, and was bubbled through 1 M methylbenzothionium hydroxide trap to collect gaseous \(^{14}\text{CO}_2\). Perfusion samples, used to determine \(^{14}\text{CO}_2\) present as bicarbonate, were removed with a syringe directly from the system without exposure to air and were placed in a 25-ml stoppered flask with a center well containing methylbenzothionium hydroxide. The sample of perfusate was acidified by addition of 9N H\(_2\)SO\(_4\) (1 ml), the flask was shaken for 1 hour, and the methylbenzothionium hydroxide trap was placed in ACS scintillation cocktail (Amersham, Arlington Heights, Ill.) to be counted.

Glycolysis and glucose oxidation were measured simultaneously by perfusing hearts with buffer containing 11 \(\mu\text{M}\) [2-\(^{3}\text{H}, \text{U-14}\text{C}\)]glucose, 1.2 \(\mu\text{M}\) palmitate, 3% albumin, and 500 microunits/ml insulin (specific activity of perfusate equaled 150,000 dpm/ml \(^{1}\text{H}\) and 150,000 dpm/ml \(^{14}\text{C}\)). Steady-state glucose oxidation was determined by quantitative measurement of \(^{14}\text{CO}_2\) production as described above. To measure glycolysis, \(^{3}\text{H}\)O in perfusate samples was separated from \(^{1}\text{H}\)glucose and \(^{14}\text{C}\)glucose as described by Rovetto et al.,\(^22\) using columns containing Dowex 1-X4 anion exchange resin (200–400 mesh, Bio-Rad Laboratories, Richmond, Calif.) suspended in 0.2 M potassium tetraborate (the volume of resin was 0.5 × 0.5 cm\(^2\)). The Dowex in the column was extensively washed with \(\text{H}_2\text{O}\) before use. A 0.2-ml volume of perfusate was then added to the column and eluted into scintillation vials with 0.8 ml \(\text{H}_2\text{O}\). After the addition of ACS scintillant, the samples were subjected to standard double-isotope counting procedures, with the windows set at 0–300 nm (\(^{1}\text{H}\) and 400–670 nm (\(^{14}\text{C}\)). The Dowex columns were found to retain 98–99.6% of the total \(^{1}\text{H}\)glucose and \(^{14}\text{C}\)glucose present in the perfusate. The \(\text{H}_2\text{O}\) (which passes through the column) was corrected for the small amount of \(^{1}\text{H}\)glucose that passed through the column. This could be accomplished because an equal amount of \(^{14}\text{C}\)glucose also passed through the column, which could be used as an internal standard for the degree of \(^{3}\text{H}\)glucose contamination in the \(\text{H}_2\text{O}\) sample. Correction was also made for the degree of spillover of \(^{14}\text{C}\) into the \(^{3}\text{H}\) counting window by measuring this degree of spillover using standards containing only \(^{14}\text{C}\)glucose.

Ischemic Perfusions

Global no-flow ischemia followed by reperfusion of isolated working hearts was performed as previously described.\(^10\),\(^13\),\(^14\) Briefly, after 15 minutes of baseline work, global ischemia was induced by clamping off left atrial and aortic flow. Hearts were maintained at 37°C throughout the ischemic period. After 30 minutes of no-flow ischemia, left atrial and aortic flow was restored, and recovery of mechanical function was monitored for a further 30 minutes. TA-3090 (0.5 \(\mu\text{M}\)), when present, was added to the perfusate 5 minutes before the onset of ischemia, or at the time of reperfusion. This concentration of TA-3090 was chosen because it was not associated with depression of myocardial PSP development or contractility under aerobic conditions (Figure 1, Table 1).

Because glycolysis during ischemia cannot be measured under conditions of no-flow ischemia in these hearts, a parallel series of hearts were subjected to a low-flow ischemia in which coronary flow was reduced to 0.5 ml/min. Hearts were initially perfused
under aerobic conditions for 40 minutes, during which time both steady-state glucose oxidation and glycolytic rates were measured. Hearts were then switched to the Langendorff mode and perfused with the same recirculating buffer at a coronary flow of 0.5 ml/min for a further 60 minutes. Hearts were perfused at this coronary flow rate to allow the $^3$H$_2$O and $^{14}$CO$_2$ produced by the heart to equilibrate with the recirculated buffer.

**Tissue Metabolites**

At the end of all perfusions, hearts were cut from the apparatus and freeze-clamped with Wollenberger clamps cooled to the temperature of liquid nitrogen. Before analysis, frozen tissue was powdered in a mortar and pestle maintained at the temperature of liquid nitrogen. A portion of the tissue was used for dry/wet ratio determination. The remainder of the tissue was used for metabolite measurements. With this ratio, as well as the total frozen ventricular weight and the weight of the dried atrial tissue, total dry weight of the heart was determined. Metabolites measured included ATP, creatine phosphate, glycogen, and lactate. Extraction of tissue lipids was carried out as previously described, and neutral lipids were separated from phospholipids by the methods of Bower and King. Triglycerides were separated from other neutral lipids as previously described using Si50-PA ($^9$C)-silica gel plates (J.T. Baker Inc., Phillipsburg, N.J.) and a solvent system that consisted of isooctane:diethyl ether:acetic acid (74:24:2 [vol/vol/vol]). Separated lipids were subsequently scraped into scintillation vials to quantitate label content of the lipids. Lactate, ATP, and creatine phosphate were extracted from ventricular tissue with perchloric acid, and measurements of ATP and creatine phosphate levels were performed using standard enzymatic assays described previously. Myocardial lactate was determined spectrophotometrically using a standard enzyme assay. Myocardial glycogen was extracted and hydrolyzed to free glucose, which was measured as described previously.

**Statistical Analysis**

Data are expressed as mean±SEM. Comparison within one group was performed using paired Student’s $t$ test. Comparison of two groups was performed using unpaired Student’s $t$ test. In experiments involving three experimental groups, two-way analysis of variance (ANOVA) was used, and comparisons between individual group means were performed using the Newman-Keuls test. Significance was set at $p<0.05$.

**Results**

**Assessment of Mechanical Function in Isolated Perfused Working Hearts Under Aerobic Conditions**

Concentration versus function curves for hearts perfused with varying concentrations of TA-3090 are depicted in Figures 1a and 1b. At a concentration of 0.5 $\mu$M, the drug had a small but significant depressant effect on heart rate, but further dose-dependent decreases in heart rate were not seen as TA-3090 concentration was increased incrementally through 20 $\mu$M (Figure 1a). In contrast, a significant effect on PSP development was not seen until TA-3090 concentration reached 5 $\mu$M (Figure 1b).

The effects of TA-3090 on PSP development, left ventricular developed pressure and peak $+ve$ and $-ve$ dp/dt in hearts paced at 250 beats/min, under conditions of varying afterload are depicted in Table 1. At a concentration of 0.5 $\mu$M, the drug had no effect on any of these three parameters. Effects on myocardial contractility were seen, however, at a concentration of 2.0 $\mu$M. At high work loads (100 mm Hg afterload) a significant decrease in both the rate of contraction and the rate of relaxation was observed.

The effects of TA-3090 on PSP, cardiac output, aortic flow, coronary flow, and oxygen consumption under aerobic conditions are given in Table 2. These parameters were measured in hearts paced at 250 beats/min. At a concentration of 0.5 $\mu$M, the drug had no effect on any of these parameters. Therefore, a concentration of 0.5 $\mu$M TA-3090 was used in hearts

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**Figure 1.** Effects of increasing concentrations of TA-3090 on heart rate (panel a) and peak systolic pressure development (panel b) in isolated working rat hearts. Hearts were perfused at an 11.5 mm Hg left atrial preload and an 80 mm Hg aortic afterload with perfusate containing 11 mM glucose, 1.2 mM palmitate, and 500 microunits/ml insulin. Values are mean±SEM of six hearts.
TABLE 1. Effects of 0.5 μM and 2.0 μM TA-3090 on Left Ventricular Developed Pressure and Left Ventricular +ve dP/dt and −ve dP/dt in Paced Hearts (250 beats/min) Subjected to Increases in Afterload

<table>
<thead>
<tr>
<th>Perfusion condition</th>
<th>Left ventricular developed pressure (mm Hg)</th>
<th>Left ventricular +ve dP/dt</th>
<th>Left ventricular −ve dP/dt</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 mm Hg aortic afterload Control</td>
<td>66.7±3.7</td>
<td>1,996±85</td>
<td>852±62</td>
</tr>
<tr>
<td>0.5 μM TA-3090</td>
<td>58.2±3.8</td>
<td>1,783±91</td>
<td>679±25</td>
</tr>
<tr>
<td>2.0 μM TA-3090</td>
<td>56.0±5.3</td>
<td>1,889±199</td>
<td>671±77</td>
</tr>
<tr>
<td>70 mm Hg aortic afterload Control</td>
<td>89.3±6.0</td>
<td>2,640±105</td>
<td>1,339±160</td>
</tr>
<tr>
<td>0.5 μM TA-3090</td>
<td>90.8±4.1</td>
<td>2,944±153</td>
<td>1,416±94</td>
</tr>
<tr>
<td>2.0 μM TA-3090</td>
<td>83.0±7.1</td>
<td>2,475±119</td>
<td>1,280±93</td>
</tr>
<tr>
<td>100 mm Hg aortic afterload Control</td>
<td>107.8±11.2</td>
<td>3,216±269</td>
<td>2,218±412</td>
</tr>
<tr>
<td>0.5 μM TA-3090</td>
<td>109.2±7.0</td>
<td>3,085±155</td>
<td>2,125±225</td>
</tr>
<tr>
<td>2.0 μM TA-3090</td>
<td>93.2±12.2</td>
<td>2,381±336*</td>
<td>1,393±331*</td>
</tr>
</tbody>
</table>

Hearts perfused with 11 mM glucose, 1.2 mM palmitate, and 500 micromunits/ml insulin were paced at 250 beats/min and subjected to a constant 11.5 mm Hg left atrial preload. Values are mean±SEM of five hearts.

*Significantly different than comparable control hearts.

The effects of TA-3090 on myocardial glucose oxidation and glycolytic rates are shown in Figures 3a and 3b. Interestingly, in the presence of the fatty acid concentrations used in these studies, glycolytic rates were markedly higher than glucose oxidation rates. This suggests a significant portion of glucose that passes through glycolysis is being excreted as lactate, as opposed to being oxidized. Under these aerobic conditions, TA-3090 (0.5 μM) significantly decreased both glucose oxidation and glycolytic rates. This occurred when glucose utilization was expressed as absolute rates (Figure 3a). If normalized for work load differences, a significant decrease in glycolysis was observed (Figure 3b).

Overall myocardial oxygen consumption and the absolute and relative amounts of total ATP production derived from palmitate and glucose are shown in Tables 2 and 3. As indicated, oxygen consumption was measured in the series of hearts that were paced at 250 beats/min, whereas ATP production was determined in spontaneously beating hearts. This was done because our experimental system did not allow simultaneous measurements of oxygen consumption and oxidative metabolism (14CO2 would be released from the enclosed system during measurements of coronary flow). As indicated, oxygen consumption, even when corrected for myocardial work load, was not affected by 0.5 μM TA-3090, despite its depressant effect on glucose utilization (Table 2). This is explained by the fact that in this model, which uses a high concentration of palmitate in the perfusate, a very high proportion of total ATP production is derived from exogenous fatty acid (92% in the absence of TA-3090) (Table 3). Thus, even major changes in glucose utilization would not be expected to affect global oxygen consumption significantly. Overall ATP production from exogenous substrates was not significantly different between the control hearts and TA-3090–treated hearts.

Reperfusion Recovery of Globally Ischemic Hearts

The effect of TA-3090 on reperfusion recovery after ischemia was determined in spontaneously beating hearts subjected to 15 minutes of aerobic perfusion, 30 minutes of global no-flow ischemia, and 30 minutes of aerobic reperfusion (Figure 4). When added to the perfusate at a concentration of 0.5 μM, 5 minutes before the onset of ischemia, the drug had a marked beneficial effect on reperfusion recovery of

TABLE 2. Effect of TA-3090 on Various Parameters of Heart Function

<table>
<thead>
<tr>
<th>Perfusion condition</th>
<th>PSP (mm Hg)</th>
<th>Cardiac output (ml/min)</th>
<th>Aortic flow (ml/min)</th>
<th>Coronary flow (ml/min)</th>
<th>O2 consumption (μmol · min⁻¹ · g dry wt⁻¹)</th>
<th>O2 consumption (μmol · min⁻¹ · [HR·PSP·10⁻³])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=10)</td>
<td>98±2</td>
<td>31.2±2.9</td>
<td>11.5±2.4</td>
<td>19.7±1.2</td>
<td>62.5±4.7</td>
<td>0.552±0.041</td>
</tr>
<tr>
<td>0.5 μM TA-3090 (n=7)</td>
<td>94±3</td>
<td>28.7±2.7</td>
<td>10.3±2.6</td>
<td>18.3±0.8</td>
<td>58.2±3.7</td>
<td>0.478±0.021</td>
</tr>
</tbody>
</table>

Hearts were paced at 250 beats/min and perfused at an 11.5 mm Hg preload and an 80 mm Hg afterload. Values are mean±SEM of numbers indicated in parentheses. PSP, peak systolic pressure; HR, heart rate.
PSP development (Figure 4). This beneficial effect was not fully explained by preischemic work load effects, since TA-3090 had no effect on systolic pressure development at this concentration under aerobic conditions (Figure 1b). The presence of TA-3090 did, however, increase the rate of decline of mechanical function, at the onset of ischemia. After the onset of no-flow ischemia, the average number of heartbeats before complete cessation of beating was 3.5±0.9 beats in hearts perfused with 0.5 μM TA-3090, compared with 7.6±0.8 beats in control hearts (p<0.05).

No beneficial effect of 0.5 μM TA-3090 on recovery of PSP was seen when the drug was added to the perfusate at the time of reperfusion (Figure 4).

In contrast to effects of TA-3090 on PSP development during reperfusion of ischemic hearts, the drug had no beneficial effect on reperfusion recovery of heart rate. A small, but nonsignificant, decrease in heart rate before ischemia did occur in hearts treated with 0.5 μM TA-3090 (Table 4). After 30 minutes of reperfusion, heart rate was not significantly different in any experimental group, although PSP and the heart rate–pressure product were significantly greater in hearts in which TA-3090 was present before ischemia.

To determine if the small but significant decrease in heart rate produced by TA-3090 preischemia could be important in the postischemic recovery of mechanical function, a correlation plot of preischemic mechanical function (heart rate×PSP) versus percent of postischemic recovery of function was plotted (Figure 5). As shown, no correlation between preischemic function and the eventual degree of recovery of PSP development was observed.

Table 5 shows the effects of 0.5 μM TA-3090 on levels of myocardial ATP, creatine phosphate, lactate, and glycogen after the ischemia/reperfusion protocol. A series of hearts were also frozen immediately after the ischemic interval, before reperfusion, for metabolite measurements. As indicated, hearts treated with TA-3090 before ischemia had significantly higher tissue glycogen levels immediately postischemia and after reperfusion than did control hearts. This suggests that TA-3090 reduced the rate of ongoing glycolysis during the ischemic inter-
TABLE 3. Effect of TA-3090 on Steady-State ATP Production From Exogenous Glucose and Palmitate in Hearts Perfused With 11 mM Glucose, 1.2 mM Palmitate, and 500 Micromolars/ml Insulin

<table>
<thead>
<tr>
<th>Perfusion condition</th>
<th>Steady-state ATP production (μmol/min/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>From glucose</td>
<td>From palmitate</td>
</tr>
<tr>
<td>Control</td>
<td>11.95 ± 3.95 (8%)</td>
</tr>
<tr>
<td>0.5 μM TA-3090</td>
<td>5.22 ± 0.97* (5%)</td>
</tr>
</tbody>
</table>

ATP production was calculated from the exogenous steady-state oxidation rates of glucose and palmitate obtained from Figures 2 and 3 based on 38 mol ATP produced/mol glucose oxidized and 129 mol ATP produced/mol palmitate oxidized. Values are mean ± SEM of six to 10 hearts in each group.

*Significantly different from control.

val. Tissue levels of lactate were, as expected, dramatically increased after ischemia and were not significantly altered by the presence of TA-3090. After 30 minutes of aerobic reperfusion, lactate levels were significantly lower in TA-3090–perfused hearts than in control hearts, probably reflecting increased lactate oxidation and tissue washout caused by enhanced mechanical performance in these hearts. Myocardial ATP concentrations were increased both immediately after ischemia and after reperfusion in TA-3090–perfused hearts. This suggests that the drug preserves tissue high-energy phosphate levels during ischemia.

Glucose Oxidation and Glycolytic Rates During Low-Flow Ischemia

The effects of 0.5 μM TA-3090 on glucose oxidation and glycolytic rates during low-flow ischemia are shown in Figure 6. Hearts were subjected to low-flow ischemia, as opposed to no-flow ischemia, to allow the 3H2O and 14C produced by the heart to equilibrate with the buffer. In this series of perfusions, glucose oxidation and glycolytic rates were expressed only as absolute values because of the absence of mechanical function. As expected, under these conditions of ischemia, glucose oxidation was essentially abolished in both control and TA-3090–treated hearts. Glycolytic rates, however, were only slightly reduced compared with aerobic glycolytic rates (Figure 3). As shown in Figure 6, TA-3090 significantly reduced glycolytic rates during ischemia, compared with ischemic control hearts.

Discussion

Despite dissimilar chemical structures, all calcium blockers share the property of inhibiting sarcolemmal

Figure 4. Effects of TA-3090 on reperfusion recovery of peak systolic pressure development of hearts subjected to a transient period of no-flow ischemia. Hearts were reperfused after 30 minutes of global no-flow ischemia as described in "Materials and Methods." TA-3090 (0.5 μM) was added either 5 minutes before ischemia or immediately on reperfusion. Spontaneously beating hearts were perfused with 11 mM glucose, 1.2 mM palmitate, and 500 micromolars/ml insulin at an 11.5 mm Hg left atrial preload and an 80 mm Hg aortic afterload. Values are mean ± SEM of five to 11 hearts in each group. *Significantly different than control hearts.

Figure 5. Plot of preischemic function vs. the percent recovery of function during reperfusion. Hearts are the same as those described in Figure 4.

Figure 6. Effects of 0.5 μM TA-3090 on rates of glucose oxidation (open bars) and glycolysis (solid bars) in isolated working rat hearts subjected to low-flow ischemia. Spontaneously beating hearts were perfused with 11 mM [2-14C, U-3H]glucose, 1.2 mM palmitate, and 500 micromolars/ml insulin as described in "Materials and Methods." Values are mean ± SEM of six control and eight TA-3090–treated hearts. Oxidative rates and glycolytic rates are expressed as a function of absolute levels of 14C or 3H produced/min, respectively. Low-flow ischemia was produced by switching hearts to a retrograde perfusion at a constant coronary flow rate of 0.5 ml/min. *Significantly different than control hearts.
TABLE 4. Effect of TA-3090 on Reperfusion Recovery of Mechanical Function in Hearts Subjected to a Transient No-Flow Ischemia

<table>
<thead>
<tr>
<th>Perfusion condition</th>
<th>HR (beats/min)</th>
<th>PSP (mm Hg)</th>
<th>HR×PSP (×10⁻¹³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preischemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>232±8</td>
<td>111±4</td>
<td>25.8±1.4</td>
</tr>
<tr>
<td>0.5 μM TA-3090</td>
<td>210±16</td>
<td>120±12</td>
<td>25.1±2.9</td>
</tr>
<tr>
<td>Postischemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>99±29*</td>
<td>51±13*</td>
<td>8.7±3.2*</td>
</tr>
<tr>
<td>0.5 μM TA-3090 added before ischemia</td>
<td>134±14*</td>
<td>128±11†</td>
<td>17.3±2.7†</td>
</tr>
<tr>
<td>0.5 μM TA-3090 added at reperfusion</td>
<td>91±43*</td>
<td>41±18*</td>
<td>8.2±5.0*</td>
</tr>
</tbody>
</table>

Hearts were reperfused for 30 minutes after 30 minutes of global ischemia. TA-3090 was added either 5 minutes before ischemia or immediately on reperfusion. Values are mean±SEM of seven to 10 hearts in each group. HR, heart rate; PSP, peak systolic pressure.

*Significantly different than preischemic control hearts.
†Significantly different than control hearts postischemia.

calcium influx through voltage-dependent channels of cardiac and smooth muscle cells.26,27 In experimental models of acute myocardial ischemia and infarction, these agents are reported to reduce infarct size not only by reducing myocardial oxygen consumption and improving collateral coronary flow, but also by decreasing calcium overload and favorably altering calcium compartmentalization during ischemia and reperfusion.1 Some authors have also suggested that the protective effects of these agents are due to their depressant effect on myocardial work load. For instance, Bourdillon and Poole-Wilson,5 using isolated perfused rabbit interventricular septa, found that high concentrations of verapamil caused reductions in developed tension, myocardial work, and rate of ATP consumption, leading to a reduction in calcium uptake and resting tension on reperfusion. These beneficial effects were seen only if depression of mechanical function was present before the ischemic interval and were not observed when the drug was added during the last 10 minutes of ischemia or at the time of reperfusion. In other experimental studies that demonstrate a beneficial effect of calcium channel antagonists on the ischemic myocardium, a significant decrease in preischemic mechanical function with the calcium channel blocker has also been observed.28–30 In this study, we demonstrate that a protective effect of the calcium channel blocker TA-3090 added before ischemia can occur at a concentration that does not decrease myocardial contractility. In addition, no correlation between preischemic function and the degree of postischemic recovery of function was observed. This suggests that, although depression of preischemic mechanical function may partially explain the beneficial effect of calcium channel blockade on the ischemic myocardium, a protective effect can nonetheless occur in the absence of a decrease in myocardial work load preschmia.

Although TA-3090 had no depressant effect on preischemic function, it is clear that a reduction in energy demand during ischemia occurred. During severe ischemia, ATP production from oxidative metabolism quickly ceases, and the primary source of ATP production is limited to glycolysis. In our study, TA-3090 significantly decreased glycolytic rates in hearts subjected to low-flow ischemia (Figure 6). Although we could not measure glycolysis during no-flow ischemia, it is probable that TA-3090 also decreased glycolysis under conditions of no-flow ischemia. In hearts subjected to low-flow ischemia, the reduction of coronary flow to 0.5 ml/min essentially abolished glucose oxidation. It is thus reasonable to assume that the effects of TA-

TABLE 5. Effect of TA-3090 on Myocardial Lactate, Glycogen, ATP, and Creatine Phosphate Levels at the End of 30 Minutes of Ischemia and After 30 Minutes of Reperfusion

<table>
<thead>
<tr>
<th>Perfusion condition</th>
<th>Glycogen (μmol glucose equivalent/g dry wt)</th>
<th>Lactate (μmol/g dry wt)</th>
<th>ATP (μmol/g dry wt)</th>
<th>Creatine phosphate (μmol/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hearts frozen at end of ischemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>18.5±2.1</td>
<td>129.3±14.2</td>
<td>6.3±0.7</td>
<td>3.0±0.4</td>
</tr>
<tr>
<td>0.5 μM TA-3090 preischemia</td>
<td>71.5±6.2*</td>
<td>126.7±2.9</td>
<td>16.5±1.4*</td>
<td>2.9±0.6</td>
</tr>
<tr>
<td>Hearts frozen at end of reperfusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>36.7±6.0</td>
<td>41.9±12.2</td>
<td>10.2±1.2</td>
<td>14.4±3.6</td>
</tr>
<tr>
<td>0.5 μM TA-3090 preischemia</td>
<td>100.4±8.3*</td>
<td>14.2±2.4</td>
<td>18.5±4.1</td>
<td>19.8±3.7</td>
</tr>
<tr>
<td>0.5 μM TA-3090</td>
<td>46.1±12.3</td>
<td>55.7±14.5</td>
<td>14.0±4.3</td>
<td>9.9±7.0</td>
</tr>
</tbody>
</table>

Hearts were reperfused for 30 minutes after 30 minutes of global ischemia. TA-3090 was added either 5 minutes before ischemia or immediately on reperfusion. Values are mean±SEM of seven to 10 hearts in each group.

*Significantly different than control hearts perfused under the same conditions.
3090 in these two models are similar. Despite the probable decrease in glycolytic rates during no-flow ischemia, ATP levels were preserved during ischemia in hearts pretreated with TA-3090 (Table 5). Because glycogenolysis was also decreased in ischemic hearts treated with TA-3090, the preservation of ATP during ischemia can only be explained by a decrease in energy demand during ischemia. A reduction in energy demand during ischemia by TA-3090 is further evidenced by the more rapid rate of heart rate loss at the onset of ischemia in TA-3090–treated hearts compared with controls. The reason for this effect is not clear, but it may occur secondary to a decrease in the activity of Ca2+-dependent ATPases or to the more rapid decrease in heart rate seen during ischemia in the TA-3090–treated hearts compared with controls. Steenenbergen et al recently demonstrated that a rise in cytosolic Ca2+ during ischemia may accelerate the depletion of cellular ATP. TA-3090 may be acting to blunt this rise in cytosolic Ca2+. It cannot be ruled out, however, that the faster decline in heart rate may also occur secondary to a TA-3090–induced decrease in glycolytic rates.

A decrease in glycolytic rates during ischemia may also decrease the accumulation of potentially harmful glycolytic products (particularly H+) during ischemia. Evidence that glycolytic product accumulation could be an important mediator of ischemic injury has been available for many years. Tennant and Wiggers were the first to show that low-flow ischemia is more damaging to the myocardium than is hypoxia. Neely and Grottyohann have demonstrated that anoxic preperfusion of globally ischemic hearts reduced lactate accumulation and improved reperfusion recovery, probably by depleting tissue glycogen. The addition of exogenous lactate to the perfusate reversed the benefits of anoxic preperfusion. The mechanism by which glycolytic product accumulation during ischemia contributes to cell injury appears to involve alterations in Ca2+ fluxes. Tani and Neely recently demonstrated that H+ ion accumulation caused by glycolytic flux in ischemic hearts may increase intracellular Na+ via the Na-H exchanger. Increased levels of intracellular Na+ at the end of ischemia were correlated with an increased Ca2+ influx during reperfusion, possibly via increased Na-Ca exchange activity. Recent studies from our laboratory have also suggested that glycolytic product accumulation during ischemia can be detrimental to subsequent reperfusion recovery of heart function. In isolated working hearts perfused with substrate concentrations identical to those used in this study, stimulation of glucose oxidation, with the pyruvate dehydrogenase activator dichloroacetate, during reperfusion of previously ischemic hearts resulted in an enhanced recovery of function. If dichloroacetate was added before ischemia, however, no beneficial effect of this agent on reperfusion recovery was observed. Dichloroacetate addition before ischemia was also accompanied by a significant increase in lactate accumulation during ischemia. A recent study by Kupriyanov et al also supports the contention that glucose utilization may be detrimental during ischemia. Taken together, these data further support the notion that part of the beneficial effect of TA-3090 on the ischemic myocardium may be related both to its ability to decrease glucose utilization and to decrease glycogen depletion during ischemia. Although we did not see a significant decrease in lactate accumulation during ischemia in our hearts, we did see a significant decrease in glycogen depletion. The possibility that tissue levels of other glycolytic products, such as H+, may have been reduced by this agent also cannot be discounted.

Previous studies by other investigators have also demonstrated that calcium antagonists can influence myocardial substrate use. Watts and coworkers used isolated pyruvate- and glucose-perfused working hearts subjected to transient global ischemia and reperfusion to demonstrate a beneficial effect of diltiazem or verapamil on reperfusion recovery when either agent was present during ischemia. Improved recovery was accompanied by preservation of high-energy phosphate stores, reduced lactate accumulation during ischemia, and reduced calcium overload after reperfusion. Diltiazem significantly reduced work load in ischemic hearts, but this effect did not fully explain the observed benefits of the drug during ischemia, since diltiazem further reduced the accumulation of lactate in potassium-arrested ischemic hearts. DeJong and Huizer, using a low coronary flow model in isolated glucose-perfused Langendorff hearts, found that nisoldipine reduces ATP catabolism during ischemia. In addition, lactate accumulation and glycogen depletion were reduced during ischemia, suggesting that glycolytic flux during ischemia was reduced by the drug. These authors suggested that effects were explained by a reduction in myocardial work, however, and an independent effect on substrate utilization was not demonstrated. These authors found that nisoldipine had no effect on glucose oxidation or lactate production under aerobic conditions. Our data also indicate that calcium channel blockade preserves myocardial ATP levels in ischemic and reperfused myocardium but, in contrast, provide evidence for a work load–independent effect of calcium channel blockade on myocardial energy substrate utilization under aerobic and ischemic conditions. The discrepancies in our studies and those of DeJong and Huizer may be explained by differences in perfusion conditions: since fatty acids strongly inhibit glucose utilization, the conditions used by DeJong and Huizer, in which glucose is used as the sole exogenous energy substrate, are likely to markedly increase glucose utilization. Under these circumstances, a direct depressant effect of calcium channel blockade on glucose utilization might not be seen. Conversely, the presence of physiologically relevant perfusate concentrations of fatty acid in our studies could have led to an “unmasking” of this depressant effect.
In our study, treatment of hearts with TA-3090 resulted in a significant decrease in glycolen depletion during ischemia, without significant effects on the accumulation of myocardial lactate. Because glycolenolysis during ischemia was decreased, we initially expected to see a decrease in lactate accumulation in TA-3090–treated hearts. However, there is not a good correlation between glycogen loss and lactate accumulation in hearts during no-flow ischemia. For instance, Neely’s laboratory\textsuperscript{15,33} has demonstrated that even if they markedly deplete glycogen stores before ischemia (from 120 to 20 μmol/g dry wt) lactate levels can still rise by over 80 μmol/g dry wt during ischemia. This suggests that much of the lactate that accumulates in the myocardium during ischemia does not have to originate from glycogen, and a portion probably originates from glucose and glycolytic intermediates already present in the heart. Therefore, a preservation of glycogen in TA-3090–treated hearts would not necessarily have to be accompanied by a decrease in tissue lactate.

In addition to reducing glycolytic rates during ischemia, TA-3090 also significantly decreased myocardial glucose utilization during aerobic perfusion. At least two potential mechanisms may be put forth to explain our observations that calcium channel blockade can reduce glucose oxidation, glycolysis, and glycogenolysis. Evidence suggests that increasing cytosolic calcium concentrations may directly promote glucose use and glycolenolysis through stimulation of phosphofructokinase\textsuperscript{35} and glycogen phosphorylase kinase (see Taegtmeyer\textsuperscript{36} for review). Calcium channel blockade may act by indirectly overcoming Ca\textsuperscript{2+} stimulation of these two key regulatory enzymes. Calcium channel blockade may also affect glucose oxidation through the activity of the pyruvate dehydrogenase (PDH) complex. This enzyme, which catalyzes the first irreversible reaction in the mitochondrial oxidative pathway (conversion of pyruvate to acetyl coenzyme A), plays a key role in the regulation of myocardial glucose oxidative rates.\textsuperscript{37,38} PDH is regulated by reversible phosphorylation through PDH kinase and PDH phosphatase.\textsuperscript{37,38} Increasing intracellular Ca\textsuperscript{2+} concentrations during mechanical systole increases PDH phosphatase activity, which leads to an increase in PDH activity.\textsuperscript{38} Therefore TA-3090 may decrease glucose oxidation by decreasing calcium stimulation of PDH activity.

The lack of depression of contractile performance by TA-3090 is an attractive property that could enhance the possible clinical applications of this agent. In aerobic hearts, the effect of TA-3090 on PSP development was less marked than that reported for other calcium channel blockers, such as diltiazem, nifedipine, or verapamil, in isolated glucose-perfused Langendorff hearts.\textsuperscript{6,8,28–30} Diltiazem, for example, the least negatively inotropic of these three agents, exhibits significant depressant effects on myocardial contractility at a concentration of 0.1 μM. Although some differences exist between Langendorff and working perfused hearts as experimental models, our data suggest that the effect of TA-3090 on contractility is less than that of diltiazem by a factor of 5. Since TA-3090 is four to five times as potent as diltiazem on the vasculature,\textsuperscript{21} our data suggest that at vasoactive concentrations, TA-3090 is unlikely to exert any deleterious effect on myocardial contractile performance. In support of this, data obtained in intact animal models has shown that at concentrations that have no effect on heart rate or contractility, TA-3090 reduces both blood pressure and systemic vascular resistance.\textsuperscript{21}

**Limitations of the Present Study**

It is possible that TA-3090 could have an effect on coronary vasomotor tone and that this could have a salutary effect on reperfusion recovery by reducing the “no-reflow” phenomenon. The drug had no significant effect on coronary flow under aerobic conditions. We did not measure coronary flow during reperfusion in these experiments, but our previous investigations suggest that coronary flow during reperfusion is directly dependent on myocardial work load in this model.\textsuperscript{10,12} It is also conceivable that the increases in intracellular calcium concentrations seen in reperfused hearts were attenuated by TA-3090. This possibility does not negate the findings of the current study, however, and we are currently undertaking further studies of TA-3090 in hearts perfused with a solution containing 45Ca\textsuperscript{2+}. Finally, recent investigations have suggested that calcium channel blockade with diltiazem reduces lipid peroxidation during ischemia and perfusion.\textsuperscript{39} We did not attempt to quantitate lipid peroxidation in these hearts but acknowledge that TA-3090, like diltiazem, could have additional effects on membrane phospholipid turnover.

**Summary**

In summary, we have shown that the calcium channel blocker TA-3090 has a beneficial effect on ATP depletion and reperfusion recovery of ischemic hearts if added before the onset of ischemia. This beneficial effect occurs in the absence of major effects on myocardial work load under aerobic conditions. In addition, we provide direct evidence that calcium channel blockers inhibit glycolytic flux under ischemic conditions. As a result, part of the beneficial action of calcium channel blockade on ischemic myocardium may occur through a reduction of both energy demand during ischemia and glycolytic product accumulation during ischemia.

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Key Words • ischemia • calcium channel blocker • glucose oxidation • heart
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