Cholesterol diet-induced hyperlipidemia impairs the cardioprotective effect of postconditioning: role of peroxynitrite

Krisztina Kupai,1,2‡ Csaba Csonka,1,2‡ Veronika Fekete,1,2 Louise Odendaal,3 Jacques van Rooyen,3 De Wet Marais,4 Tamás Csont,1,2 and Péter Ferdinandy1,2

1Cardiovascular Research Group, Department of Biochemistry, University of Szeged, and 2PharmaHungary Group, Szeged, Hungary; 3Experimental Anti-Oxidant Research Group, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Cape Town; 4Nutritional Intervention Research Unit, Medical Research Council, Cape Town, South Africa

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Kupai K, Csonka C, Fekete V, Odendaal L, van Rooyen J, Marais DW, Csont T, Ferdinandy P. Cholesterol diet-induced hyperlipidemia impairs the cardioprotective effect of postconditioning: role of peroxynitrite. Am J Physiol Heart Circ Physiol 297:H1729–H1735, 2009. First published September 4, 2009; doi:10.1152/ajpheart.00484.2009.—The aim of the present study was to investigate if hyperlipidemia interferes with the infarct size-limiting effect of postconditioning and to study the involvement of peroxynitrite in this phenomenon. Rats were fed a 2% cholesterol-enriched or normal diet for 12 wk. Infarct size by triphenyltetrazolium chloride staining was measured in hearts isolated from both groups and subjected to 30 min coronary occlusion followed by 120 min reperfusion with or without the postconditioning protocol induced by six cycles of 10 s coronary occlusion and 10 s reperfusion at the onset of the reperfusion. Postconditioning significantly decreased infarct size in the normolipidemic but not in the hyperlipidemic group. Postconditioning increased cardiac 3-nitrotyrosine concentration (a marker for peroxynitrite formation) in the normal but not in the cholesterol-fed group when measured at the 5th min of reperfusion. Next, we tested if the postconditioning-induced acute increase in peroxynitrite is involved in the cardioprotection in normolipidemic animals in separate experiments. Postconditioning failed to decrease infarct size in the presence of the peroxynitrite decomposition catalyst 5,10,15,20-tetrakis-[4-sulfonatophenyl]-porphyrinato-iron [III] (20 mg/l) in normolipidemic animals. We conclude that an early increase in myocardial peroxynitrite formation triggers the cardioprotective effect of postconditioning-induced early increase in myocardial peroxynitrite formation.

postconditioning; infarct; cholesterol; heart; peroxynitrite

ISCHEMIC HEART DISEASE is the leading cause of death in the industrialized world. Although ischemic heart disease in humans is a complex disorder caused by or associated with other systemic diseases and conditions, most experimental studies on cardioprotection have been undertaken in healthy juvenile animal models, in which ischemia-reperfusion is imposed in the absence of other disease processes and risk factors, including hyperlipidemia, atherosclerosis, hypertension, diabetes, insulin resistance, heart failure, and aging (9). In these diseases and aging, the pathological processes are associated with fundamental molecular alterations that can potentially affect the development of ischemia-reperfusion injury per se and responses to cardioprotective interventions such as ischemic preconditioning, i.e., brief exposure to ischemia-reperfusion before sustained ischemia (21), and postconditioning, i.e., brief repetitive episodes of ischemia-reperfusion at the immediate onset of reperfusion (22) (see, for a recent extensive review, Ref. 9).

Although existing data in the literature are still somewhat contradicting, the majority of the studies show that experimental hyperlipidemia independently from the development of coronary atherosclerosis interferes with the cardioprotective effect of preconditioning (see, for reviews, Refs. 5, 9, and 10). The loss of preconditioning has been shown in hypercholesterolemic humans as well (32). However, very little is known about the effect of postconditioning in hyperlipidemia. Iliodromitis et al. (15) have recently shown that the infarct size-limiting effect of postconditioning is lost in rabbits with experimental hyperlipidemia and atherosclerosis, but the paper by Donato et al. (4) has not confirmed this result.

The mechanisms by which hyperlipidemia may interfere with cardioprotective mechanisms are still not known; however, a decrease in cardiac nitric oxide content because of altered nitrosative stress has been observed (3, 11, 14, 31). Nitrosative stress is induced by formation of peroxynitrite by the nonenzymatic reaction of nitric oxide and superoxide (6, 25). We and others have previously shown that an early increase in myocardial peroxynitrite formation triggers the development of cardioprotection by preconditioning (1, 2, 8, 18). However, it is not known if peroxynitrite-induced nitrosative stress contributes to postconditioning and if hyperlipidemia via alteration of nitrosative stress may lead to the loss of the cardioprotective effect of postconditioning.

Therefore, here we examined if 1) experimental hyperlipidemia induced by cholesterol-enriched diet interferes with the cardioprotective effect of postconditioning, 2) peroxynitrite-induced nitrosative stress may trigger the cardioprotective effect of postconditioning, and 3) alteration of nitrosative stress signal by experimental hyperlipidemia may contribute to the possible loss of postconditioning effect.

MATERIALS AND METHODS

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (National Institutes of Health publication 85–23, revised 1996), and it was approved by a local animal ethics committee of the University of Szeged.

Experimental design and infarct size measurements. Six-week-old male Wistar rats were fed normal (n = 61) or 2% cholesterol-enriched rat chow (n = 58) for 12 wk. Wistar rats were chosen for the study since this species shows a moderate increase in serum cholesterol and...
triglyceride level due to a high-cholesterol diet and no substantial atherosclerosis develops; therefore, the direct myocardial effect of hyperlipidemia, independent from atherosclerosis, can be studied in this model (11, 24). At the end of the 12-wk diet, serum cholesterol and triglyceride were measured. In separate experiments, cholesterol (n = 7–8) and free fatty acid levels (n = 4 in each group) were measured in myocardial tissue samples from normolipidemic and hyperlipidemic animals.

At the end of the diet period, animals were anesthetized with diethyl ether and given 500 U/kg heparin. Hearts were isolated and perfused with Krebs-Henseleit buffer according to Langendorff with constant pressure as described (24). Regional ischemia was induced by 30 min coronary occlusion followed by 120 min reperfusion. Postconditioning was induced by six consecutive cycles of 10 s coronary occlusion and 10 s reperfusion immediately at the onset of reperfusion (Fig. 1). Cardiac electrogram was monitored during the study to measure heart rate and incidence of reperfusion-induced ventricular fibrillation (VF). At the end of the 120 min reperfusion, infarct size was determined by triphenyltetrazolium chloride staining and evaluated by planimetry (Infarct size 1.0; Pharmahungary, Szeged, Hungary). Infarct size was expressed in percentage of area at risk.

In separate experiments, hearts from control and cholesterol-fed groups were subjected to 30 min coronary occlusion followed by 5 min reperfusion with or without of postconditioning, and left ventricular tissue was sampled, homogenized, and used for biochemical measurements (Fig. 1).

To check if peroxynitrite plays a role in the cardioprotective mechanism of postconditioning, in separate experiments, normolipidemic hearts were subjected to 30 min regional ischemia followed by 30 min coronary occlusion and 120 min reperfusion. Postconditioning was induced by six consecutive cycles of 10 s coronary occlusion and 10 s reperfusion immediately at the onset of reperfusion. At the end of the 120-min reperfusion, infarct size was measured as described above.

In a separate set of experiments, to check if a stronger stimulus of postconditioning could postcondition the heart, hearts were isolated from normolipidemic and hyperlipidemic groups and subjected to 30 min coronary occlusion and 120 min reperfusion with or without a postconditioning protocol of 12 cycles of 10 s coronary occlusion and 10 s reperfusion. At the end of the 120-min reperfusion, infarct size was determined as mentioned above.

Measurement of lipids. Serum cholesterol and triglyceride were measured by a colorimetric assay (Triglyceride PAP and Cholesterol PAP assay; Diagnosticum). Tissue cholesterol was measured (Cholesterol/Cholesterol Ester Quantification kit; BioVision) from ventricular homogenates. For measurements of cardiac free fatty acids, ventricular homogenates were extracted with chloroform-methanol (18 ml; 2:1; vol/vol) according to a modified method of Folch et al. (12). All of the extraction solvents contained 0.01% butylated hydroxytoluene as an antioxidant. Free fatty acids were separated from the other fraction by thin-layer chromatography (TLC) on precoated silica gel 60 plates (10 × 10 cm) without a fluorescence indicator (1.05721, Merck) using the solvent system petroleum benzene (bp 40–60 °C)-diethyl ether (peroxide free)-acetic acid (90:30:1, by vol) as solvent (13). The lipid bands containing phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, and sphingomyelin were visualized with long-wave ultraviolet light after spraying the plates with chloroform-methanol (1:1, by vol) containing 2,5-bis-(5′-tert-butylbenzoxazolyl-[2′][2′][2]thiophene (10 mg/100 ml; Sigma Chemical).

Assessment of cardiac peroxynitrite-induced nitrosative stress. Cardiac free and protein-bound 3-nitrotyrosine content, as markers for peroxynitrite-induced nitrosative stress, were measured at the 5th min of reperfusion.

Cardiac free 3-nitrotyrosine level was measured by enzyme-linked immunosorbent assay (ELISA; Cayman Chemical) from control and

**Fig. 1.** Experimental protocol. Control and 2% cholesterol-enriched chow-fed animals were subjected to 30 min coronary occlusion and 120 min reperfusion. Postconditioning was induced by 6 × 10 s cycles of coronary occlusion and reperfusion. Infarct size was measured at the 120th min of reperfusion. In separate experiments, hearts were frozen in liquid nitrogen at the 5th min of reperfusion for 3-nitrotyrosine measurements. In separate groups of normolipidemic hearts, the peroxynitrite decomposition catalyst 5,10,15,20-tetrakis-4-sulfonatophenyl)-porphyrinato-iron[III] (FeTPPS; 20 mg/l, n = 10). The dose of 20 mg/L FeTPPS was selected according to our previous studies (7, 24). FeTPPS is a ferric porphyrin complex that catalytically isomerizes peroxynitrite to nitrate. FeTPPS does not directly react with superoxide or nitric oxide; therefore, it is considered as a selective peroxynitrite decomposition catalyst (17, 20, 29). Krebs-Henseleit solution contained FeTPPS only during the last 20 s of the 30-min ischemic period and during postconditioning. At the end of the 120-min reperfusion period, infarct size was measured as described above.

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Cardiac free 3-nitrotyrosine level was measured by enzyme-linked immunosorbent assay (ELISA; Cayman Chemical) from control and
cholesterol-fed heart tissue samples. Briefly, supernatants of ventricu-
lar tissue homogenate was incubated overnight with anti-nitroty-
orosine rabbit IgG specific to free 3-nitrotyrosine and nitrotyrosine
acetylcholinesterase tracer in precoated (mouse anti-rabbit IgG) mi-
croplates followed by development with Ellman’s reagent. Free nit-
rotyrosine content was normalized to protein content of cardiac homogenate and expressed as nanograms per milligram protein.

To measure the abundance of 3-nitrotyrosine-protein adducts, we
performed SDS-PAGE Western blot. Heart tissues were homogenized
and centrifuged. Protein concentrations of supernatants were mea-
sured by the bicinchoninic acid assay. Equal amounts (20 μg) of
proteins were separated by 10% SDS-PAGE and transferred to nitro-
cellulose membrane (Amersham), and the blot was blocked in Tris-
buffered saline/Tween 20 supplemented with 5% nonfat dry milk
overnight. The primary 3-nitrotyrosine antibody (MAB5404; Chemi-
on International) was used at the manufacturer-recommended dilu-
tion. Membrane was developed with an enhanced chemiluminescence
kit (ECL Plus; GE Healthcare), exposed to X-ray film, and scanned.

Statistical analysis. Results were expressed as means ± SE when
appropriate. Differences among means were analyzed by using one-
way ANOVA followed by Tukey’s post hoc test. The incidence of VF
was analyzed by Fisher’s exact test. Differences were considered
significant at \( P < 0.05 \).

RESULTS

At the end of the 12-wk diet, the body weight of the
animals were 400–580 g, and there was no significant
difference between groups. High-cholesterol diet did not
affect basal hemodynamic parameters except heart rate,
which was lower in the hyperlipidemic group at the end of
reperfusion after 12 × 10 s cycles postconditioning. The
diet did not affect the area at risk (Fig. 2B and see Table 3).

In the cholesterol-fed group, plasma cholesterol, triglyc-
eride, heart weight, and tissue dihomo-γ-linolenic acid were
increased significantly (Tables 1 and 2).

To examine if experimental hyperlipidemia interferes
with the cardioprotective effect of postconditioning, infarct size and
the incidence of reperfusion-induced VF were assessed in rat
hearts with coronary occlusion in both normolipidemic and
hyperlipidemic rats. Postconditioning with 6 × 10 s cycles
significantly decreased infarct size and the incidence of VF in
hearts of rats with normolipidemic diet. However, in heart of
cholesterol-fed animals, infarct size and incidence of VF were
not changed by 6 × 10 s postconditioning (Figs. 2A and 3).
Neither postconditioning nor cholesterol diet affected coronary
flow or area at risk significantly (Tables 1 and 3).

Next, we studied if postconditioning altered myocardial nitro-
sative stress at early reperfusion in normol and cholesterol-fed
animals. Therefore, in separate experiments, we measured free
and protein-bound 3-nitrotyrosine, a marker for peroxynitrite-
induced nitrosative stress, by ELISA and Western blot, respec-
tively, in left ventricular tissue samples at 5 min of reperfusion
after postconditioning (Fig. 4, A and B). Postconditioning
significantly increased peroxynitrite-induced nitrotyrosine for-
mation in normolipidemic hearts, but not in hyperlipidemic
conditions (Fig. 4, A and B).

To test if the early increase in nitrosative stress signal
observed in normolipidemic animals is a necessary trigger for

the development of cardioprotection by postconditioning, in
separate experiments, postconditioning was induced in the
presence of a peroxynitrite decomposition catalyst FeTPPS in
normolipidemic animals. Although FeTPPS did not affect
infarct size significantly in the nonpostconditioned group, it
abolished the infarct size-limiting effect of postconditioning
(Fig. 5).

Finally, to test if a more potent postconditioning stimulus
could protect the hyperlipidemic heart, in a separate set of
experiments, postconditioning with 12 × 10 s cycles of brief
ischemia-reperfusion was applied. However, this postcondi-
tioning protocol failed to reduce infarct size not only in
hyperlipidemic but also in normolipidemic hearts. Therefore,
no additional biochemical or pharmacological studies were
performed in these groups (see Table 3).

DISCUSSION

We have shown here that the infarct size-limiting effect of
postconditioning was lost in hearts of hyperlipidemic rats.
Furthermore, we have shown that cardiac nitrotyrosine content
was increased during early reperfusion after postconditioning,
which was not seen in hyperlipidemic hearts. Finally, we have
shown that postconditioning in the presence of the peroxyni-
trite decomposition catalyst FeTPPS failed to reduce infarct
size in normal hearts. This is the first demonstration that an
early increase in peroxynitrite-induced nitrosative stress after
postconditioning is involved in the triggering mechanism of cardioprotection by postconditioning and that, in hyperlipidemia, the absence of this mechanism may contribute to the loss of postconditioning in hyperlipidemia.

Our present results confirm that of Iliodromitis et al. (15) showing that the infarct size-limiting effect of postconditioning is lost in rabbits with experimental hyperlipidemia and atherosclerosis. Another study by Zhao et al. (36) showed in minipigs that postconditioning under normolipidemic condition can reduce the area of no-reflow and necrosis area, while postconditioning under hypercholesterolemic condition cannot. In contrast, Donato et al. (4) showed that ischemic postconditioning reduces infarct size by activation of A1 receptors and K+ (ATP) channels in both normal and hypercholesterolemic rabbits. The discrepancies can be attributed to differences in experimental hyperlipidemia and the presence of coronary atherosclerosis, which may interfere with the severity of coronary occlusion per se. Therefore, hyperlipidemic rats that do not develop significant atherosclerosis seem to be a more suitable model to study the direct myocardial effect of hyperlipidemia on cardioprotective mechanisms. Therefore, we conclude that, similarly to the effect of preconditioning, the effect of postconditioning is also influenced by hyperlipidemia (see, for review, Ref. 9).

Our present results show that postconditioning increases formation of cardiac 3-nitrotyrosine, a marker for peroxynitrite-induced nitrosative stress, at early reperfusion; however, the increased peroxynitrite formation was not observed in peroxynitrite-induced nitrosative stress, at early reperfusion; however, the effect of preconditioning, the effect of postconditioning is involved in the triggering mechanism of the cardioprotective effect of preconditioning (1, 2), it was plausible to speculate that peroxynitrite may be also involved in the triggering mechanism of postconditioning. To test this hypothesis, in normal animals, postconditioning was performed in the presence of a peroxynitrite decomposition catalyst FeTPPS at a dose that significantly reduced peroxynitrite-induced myocardial effects in different models (7, 20). We have found here that the infarct size-limiting effect of postconditioning was abolished in the presence of FeTPPS, which shows for the first time that increased nitrosative stress at early reperfusion after postconditioning is necessary to trigger its cardioprotective effect. This is another important example of the physiological regulatory role of mild nitrosative stress (6, 25). In isolated adult rat cardiac myocytes, Wang et al. (34) suggested that hypoxic postconditioning is partly attributable to the reduced peroxynitrite formation following reoxygenation. The discrepancy may be due to the fact that they measured peroxynitrite late in

<table>
<thead>
<tr>
<th>Before ischemia</th>
<th>Coronary flow, ml/min</th>
<th>Heart rate, beats/min</th>
<th>Heart wt g</th>
<th>Body wt g</th>
<th>Heart wt/body wt %</th>
<th>End of ischemia</th>
<th>Coronary flow, ml/min</th>
<th>Heart rate, beats/min</th>
<th>Heart wt/body wt %</th>
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<tbody>
<tr>
<td>Normolipemic</td>
<td>Control</td>
<td>Post (6 x 10s)</td>
<td>Control</td>
<td>Post (6 x 10s)</td>
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<td>Post (6 x 10s)</td>
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<td>Post (6 x 10s)</td>
<td>Control</td>
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<tr>
<td></td>
<td>20.5±1.5</td>
<td>17.7±1.2</td>
<td>382±49</td>
<td>332±30</td>
<td>1.90±0.15</td>
<td>1.78±0.07</td>
<td>527±17</td>
<td>435±11</td>
<td>0.38±0.02</td>
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<td></td>
<td>Hyperlipemic</td>
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<td>Control</td>
<td>Post (6 x 10s)</td>
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<td></td>
<td>19.4±1.1</td>
<td>17.0±0.9</td>
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<td>352±31</td>
<td>251±17</td>
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<td>2.11±0.06*</td>
<td>2.13±0.11*</td>
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<td>500±23</td>
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<td>0.43±0.02</td>
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Values are means ± SE. Post, postconditioning induced by 6 x 10 s cycles of ischemia and reperfusion. *P < 0.05 vs. normolipemic.

Table 2. Influence of cholesterol-enriched diet on lipid profile in rats

<table>
<thead>
<tr>
<th>Lipid Profile</th>
<th>Normolipemic</th>
<th>Hyperlipemic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum cholesterol, mmol/l</td>
<td>2.57±0.20</td>
<td>3.05±0.10*</td>
</tr>
<tr>
<td>Serum triglyceride, mmol/l</td>
<td>1.47±0.10</td>
<td>2.15±0.20*</td>
</tr>
<tr>
<td>Myocardial tissue cholesterol, μg/mg</td>
<td>0.88±0.02</td>
<td>0.93±0.10</td>
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<tr>
<td>Myocardial free fatty acid content</td>
<td></td>
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</tr>
<tr>
<td>DHGLA, μg/g</td>
<td>4.13±2.0</td>
<td>16.3±4.1*</td>
</tr>
<tr>
<td>Palmitic acid, μg/g</td>
<td>178±50</td>
<td>316±50</td>
</tr>
<tr>
<td>Stearic acid, μg/g</td>
<td>162±30</td>
<td>206±30</td>
</tr>
</tbody>
</table>

Values are means ± SE. DHGLA, dihomo-γ-linolenic acid. *P < 0.05 vs. normolipemic.
reoxygenation and used a different model and postconditioning trigger.

On the other hand, the role of reactive oxygen species (ROS), including peroxynitrite in cardioprotection including postconditioning, is still not clear; furthermore, still little is known on the balance between the detrimental and protective effects of peroxynitrite (see, for reviews, Refs. 6, 8, 19, 25, 26). It has been shown that oxidative/nitrosative stress is involved in myocardial ischemia-reperfusion injury (35) and that peroxynitrite decomposition catalysts provide significant cardioprotection against myocardial/reperfusion injury (16). However, recent studies suggest that some ROS species at low concentrations could protect ischemic hearts (27). ROS scavengers N-acetyl-L-cysteine or mercaptopropionyl glycine given at the beginning of reperfusion abolished postconditioning-induced protection (30). Nossuli et al. (23, 30) showed that, in a feline model of coronary occlusion/reperfusion, intraventricular infusion of authentic peroxynitrite (1 μmol/l) 10 min

Table 3. Cardiac functional parameters in normal and hyperlipidemic rat hearts

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<thead>
<tr>
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<th>Normolipidemic</th>
<th>Hyperlipidemic</th>
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<tbody>
<tr>
<td></td>
<td>Control Post (12 × 10 s)</td>
<td>Control Post (12 × 10 s)</td>
</tr>
<tr>
<td><strong>Before ischemia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary flow, ml/min</td>
<td>20.4±0.9</td>
<td>23.9±2.1</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>375±36</td>
<td>345±31</td>
</tr>
<tr>
<td>Heart wt, g</td>
<td>1.79±0.08</td>
<td>2.11±0.16</td>
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<tr>
<td>Body wt, g</td>
<td>556±14</td>
<td>572±26</td>
</tr>
<tr>
<td>Heart wt/body wt, %</td>
<td>0.32±0.01</td>
<td>0.37±0.02</td>
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<tr>
<td><strong>End of ischemia</strong></td>
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<tr>
<td>Coronary flow, ml/min</td>
<td>11.0±0.9</td>
<td>12.9±1.5</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>308±27</td>
<td>353±32</td>
</tr>
<tr>
<td><strong>End of reperfusion</strong></td>
<td></td>
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<tr>
<td>Coronary flow, ml/min</td>
<td>16.8±1.8</td>
<td>16.0±1.5</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>367±33</td>
<td>345±32</td>
</tr>
<tr>
<td>Infarct size, %</td>
<td>31.3±4.7</td>
<td>31.9±3.4</td>
</tr>
<tr>
<td>Area at risk, %</td>
<td>40.0±3.9</td>
<td>40.4±3.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. Post, postconditioning induced by 12 × 10 s cycles of ischemia and reperfusion. P < 0.05 vs. normolipidemic (*) and vs. hyperlipidemic (†).

Fig. 4. Cardiac 3-nitrotyrosine content, a marker of endogenous peroxynitrite-induced nitrosative stress. Free 3-nitrotyrosine assayed by enzyme-linked immunosorbent assay (ELISA; A) and protein-bound 3-nitrotyrosine by Western blot (B) in control (Control and Control Post) and cholesterol-fed (Chol and Chol Post) groups. *P < 0.05 vs. control; n = 10–13 in each group.

Fig. 5. Myocardial injury after 30 min of regional ischemia and 120 min of reperfusion: myocardial AAR (B) and IS/AAR (A). Infarct size measured at the end of 120 min reperfusion with (Control Post) or without 6 × 10 s postconditioning (Control) in the presence or absence of 20 mg/l FeTPPS, a peroxynitrite decomposition catalyst. *P < 0.05 vs. control; n = 6–10 in each group. †, Individual values; ‡, average values.
before reperfusion was associated with a reduction in infarct size. Furthermore, it has been shown that peroxynitrite regulates mitogen-activated protein kinases, which are also involved in the mechanism of postconditioning (19, 28).

Since we have shown here that, in hyperlipidemia, postconditioning with six brief cycles of ischemia-reperfusion were unable to induce cardioprotection, it was feasible to speculate that this might be due to an increased threshold for postconditioning triggers in hyperlipidemia. Therefore, we tested if an increased postconditioning stimulus of 12 brief cycles of coronary occlusion/reperfusion could postcondition the hyperlipidemic heart, but found that this protocol was unable to induce postconditioning in both hyperlipidemic and normolipidemic animals. This suggests that the loss of postconditioning in hyperlipidemia is not due to an increased threshold for triggers of postconditioning, but possibly due to the disruption of the cardioprotective cellular pathways (9).

Our current findings should be interpreted within the constraints of potential limitations. Although FeTPPS is thought to be specific to peroxynitrite, it cannot be excluded that it reacts with nonperoxynitrite species as well. Because of technical limitations to measure local concentrations and cellular sources of peroxynitrite (6), nitrosative stress in the coronary endothelium, endocardial endothelium, cardiac nerves, fibroblasts, and cardiac myocytes could all contribute to changes in nitrotyrosine levels and to the cardioprotective effect of postconditioning. Furthermore, we cannot exclude that nitrotyrosine can be formed by peroxynitrite-independent pathways as well, for example, via the actions of peroxidasises in the presence of nitrite.

In conclusion, this is the first demonstration that nitrosative stress is involved in the triggering mechanism of postconditioning and that hyperlipidemia leads to the loss of the cardioprotective effect of postconditioning, at least in part due to deterioration of the nitrosative trigger. Furthermore, we emphasize the importance of preclinical studies that examine cardioprotective mechanisms specifically in relation to complicating disease states such as hyperlipidemia to maximize the likelihood of identifying rational approaches to therapeutic protection of the ischemic heart in the presence of risk factors.

GRANTS

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