Ischemic-reperfused isolated working mouse hearts: membrane damage and type IIA phospholipase A2

LEON J. DE WINDT, JODIL WILLEM, THEO H. M. ROEMEN, WILL A. COUMANS, ROBERT S. RENEMAN, GER J. VAN DER VUSSE, AND MARC VAN BILSEN

Department of Physiology, Cardiovascular Research Institute Maastricht, Maastricht University, 6200 MD Maastricht, The Netherlands

Received 17 May 2000; accepted in final form 23 January 2001

---

De Windt, Leon J., Jodil Willems, Theo H. M. Roemen, Will A. Coumans, Robert S. Reneman, Ger J. Van Der Vusse, and Marc Van Bilsen. Ischemic-reperfused isolated working mouse hearts: membrane damage and type IIA phospholipase A2. Am J Physiol Heart Circ Physiol 280: H2572–H2580, 2001.—For the murine heart the relationships between ischemia-reperfusion-induced loss of cardiac function, enzyme release, high-energy phosphate (HEP), and membrane phospholipid metabolism are ill-defined. Accordingly, isolated ejection murine hearts were subjected to varying periods of ischemia, whether or not followed by reperfusion. On reperfusion, hemodynamic function was almost completely restored after 10 min of ischemia (83 ± 14% recovery of cardiac output (CO), but was severely depressed after 15 and 20 min of ischemia (40 ± 24 and 31 ± 24% recovery of CO, respectively). Reperfusion was associated with partial recovery of HEP stores and enhanced degradation of phospholipids as indicated by the accumulation of fatty acids (FA). Myocardial FA content and enzyme release during reperfusion were correlated (r = 0.70), suggesting that membrane phospholipid degradation and cellular damage are closely related phenomena. To investigate the role of type IIA secretory phospholipase A2 (sPLA2) in this process, hearts from wild-type and sPLA2-deficient mice were subjected to ischemia-reperfusion. Posts ischemic functional recovery, ATP depletion, enzyme release, and FA accumulation were not significantly different between wild-type and sPLA2-deficient hearts. These findings argue against a prominent role of type IIA sPLA2 in the development of irreversible cell damage in the ischemic-reperfused murine myocardium.

---

The mouse is being increasingly used for the study of molecular mechanisms of cardiac dysfunction after ischemia-reperfusion due to the widespread availability of transgenic and gene-targeted models. The isolated heart preparation is particularly suitable to assess the impact of ischemia-reperfusion on functional and biochemical performance independent of potentially compensatory extracardiac factors. Ischemic tolerance of the isolated murine heart was found to vary considerably among various studies (3, 12, 17, 20, 21, 26, 36).
To explore the relationship among hemodynamic function, cell necrosis, energy status, and membrane damage in the murine heart in more detail, the severity of the ischemic insult was modulated by varying the duration of flow deprivation before reinstallation of flow. For these studies we used isolated hearts from Swiss mice, the functional characteristics of which were explored under normoxic conditions in a previous study (9). The isolated hearts from the wild-type C57BL/Ks strain and the type IIa sPLA₂-deficient C57BL/6 strain were then subjected to ischemia-reperfusion to assess the potential contribution of this enzyme in ischemia-reperfusion-induced cardiac injury and loss of function.

**MATERIALS AND METHODS**

**Chemicals.** Except for D(+)-glucose and pyruvate (Sigma; St. Louis, MO), all chemicals used for the Krebs-Henseleit solution were of the highest grade available and purchased from Merck (Darmstadt, Germany). Insulin was purchased from Novo Nordisk (Bagsvaerd, Denmark).

**Animals and surgery.** Adult female Swiss mice (Iffa Credo; Lyon, France) 3–4 mo of age were used for studies in which the duration of ischemia was varied. To assess the role of type IIa sPLA₂ in ischemia-reperfusion-induced cardiac injury adult male mice of the two closely related inbred C57/Bl substrains, namely C57BL/Ks (wild-type C57/Bl) and C57BL/6 (mutated pla2ga gene; B&K, Hull, UK), were used (15, 18, 24). The animals were allowed to adjust to the new housing conditions for 2 wk before experimental use. All mice were kept under standard housing conditions with an artificial 12-h light cycle with free access to standard rodent food (Diet SRM-A, Hope Farms; Woerden, The Netherlands) and tap water. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Maastricht University.

**PCR genotyping.** To confirm thymidine insertional mutation in exon 3 of the pla2ga gene in the inbred C57BL/6 strain and its absence in the inbred C57BL/Ks strain, exon 3 was amplified from a number of individual mice by PCR as described previously (14, 15, 19). Because the thymidine insertion disrupts a BamHI site in exon 3 of the Pla2ga gene, PCR products were digested with BamHI before gel electrophoresis. Briefly, genomic DNA was isolated from tails using a Qiamp genomic DNA prep kit (Qiagen; Leusden, The Netherlands). PCR analysis of type IIa sPLA₂ exon 3 was performed using the following primers: 5'-primer (5' -CTGGCT TTCTTCTCCTGTCAGCCTGGCC-3'), 3'-primer (5' -GGAAC CACCTGGGACACTGAGATTG-3'). Full-length genomic DNA products from both strains were of the expected length of 500 bp. The C57BL/6 exon 3 PCR fragments could not be digested by BamHI, indicating disruption of the wild-type BamHI site, whereas digestion of the C57BL/Ks PCR products resulted in two cleavage signals of 300 and 200 bp, respectively (data not shown). Later in this paper C57BL/Ks and C57BL/6 mice are referred to as sPLA₂(+/+) and sPLA₂(−/−) mice, respectively.

**Isolated ejecting mouse heart preparation.** The isolated ejecting mouse heart preparation used in the present study has been described in detail before (9). Briefly, animals were anesthetized with 50 mg/kg ip pentobarbital sodium (Nembutal, Sanofi-Sante; Maassluis, The Netherlands). After thoracotomy, the hearts were quickly excised and transferred to ice-chilled perfusion buffer (for composition see below). The ascending aorta was cannulated with a cannula matching the hemodynamic impedance characteristics of the murine ascending aorta (9). Retrograde perfusion at a perfusion pressure of 50 mmHg was started immediately after which the hearts resumed spontaneous beating. The left atrium was cannulated with a cannula through one of the lung veins. The recirculating modified Krebs-Henseleit perfusion buffer, pre-filtered by a microfilter (0.45 μm diameter, Millipore), had the following composition: 118 mM NaCl, 4.7 mM KCl, 3.0 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, 0.5 mM Na-EDTA, 10 mM D(+)-glucose, 1.5 mM sodium pyruvate, and 5 U/I insulin. The buffer was continuously gassed with 95% O₂-5% CO₂. Care was taken to maintain the temperature of the perfusate, and thus the heart, at 38.5°C.

**Hemodynamic data.** All hemodynamic variables were continuously recorded on a personal computer, using specialized software (Hemodynamic Data Acquisition System, Technical Department, Maastricht University), allowing the on-line acquisition, presentation, and calculation of left atrial filling flow, aortic flow (AOF), left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), diastolic aortic pressure, and the first maximal and minimal derivatives of left ventricular pressure (LV dP/dt max and LV dP/dt min). Left ventricular developed pressure (LVEDP) was defined as the difference between LVSP and LVEDP. Cardiac output (CO) was defined as the sum of AOFP and coronary flow (CF). CF was determined from the difference between AOFP as measured by an 1 N in-line aortic flow probe and left atrial filling flow, as measured by a 2 N in-line flow probe placed in the left atrial inflow tract. Calculated CF data were periodically checked by timed collection of the coronary perfusate. CF data were used to calculate coronary resistance, which was defined as aortic pressure divided by CF normalized for individual heart weights.

**Experimental protocol.** After an initial 10-min retrograde stabilization period (perfusion pressure 50 mmHg), antegrade perfusion was started by opening the left atrial conduit. Left atrial filling pressure was set at 10 mmHg, whereas diastolic aortic pressure was kept at 50 mmHg. Except for the ischemic period and the first 5 min of reperfusion, the hearts were paced artificially throughout the whole experiment at a frequency of 380 beats/min, a rate slightly higher than the intrinsic rate of the isolated hearts of the mouse strains tested.

The hearts were normoxically perfused for 20 min (preischemic period). Just before the ischemic period, the water-jacketed chamber was filled with warm perfusate solution (38.5°C) until the heart was completely submerged, pacing was stopped, and the aortic and atrial lines were clamped to create normothermic, global ischemia. After the ischemic period, the water-jacketed chamber was emptied, and hearts were reperfused retrogradely at a perfusion pressure of 50 mmHg for 10 min. Subsequently, the left atrial conduit was reopened, and the hearts were allowed to work in the antegrade mode at a preload pressure of 10 mmHg and a diastolic aortic pressure of 50 mmHg for an additional 50 min. In the first series of experiments, the hearts of the Swiss mice were subjected to either 10, 15, or 20 min of ischemia, whether or not followed by reperfusion. As potential strain differences have to be appreciated, the critical time duration whether or not followed by reperfusion.
membrane damage in ischemic-reperfused mouse hearts

Table 1. Preischemic hemodynamic values measured in isolated, left ventricular ejecting murine hearts from Swiss, C57BL/ks [sPLA$_2$ (+/+)]) and C57BL/6 [sPLA$_2$ (−/−)] mice

<table>
<thead>
<tr>
<th></th>
<th>Swiss</th>
<th>C57BL/6/sPLA$_2$ (+/+)</th>
<th>C57BL/6/sPLA$_2$ (−/−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HW/BW, mg/g</td>
<td>7.9 ± 0.6</td>
<td>8.6 ± 0.3</td>
<td>8.3 ± 0.5</td>
</tr>
<tr>
<td>CO, ml·min$^{-1}$·g$^{-1}$</td>
<td>50.0 ± 9.0</td>
<td>49.0 ± 8.9</td>
<td>52.3 ± 11.0</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>86 ± 9</td>
<td>85 ± 9</td>
<td>97 ± 15</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>7 ± 4</td>
<td>7 ± 4</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>LVDP, mmHg</td>
<td>77 ± 9</td>
<td>78 ± 9</td>
<td>91 ± 12</td>
</tr>
<tr>
<td>LV dP/dt$_{max}$, mmHg/s</td>
<td>5,652 ± 831</td>
<td>6,461 ± 1,398</td>
<td>8,578 ± 1,734†</td>
</tr>
<tr>
<td>LV dP/dt$_{min}$, mmHg/s</td>
<td>−3,054 ± 415</td>
<td>−3,435 ± 434</td>
<td>−4,102 ± 545†</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD (n = 9–13 hearts). Isolated hearts were perfused at a pre- and afterload pressure of 10 and 50 mmHg, respectively. Hearts were paced at 380 beats/min. HW, heart weight; BW, body weight; CO, cardiac output; CF, coronary flow; SV, stroke volume; LVDP, left ventricular developed pressure; LVSP, left ventricular systolic pressure, LVEDP, left ventricular end-diastolic pressure; LV dP/dt$_{max}$, positive maximal first derivative of left ventricular pressure; LV dP/dt$_{min}$, negative maximal first derivative of left ventricular pressure. * $P < 0.05$ vs. Swiss mice, † $P < 0.05$ vs. sPLA$_2$ (+/+).

Preischemic cardiac function. Hemodynamic function of the normoxically perfused isolated hearts of the Swiss outbred strain and the two C57BL substrains are presented in Table 1. Heart weight-to-body weight ratios and CO, normalized to heart weight, were comparable for each (sub)strain (Table 1). In hearts from C57BL/Ks mice, CF was significantly lower than in hearts from Swiss mice. CF and LVDP tended to be higher in the hearts from C57BL/6 mice compared with C57BL/Ks mice, but the differences were not significant (Table 1). LV dP/dt$_{max}$ and LV dP/dt$_{min}$, however, were significantly higher in C57BL/6 than in C57BL/Ks hearts.

Functional recovery. To test the ischemic tolerance of the hearts of Swiss mice the duration of the ischemic episode was varied. Following 10 min of global ischemia and 60 min of reperfusion, functional recovery was almost complete (Fig. 1). CO recovered to 83 ± 14% of its preischemic value ($P < 0.05$ vs. preischemia), and CF returned to preischemic values (Fig. 2). The recovery of LVDP, LV dP/dt$_{max}$, and LV dP/dt$_{min}$ ranged from 80 to 90% of their preischemic values (Fig. 2).

Extending the ischemic duration from 10 to 15 min resulted in a markedly depressed recovery of CO (Figs. 1 and 2). Postischemic CO and LVDP amounted to 40 ± 24 and 62 ± 18% of their respective preischemic values ($P < 0.05$ vs. preischemia). Recovery of LV dP/dt$_{max}$ and LV dP/dt$_{min}$ was also more severely depressed after 15 min of ischemia. Functional recovery was severely impaired after 20 min of ischemia. CO recovered to only 31 ± 24% of its preischemic value (Figs. 1 and 2). Additionally, CF remained seriously depressed during the entire reperfusion phase. Because of a marked rise in end-diastolic pressure and a reduction in systolic function, LVDP recovered to only 28 ± 21% of its preischemic value (Fig. 2).

Enzyme and lactate release. Preischemic LDH release was low in all groups and amounted to 166 ± 9 mU·min$^{-1}$·g$^{-1}$. Following 10 min of global ischemia, a small additional release of LDH was observed during reperfusion. The specific activity of lactate dehydrogenase (LDH) activity in coronary effluent samples was determined for each group (final concentration 3%).

Biochemical analysis. LDH and lactate content in the coronary perfusate were assessed spectrophotometrically using a Cobas Bio autoanalyzer as described earlier (1, 2). Tissue contents of adenosine and guanine nucleotides, inosine monophosphate, and (oxy)purines were determined by high-performance liquid chromatography (Varian Vista 5500 HPLC) as previously described (30). The determination of cardiac FA, PL, and triacylglycerols (TG) was performed as described in detail earlier (29, 34).

Statistical analysis. The results are presented as means ± SD. All statistical analyses were performed using InStat 3.0 software (GraphPad Software; San Diego, CA). Changes in the hemodynamic parameters in time were statistically analyzed by repeated measures ANOVA with Dunnett’s post hoc correction test for multiple comparisons. Differences between values of hemodynamic and biochemical parameters of experimental groups were analyzed using one-way ANOVA, followed by Tukey’s test. Linear regression was performed with the least squares method and the Pearson rank correlation coefficient ($r$) was used to estimate the strength of the relation between the two variables. In all tests, significance was accepted at $P < 0.05$.

RESULTS

Preischemic cardiac function. Hemodynamic function of the normoxically perfused isolated hearts of the Swiss outbred strain and the two C57BL substrains were comparable for each (sub)strain (Table 1). In hearts from C57BL/Ks mice, CF was significantly lower than in hearts from Swiss mice. CF and LVDP tended to be higher in the hearts from C57BL/6 mice compared with C57BL/Ks mice, but the differences were not significant (Table 1). LV dP/dt$_{max}$ and LV dP/dt$_{min}$, however, were significantly higher in C57BL/6 than in C57BL/Ks hearts.
The tissue content of adenine nucleotide degradation products, i.e., adenosine, inosine, hypoxanthine, and xanthine, was very low before ischemia (Table 2).

During 10 and 15 min of ischemia, tissue ATP content progressively decreased, whereas ADP and AMP levels increased (Fig. 3). Extending the ischemic duration to 20 min was associated with further reductions in ATP, a decrease in ADP content and a marked increase in tissue AMP levels. Adenine nucleotide degradation products, in particular adenosine and inosine, increased as a function of the duration of ischemia (Table 2).

Restoration of flow for 60 min resulted in an almost complete recovery of tissue ATP levels after 10 min of ischemia. In contrast, after an ischemic insult of 15 or 20 min only partial restoration of tissue ATP content was observed (Fig. 3). Tissue ADP and AMP levels returned to preischemic values irrespective of the duration of the preceding ischemic episode (Fig. 3). Restoration of coronary flow after 10 as well as 15 min of ischemia resulted in a removal of adenine degradation products that had accumulated in the flow-deprived tissue. However, in hearts subjected to 20 min of ischemia and 60 min of reperfusion, the tissue content of (oxy)purines remained elevated (Table 2). The tissue content of lactate in reperfused hearts was found to be 15.4 ± 11.3, 21.0 ± 9.6, and 41.1 ± 24.8 μmol/g dry wt after 10, 15, and 20 min of ischemia, respectively. The finding of markedly enhanced tissue levels of lactate and adenine degradation products in hearts subjected to 20 min of ischemia plus reperfusion corroborates the lack of adequate recovery of coronary flow during reperfusion as observed in this experimental group.

Tissue FA, TG, and PL. The preischemic tissue content of FA, PL, and TG in the hearts of Swiss mice and their relative FA composition are shown in Table 3. The total amount of FA esterified to the glycerol backbone via an ether bond (plasmalogens) averaged 3.6% of the total PL pool. Palmitic (16:0), stearic (18:0), linoleic (18:1), and docosahexaenoic acid (22:6) were the most abundant fatty acyl moieties in the FA as well as in the PL pool, each species accounting for 10–20% of total FA present (Table 3). The polyunsaturated FA arachidonic acid (20:4) accounted for 6.7 ± 0.7 and 4.0 ± 0.4% of total fatty acyl moieties in the PL and FA pool, respectively (Table 3). Cardiac TG mainly consisted of 16:0, 18:1, and 18:2, each representing about 30% of all TG fatty acyl moieties. In contrast to the FA and PL pool, 18:0, 20:4, and 22:6 accounted for only a small percentage of FA esterified in the TG pool (Table 3).

Irrespective of the duration of the ischemic insult, the tissue content of TG and PL did not change significantly during ischemia or the subsequent period of reperfusion (data not shown). The total cardiac (unesterified) FA content, however, transiently declined during ischemia. This initial reduction in total FA content was associated with marked changes in the percent mole of individual FA species (Table 4). In general, the percent mole of saturated FA species increased, whereas the percent mole of polyunsaturated FAs declined. In this respect, the shift in the percent mole of

![Graph](http://ajpheart.physiology.org/)

**Fig. 2.** Percentage recovery of CO, coronary flow (CF), left ventricular developed pressure (LVDP), and first maximal and minimal derivative of left ventricular pressure (LV dP/dt max and LV dP/dt min, respectively) after 60 min of reperfusion after 10 (open bars), 15 (hatched bars), and 20 min (solid bars) of ischemia. Data are expressed as percentage of corresponding values measured before induction of ischemia and means ± SD (n = 10–11 per group). *P < 0.05 vs. preischemic value. †P < 0.05 vs. hearts subjected to 10 min of ischemia plus 60 min of reperfusion.
16:0 and 22:6 was most pronounced. Reperfusion itself was associated with a marked increase in tissue FA content (Table 4). Accumulation of FA was most pronounced in reperfused hearts previously subjected to 20 min of ischemia. Irrespective of the duration of the preceding ischemic episode, reperfusion was accompanied by a substantial rise in the tissue content of 18:2, 20:4, and 22:6, both in absolute amounts and in the percentage of contribution to the total FA pool (Table 4).

Involvement of type IIA sPLA₂. To test whether endogenous cardiac type IIA sPLA₂ might play a role in ischemia-reperfusion-induced cardiac damage, a functional and biochemical analysis was performed on ischemia-reperfused isolated ejecting hearts of sPLA₂(1/1) and sPLA₂(2/2) C57/BL substrains. Following 17.5 min of global, normothermic ischemia and 60 min of reperfusion, CO recovered to 39 ± 25% and 37 ± 23% of the corresponding preischemic values in sPLA₂(2/2) and sPLA₂(1/1) hearts, respectively (Fig. 4). CF recovered to preischemic values in both groups. In contrast, postischemic LVDP, LV dP/dt max, and LV dP/dt min were substantially reduced, but to similar extents in sPLA₂(2/2) and sPLA₂(1/1) hearts (Fig. 4).

No significant differences in postischemic irreversible cell injury were observed in sPLA₂(1/1) and sPLA₂(2/2) hearts. Preischemic LDH release was low and comparable in both sPLA₂(2/2) and sPLA₂(1/1) hearts (0.15 ± 0.05 and 0.16 ± 0.11 U·min⁻¹·g⁻¹, respectively; not significant, NS). Cumulative LDH re-

### Table 2. Tissue content of adenine nucleotide degradation products in preischemic, ischemic, and ischemic-reperfused Swiss mouse hearts

<table>
<thead>
<tr>
<th></th>
<th>Pre-I (n = 6)</th>
<th>10 min I (n = 6)</th>
<th>10 min I + 60 min R (n = 10)</th>
<th>15 min I (n = 6)</th>
<th>15 min I + 60 min R (n = 9)</th>
<th>20 min I (n = 5)</th>
<th>20 min I + 60 min R (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.07 ± 0.02</td>
<td>0.82 ± 0.35*</td>
<td>0.18 ± 0.22†</td>
<td>1.07 ± 0.57*</td>
<td>0.06 ± 0.09†</td>
<td>1.87 ± 0.45*</td>
<td>0.35 ± 0.20†</td>
</tr>
<tr>
<td>I</td>
<td>ND</td>
<td>0.71 ± 0.26*</td>
<td>0.01 ± 0.03†</td>
<td>1.27 ± 0.65*</td>
<td>0.29 ± 0.44†</td>
<td>1.85 ± 0.38*</td>
<td>1.09 ± 1.29</td>
</tr>
<tr>
<td>HX</td>
<td>0.04 ± 0.02</td>
<td>0.21 ± 0.04*</td>
<td>0.03 ± 0.05†</td>
<td>0.34 ± 0.08*</td>
<td>0.11 ± 0.23†</td>
<td>0.48 ± 0.14*</td>
<td>0.40 ± 0.43</td>
</tr>
<tr>
<td>X</td>
<td>ND</td>
<td>0.02 ± 0.02</td>
<td>0.08 ± 0.16</td>
<td>0.08 ± 0.07</td>
<td>0.04 ± 0.10</td>
<td>0.21 ± 0.12</td>
<td>0.15 ± 0.28</td>
</tr>
<tr>
<td>Total</td>
<td>0.11 ± 0.04</td>
<td>1.76 ± 0.51*</td>
<td>0.30 ± 0.41†</td>
<td>2.76 ± 1.34*</td>
<td>0.50 ± 0.77†</td>
<td>4.41 ± 0.72*</td>
<td>1.98 ± 1.90†</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD. Presented are individual purines in μmol/g dry wt ventricular tissue. n indicates number of hearts analyzed. Pre-I, Preischemia; I, ischemia; R, reperfusion. Total refers to the sum of adenosine (A), inosine (I), hypoxanthine (HX), and xanthine (X) (expressed as μmol/g dry wt). *P < 0.05 vs. preischemic value, †P < 0.05 vs. corresponding end-ischemic value. ND, not detectable.
and 171
6
6
mic values of ventricular ATP content in sPLA2(−/−) and sPLA2(+/+) hearts, respectively. The preischemic TG content amounted to 31 ± 7 and 36 ± 10 μmol/g dry wt for sPLA2(−/−) and sPLA2(+/+) hearts, respectively. The tissue content as well as the fatty acyl composition of both esterified lipid pools did not differ between preischemic hearts from both strains, nor did they change during ischemia-reperfusion (data not shown).

Preischemic unesterified FA content in hearts of sPLA2(−/−) and sPLA2(+/+) mice amounted to 334 ± 127 and 198 ± 80 nmol/g dry wt, respectively (NS). In the reperfused hearts of both sPLA2(−/−) and sPLA2(+/+) mice, significant amounts of FA accumulated. However, between the two groups, significant differences in total FA content were not observed (Fig. 5). Significant differences in the tissue content of 20:4, a sensitive marker reflecting membrane PL degradation, were also not found (Fig. 5).

DISCUSSION

In the present study, the ischemia tolerance of the murine heart defined as functional and biochemical changes was first investigated. It is demonstrated that hearts from the Swiss strain tolerate 10 min of ischemia well, as evidenced by a near-complete recovery of hearts, respectively. The preischemic TG content amounted to 31 ± 7 and 36 ± 10 μmol/g dry wt for sPLA2(−/−) and sPLA2(+/+) hearts, respectively. The tissue content as well as the fatty acyl composition of both esterified lipid pools did not differ between preischemic hearts from both strains, nor did they change during ischemia-reperfusion (data not shown).

Preischemic unesterified FA content in hearts of sPLA2(−/−) and sPLA2(+/+) mice amounted to 334 ± 127 and 198 ± 80 nmol/g dry wt, respectively (NS). In the reperfused hearts of both sPLA2(−/−) and sPLA2(+/+) mice, significant amounts of FA accumulated. However, between the two groups, significant differences in total FA content were not observed (Fig. 5). Significant differences in the tissue content of 20:4, a sensitive marker reflecting membrane PL degradation, were also not found (Fig. 5).

DISCUSSION

In the present study, the ischemia tolerance of the murine heart defined as functional and biochemical changes was first investigated. It is demonstrated that hearts from the Swiss strain tolerate 10 min of ischemia well, as evidenced by a near-complete recovery of hearts, respectively. The preischemic TG content amounted to 31 ± 7 and 36 ± 10 μmol/g dry wt for sPLA2(−/−) and sPLA2(+/+) hearts, respectively. The tissue content as well as the fatty acyl composition of both esterified lipid pools did not differ between preischemic hearts from both strains, nor did they change during ischemia-reperfusion (data not shown).

Preischemic unesterified FA content in hearts of sPLA2(−/−) and sPLA2(+/+) mice amounted to 334 ± 127 and 198 ± 80 nmol/g dry wt, respectively (NS). In the reperfused hearts of both sPLA2(−/−) and sPLA2(+/+) mice, significant amounts of FA accumulated. However, between the two groups, significant differences in total FA content were not observed (Fig. 5). Significant differences in the tissue content of 20:4, a sensitive marker reflecting membrane PL degradation, were also not found (Fig. 5).

DISCUSSION

In the present study, the ischemia tolerance of the murine heart defined as functional and biochemical changes was first investigated. It is demonstrated that hearts from the Swiss strain tolerate 10 min of ischemia well, as evidenced by a near-complete recovery of hearts, respectively. The preischemic TG content amounted to 31 ± 7 and 36 ± 10 μmol/g dry wt for sPLA2(−/−) and sPLA2(+/+) hearts, respectively. The tissue content as well as the fatty acyl composition of both esterified lipid pools did not differ between preischemic hearts from both strains, nor did they change during ischemia-reperfusion (data not shown).

Preischemic unesterified FA content in hearts of sPLA2(−/−) and sPLA2(+/+) mice amounted to 334 ± 127 and 198 ± 80 nmol/g dry wt, respectively (NS). In the reperfused hearts of both sPLA2(−/−) and sPLA2(+/+) mice, significant amounts of FA accumulated. However, between the two groups, significant differences in total FA content were not observed (Fig. 5). Significant differences in the tissue content of 20:4, a sensitive marker reflecting membrane PL degradation, were also not found (Fig. 5).

DISCUSSION

In the present study, the ischemia tolerance of the murine heart defined as functional and biochemical changes was first investigated. It is demonstrated that hearts from the Swiss strain tolerate 10 min of ischemia well, as evidenced by a near-complete recovery of hearts, respectively. The preischemic TG content amounted to 31 ± 7 and 36 ± 10 μmol/g dry wt for sPLA2(−/−) and sPLA2(+/+) hearts, respectively. The tissue content as well as the fatty acyl composition of both esterified lipid pools did not differ between preischemic hearts from both strains, nor did they change during ischemia-reperfusion (data not shown).

Preischemic unesterified FA content in hearts of sPLA2(−/−) and sPLA2(+/+) mice amounted to 334 ± 127 and 198 ± 80 nmol/g dry wt, respectively (NS). In the reperfused hearts of both sPLA2(−/−) and sPLA2(+/+) mice, significant amounts of FA accumulated. However, between the two groups, significant differences in total FA content were not observed (Fig. 5). Significant differences in the tissue content of 20:4, a sensitive marker reflecting membrane PL degradation, were also not found (Fig. 5).

DISCUSSION

In the present study, the ischemia tolerance of the murine heart defined as functional and biochemical changes was first investigated. It is demonstrated that hearts from the Swiss strain tolerate 10 min of ischemia well, as evidenced by a near-complete recovery of
imperative function in the Langendorff-perfused mouse heart in these studies ranged from 20 min (20) to 50 min (17). Factors likely to play a role in the spread in the observations are variations in nominally free Ca\(^{2+}\) concentrations in the perfusate, choice of substrates, temperature of the isolated hearts during global ischemia, differences in workload (perfusion pressure, retrograde versus antegrade perfusion), and strain differences. The present findings are in line with the earlier expressed notion of a higher susceptibility of the mouse heart to ischemia-reperfusion-induced damage compared with other species, such as the rat (29).

Energy metabolism and lipid homeostasis. Prolonged duration of ischemia results in a substantial reduction of ATP (<25% of preischemic value) and accumulation of AMP and (oxy)purines, mainly adenosine and inosine in the myocardium. Comparable findings were obtained in the isolated working rat heart subjected to varying periods of ischemia-reperfusion (30). The increase of ATP levels after restoration of flow indicates that mitochondrial ATP production is not seriously compromised in the surviving cells of the postischemic heart. The data suggest that the reduced total adenine nucleotide pool (sum of ATP, ADP, and AMP) at the end of reperfusion results mainly from washout of its degradation products, which prevents fast resynthesis of the parent adenine nucleotides.

The decrease in tissue FA levels during initial ischemia might point to subtle changes in the turnover of PL and TG in the initial ischemic phase. This finding is in accordance with previous observations in the ischemic rat heart, in which the transient decline in tissue FA content was associated with the accumulation of glyceral, which indicates increased TG-FA cycling (29, 32). The observation that the arachidonoyl content of the myocardial (unesterified) FA pool increased when the duration of ischemia is prolonged, is in favor of a switch from increased TG cycling to enhanced deacylation and/or decreased reacylation of the PL pool during more prolonged ischemia (Table 4) (5, 29). The substantial contribution of polyunsaturated FA such as arachidonic acid and docosahexaenoic acid relative to saturated FA is in agreement with an accelerated net degradation of the cardiac PL pool during the reperfusion phase (5, 8, 27, 29) and is indicative for phospholipase \(A_2\)-mediated membrane PL degradation.

Fatty acid accumulation, sPLA\(_2\), and irreversible cell damage. The release of the cytosolic enzyme LDH during reperfusion was taken as a measure of the loss of cellular integrity. The strong positive correlation \((r = 0.78)\) between the amount of LDH released during reperfusion and ventricular arachidonic acid accumulation during reperfusion supports the notion of a causative relation between accelerated PL degradation during ischemia-reperfusion and irreversible cell damage (13, 29, 32, 33).

To test whether type IIA sPLA\(_2\) known to be present in cardiac myocytes (10) plays a prominent role in postischemic cardiac PL degradation, the ischemia-reperfusion tolerance of type IIA sPLA\(_2\)-deficient mice was investigated. This mouse model already enabled
investigators to explore the role of this sPLA₂ isoform in prostaglandin synthesis (22, 28), in intestinal tumorigenesis (7) and the gastric epithelial apoptotic response to *Helicobacter* infection (35). We hypothesized that the absence of type IIA sPLA₂ activity would attenuate myocardial ischemia-reperfusion-induced membrane PL degradation and thus irreversible cell damage and improve postischemic functional recovery.

Type IIA sPLA₂ has been implicated as a candidate for cardiac ischemia-reperfusion-induced membrane PL degradation because of its Ca²⁺ dependency and nonspecific hydrolytic action toward the acylglycerol bonds of PL (23, 29, 31). Previous studies (8, 27, 32) have demonstrated that antibodies that recognize type II sPLA₂ and pharmacological inhibition of type IIA sPLA₂ resulted in a reduction of both ischemia-reperfusion-induced membrane degradation and cellular damage of the rat heart.

A potential experimental caveat might be that some differences in preischemic hemodynamic function were apparent between the experimental groups (see Table 1). The two inbred substrains were specifically selected for their high degree of genetic similarity (15, 18, 24). It cannot be fully excluded, however, that the remaining genetic differences might account for undetermined phenotypical differences affecting hemodynamic function and/or cardiac ischemia-reperfusion susceptibility between the two substrains.

The present study clearly shows that postischemic cardiac accumulation of total unesterified FA in general and arachidonic acid in particular did not differ between the two substrains, indicating that a crucial role of type IIA sPLA₂ in ischemia-reperfusion-induced myocardial cell damage is less likely. One implication of the current observation is that PLA₂β other than type IIA sPLA₂ must be responsible for the enhanced PL degradation in the transiently ischemic heart. Indeed, previous studies employing synthetic PLA₂ inhibitors strongly suggest that multiple PLA₂ types are likely to be involved (13). In this context, the recent identification of another low-molecular-weight, Ca²⁺-dependent sPLA₂ isofrom (type V) with limited substrate preference but abundantly present in the heart is of interest (4, 23). It is feasible that type IIA and type V sPLA₂ fulfill redundant functions in the heart as far as ischemia-reperfusion-induced PL degradation is concerned.

In conclusion, the isolated, antegradeley perfused, ejecting mouse heart model as described in the present study allows detailed evaluation of the functional and metabolic consequences of transient ischemia. Furthermore, the findings in the present study provide evidence against a decisive role of type IIA sPLA₂ in ischemia-reperfusion-induced PL degradation, cellular injury and cardiac dysfunction.

This work was supported by Grant 900–516–160 of The Netherlands Foundation of Scientific Research (NWO). M. van Bilsen is an Established Investigator of The Netherlands Heart Foundation.

**REFERENCES**


