Regional myocardial ischemia-induced activation of MAPKs is associated with subcellular redistribution of caveolin and cholesterol

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Although rapid activation of ERK and p38 MAPK by myocardial ischemia-reperfusion is well established (2, 13, 25, 43), the role of these kinases in the modulation of injury is unclear (2, 13, 42, 33). Differential MAPK compartmentation and duration of activation may indicate whether these kinases are cardioprotective or detrimental (16, 47). Cardiac ischemia-reperfusion-mediated MAPK activation has been well documented in cytosolic and nuclear fractions (3, 12, 13, 29, 32), and although we recently showed that ischemia-reperfusion also modulates these kinases in cardiac membrane fractions (3, 32), MAPK activation in myocardial membranes has not been fully explored.

Membrane signaling can be further compartmentalized into lipid rafts, which are low-density, detergent-resistant membrane domains enriched in cholesterol. Caveolae, a subset of lipid rafts, contain the scaffolding protein caveolin. Three caveolin isoforms are expressed in the heart: caveolin-1 and -2 in endothelial and smooth muscle cells and the predominant cardiac isoform caveolin-3, which is expressed in cardiomyocytes (1, 22). Although numerous signaling molecules are located in caveolae (1, 34), the exact role of these membrane domains in signal transduction is not known. Caveolae may negatively regulate signaling molecules through caveolin binding (9, 10) or may concentrate signaling molecules for more efficient activation (8, 43). ERK has been found in cardiomyocyte caveolae/lipid rafts (24, 34), but whether localization of caveolae results in myocardial ERK inhibition (48) or activation (24, 34) is unclear. Moreover, the role of caveolae/lipid rafts in cardiac p38 MAPK signaling has not been studied.

Several myocardial pathologies are associated with alterations in subcellular caveolin expression. Elevated caveolin-1 and -3 levels have been reported in pressure-overloaded and failing hearts (20, 44), whereas reduced cardiac caveolin-1 and -3 expression has been reported in cardiac hypertrophy, heart failure, and chronic hypoxia (17, 28, 37). Caveolin-1 levels are also altered in renal failure and pulmonary hypertension (18, 51). Changes in the amount and distribution of cholesterol, another major component of caveolar membranes, have also been reported. Roulin et al. (33) showed increased cholesterol content in mitochondria after ischemia-reperfusion in pigs, whereas Venter et al. (45) subsequently reported that myocardial ischemia in rats causes a translocation of cholesterol from the membrane to mitochondria. Because subcellular caveolin and cholesterol levels are altered in various myocardial disease states, it is possible that myocardial ischemia-reperfusion may modulate MAPK signaling in caveolae/lipid rafts. The purpose of this study was to determine the effect of in vivo myocardial ischemia-reperfusion on the level and distribution of caveolin-1 and -3 and cholesterol, as well as MAPK activation in caveolin-enriched fractions.

Methods

All animals received humane care according to “The Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the National Institutes of Health (National Institutes of Health) Guide for the Care and Use of Laboratory Animals (NIH Publication No. 86-23, Revised 1996). Animals were used in compliance with an animal protocol approved by the University of Kentucky Institutional Animal Care and Use Committee.

In vivo ischemia-reperfusion. Adult male Sprague-Dawley rats (Charles-River, Portage, MI; 350–399 g body wt) were subjected to in vivo ischemia-reperfusion as described previously (3). The rats were anesthetized with an intraperitoneal injection of ketamine (60 mg/kg) and xylazine (6–9 mg/kg) and supplemental doses of ketamine as needed. A tracheotomy was performed, and the animal was connected to a respirator (2). The lungs were ventilated with 100% oxygen at a rate of 100 breaths/min. Body temperature was maintained at 37°C with a water-wetted heating pad. Anesthesia was maintained using a 3% isoflurane-in-oxygen mixture.

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to a small animal ventilator (model 683, Harvard Apparatus, South Natick, MA). After a median sternotomy, a 6-0 prolene suture was passed below the left coronary artery, and the ends of the suture were fed through a short length of propylene tubing to form a snare. After 30 min of recovery from the surgical procedures, experimental protocols were initiated. For induction of regional myocardial ischemia, the snare was pulled up and clamped onto the epicardial surface. After 25 min of regional ischemia, the occlusion was released and the heart was reperfused.

**Experimental protocols.** The hearts were treated as follows: 1) no treatment (normal, n = 4), 2) 25 min of ischemia followed by 10 min of reperfusion (n = 5), 3) 25 min of ischemia followed by 2 h of reperfusion (n = 4), and 4) 2-h reperfusion time-matched control (n = 4). Rats in the normal and time-matched control groups underwent sham surgery, including placement of the coronary occluder. No deaths were associated with any of the protocols. In the ischemia-reperfusion groups, the ischemic zone of the heart (~40% of the left ventricle), defined as the cyanotic area during occlusion, was demarcated with an epicardial 4-0 prolene suture around the perimeter. The rest of the left ventricle was considered the nonischemic zone. Bordering ischemic and nonischemic tissue located within 2–3 mm of this suture was discarded on tissue collection.

**Purification of caveolin-rich membrane fractions.** Caveolin-enriched fractions were isolated from cardiac tissue by the detergent-free carbonate procedure of Song et al. (40). Cardiac tissue (0.25–0.35 g) was placed in buffer (500 mM sodium carbonate, 1 mM EDTA, 1 mM dithiothreitol, 1 mM sodium vanadate, 0.1 mg/ml PMSF, 45 μg/μl aprotinin, and 0.5 mM β-glycerophosphate, pH 11) and homogenized with a Polytron (three 15-s bursts). The sample was sonicated (six 10-s bursts), and the homogenate was adjusted to 40% sucrose by the addition of an equal volume of 80% sucrose in MBS buffer (25 mM MES, pH 6.5, 0.15 M NaCl, 1 mM sodium vanadate, 0.1 mg/ml PMSF, and 45 μg/μl aprotinin). The sample was placed in a centrifuge tube and overlaid with 4 ml of 5% and 3 ml of 35% sucrose fractions 1–3 in the nonischemic zone by 82%. The cholesterol content in the ischemic and nonischemic zones, respectively. The ischemia-reperfusion-induced changes in caveolin-3 distribution were not accompanied by changes in the total expression of caveolin-3. Caveolin-1, an endothelial/smooth muscle cell caveolar marker, was found exclusively in caveolae/lipid raft fractions 4–7 in normal heart, and this distribution pattern did not change with ischemia-reperfusion (Fig. 1D). However, caveolin-1 levels were significantly reduced by 59% and 55% in the ischemic and nonischemic zones, respectively (Fig. 1, D and E).

In normal heart, 70% of the total cholesterol was found in fractions 4–7, 18% in fractions 8–10, and 12% in fractions 1–3 (Fig. 1F). Ischemia-reperfusion reduced the amount of cholesterol in fractions 1–3 of the ischemic zone by 89% and in the nonischemic zone by 82%. The cholesterol content in fractions 4–7 was also significantly reduced by 58% and 51% in the ischemic and nonischemic zones, respectively, after 10 min of reperfusion. No difference in cholesterol levels was observed among the groups in fractions 8–10. The total amount of cholesterol was significantly reduced from 0.237 ± 0.012 μg/μg protein in normal heart to 0.097 ± 0.019 and 0.152 ± 0.014 μg/μg protein in ischemic and nonischemic zones, respectively.

**RESULTS**

Baseline heart rate and mean arterial pressure were not different among the groups. These parameters declined significantly after 10 min of reperfusion, whereas only mean arterial pressure was significantly lower than baseline after 2 h of reperfusion.

**Short-reperfusion protocol.** Myocardial density gradient fractions from the short-reperfusion protocol are characterized in Fig. 1. Total myocardial protein in the normal heart increased progressively from the light to the heavy fractions, with the most protein in fractions 8–10 (Fig. 1A). This pattern was not altered by ischemia-reperfusion. Distribution of the cardiomyocyte caveolar marker caveolin-3 is shown in Fig. 1, B and C. In normal heart, caveolin-3 was concentrated in fractions 5 and 6, with fractions 4 and 7 also containing a significant amount of this protein (Fig. 1B). Considerably less caveolin-3 was found in fractions 8–10 than in fractions 4–7, and no immunoreactivity was detected in fractions 1–3. Because caveolin-3 was located primarily in fractions 4–7, the densitometric results were pooled into light caveolae/lipid raft fractions 4–7 and heavy fractions 8–10. In the normal heart, 90% of caveolin-3 immunoreactivity was detected in fractions 4–7 and the remaining 10% in fractions 8–10 (Fig. 1C). Ischemia-reperfusion significantly reduced ischemic and nonischemic zone caveolin-3 protein in fractions 4–7 to 60%. In fractions 8–10, caveolin-3 was increased by 3.3- and 2.4-fold in the ischemic and nonischemic zones, respectively. The ischemia-reperfusion-induced changes in caveolin-3 distribution were not accompanied by changes in the total expression of caveolin-3. Caveolin-1, an endothelial/smooth muscle cell caveolar marker, was found exclusively in caveolae/lipid raft fractions 4–6 in normal heart, and this distribution pattern did not change with ischemia-reperfusion (Fig. 1D). However, caveolin-1 levels were significantly reduced by 59% and 55% in the ischemic and nonischemic zones, respectively (Fig. 1, D and E).

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**Distribution of the ERK isoforms in the short-reperfusion protocol is shown in Fig. 2. In normal heart, 67% of total p44 ERK protein was located in fractions 8–10 and the remaining 33% in fractions 4–7 (Fig. 2, A and B). No p44 ERK immunoreactivity was detected in fractions 1–3. Although the expression of p44 in ischemic zone fractions 4–7 was not altered, p44 immunoreactivity in these light fractions was increased by

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**Statistical analysis.** Values are means ± SE. Statistical significance among the groups within the short- or long-reperfusion protocol was determined using a one-way ANOVA followed by Tukey’s post hoc test. Statistical significance was defined as P < 0.05.
43% in the nonischemic zone. The amount of p44 ERK protein in fractions 8–10 was not significantly altered by ischemia-reperfusion. The bulk (83%) of p42 ERK protein was also found in fractions 8–10 and 17% in fractions 4–7 (Fig. 2, A and C). No p42 immunoreactivity was detected in fractions 1–3. Although ischemia-reperfusion did not alter the amount of p42 protein in fractions 4–7, a significant reduction in this ERK isoform was observed in fractions 8–10.

Figure 3 shows the activation of p44/p42 ERKs after 10 min of reperfusion. The level of p44 ERK phosphorylation was very low in the normal heart (Fig. 3, A and B). Phosphorylated p44 ERK was increased by fivefold in the light and heavy fractions of the ischemic zone compared with the normal heart. Activation of this isoform was increased by 2.6-fold in the nonischemic light fractions. The level of p42 phosphorylation was also very low in the normal heart, and no difference in phosphorylated p42 was detected in the nonischemic zone (Fig. 3, A and C). A significant 4.6-fold increase in the phosphorylation of p42 ERK occurred in the ischemic zone light fractions. This ERK isoform was also activated by 3.5-fold in the heavier fractions from the ischemic zone compared with the normal heart.

The distribution and activation of p38 MAPK after 10 min of reperfusion are shown in Fig. 4. In the normal heart, the distribution pattern of p38α was more even than that of the ERKs: 56% in the heavy fractions 8–10 and 44% in light fractions 4–7 (Fig. 4, A and B). In fractions 1–3, p38α protein was not consistently detected. The amount of total p38α protein in fractions 4–7 of the ischemic zone was significantly reduced by 45%, whereas expression of this protein was not altered in heavy fractions 8–10. No significant p38 MAPK activation in the light or heavy fractions occurred after 10 min of reperfusion (Fig. 4, C and D).

Long-reperfusion protocol. Myocardial samples from the long-reperfusion protocol are characterized in Fig. 5. The presence of the bulk of myocardial protein in the time-matched control in fractions 8–10 did not change with ischemia-reperfusion (Fig. 5A). After 2 h of reperfusion, 60% of caveolin-3 was present in fractions 4–7, similar to the 10-min reperfusion samples. The time-matched control showed a similar pattern (Fig. 5, B and C). Caveolin-1 was located only in fractions 4–6 in the time-matched control and long-reperfusion samples, and there was no difference in caveolin-1 immunoreactivity among the groups (Fig. 5, D and E). The distribution of cholesterol among the fractions is shown in Fig. 5F. In the time-matched control, 50% of the total cholesterol was in fractions 4–7, 38% in fractions 8–10, and 12% in fractions 1–3. After 2 h of reperfusion, cholesterol levels were significantly reduced in ischemic zone fractions 8–10. In addition, the cholesterol content in the light fractions was
significantly reduced in the ischemic and nonischemic zones after 2 h compared with the corresponding 10-min reperfusion samples.

The distribution of the ERK isoforms after long-duration reperfusion is depicted in Fig. 6. In the time-matched control, p44 ERK was concentrated in fractions 8–10 (76%) and the remaining 24% was detected in fractions 4–7 (Fig. 6, A and B). Immunoreactivity of p44 was increased by 83% and 56% in ischemic zone fractions 4–7 compared with the time-matched control and nonischemic zone, respectively. Long-duration ischemia-reperfusion did not alter the amount of p44 protein in fractions 8–10. The bulk (72%) of p42 ERK was also located in heavy fractions 8–10 in the time-matched control, and 28% was found in light fractions 4–7 (Fig. 6, A and C). The amount of this MAPK in the heavy fractions was decreased by 27% in the ischemic zone compared with the time-matched control. By contrast, p42 immunoreactivity in nonischemic zone fractions 8–10 was increased by 23%. There was no difference in p42 protein levels in light fractions 4–7 during long-duration ischemia-reperfusion.

The level of p44 ERK phosphorylation in fractions 4–7 was increased by 2.5-fold in the ischemic zone compared with the time-matched control (Fig. 7, A and B). Phosphorylated p44 ERK levels were also increased by 1.9-fold in the ischemic zone light fractions compared with the nonischemic zone. The amount of phosphorylated p44 ERK in the heavy fractions was not different among the groups. The phosphorylated p42 levels

![Fig. 2. Distribution of ERKs after 10 min of reperfusion.](image)

A: representative Western blots depicting p44/p42 ERK expression in normal, ischemic zone, and nonischemic zone samples. B and C: densitometric summary of p44 and p42 protein in normal, nonischemic, and ischemic samples. Results were combined into light fractions 4–7 and heavy fractions 8–10. *P < 0.05 vs. normal. +P < 0.05 vs. nonischemic zone.

![Fig. 3. Activation of ERKs in the short-reperfusion protocol.](image)

A: representative Western blot utilizing an antibody that detects active, dually phosphorylated form of p44/p42 ERKs (p-ERK). B and C: densitometric summary of p44 and p42 activation in normal, ischemic, and nonischemic samples. Results were pooled into light fractions 4–7 and heavy fractions 8–10. *P < 0.05 vs. normal. +P < 0.05 vs. nonischemic zone.
in the time-matched control and nonischemic zone fractions 4–7 were similar, and p42 activation was increased by 2.5-fold in these fractions in the ischemic zone (Fig. 7, A and C). The level of p42 phosphorylation was increased by 1.7-fold in the ischemic zone and 1.6-fold in the nonischemic zone heavy fractions compared with the time-matched control.

In the time-matched control, 62% of p38α/H9251 immunoreactivity was located in fractions 8–10 and the remaining 38% in fractions 4–7; this distribution did not change with long-duration ischemia-reperfusion (Fig. 8, A and B). In the ischemic zones, phosphorylated p38 levels in the light and heavy fractions were not different from the time-matched control. However, phosphorylated p38 MAPK increased by 1.6-fold in nonischemic zone light buoyant and heavy fractions 8–10 compared with the time-matched control (Fig. 8, C and D).

**DISCUSSION**

In the present study, in vivo myocardial ischemia followed by 10 min of reperfusion was associated with a redistribution of caveolin-3, one of the main components of cardiomyocyte caveolae, from light buoyant to heavy fractions. Short-duration reperfusion also reduced caveolin-1, which is found in endothelial cell caveolae, and cholesterol, which is found in the light fractions. The ERK isoforms were activated in the light buoyant fractions in the ischemic zone after 10 min of reperfusion. By contrast, p38 MAPK was activated in the nonischemic zone light buoyant and heavy fractions after 2 h of reperfusion. These results suggest that myocardial stressors, such as ischemia-reperfusion, change the caveolin and cholesterol content of caveolar membranes, which may lead to alterations in subcellular signaling.

Although caveolin is known to be a membrane-associated protein that is resistant to detergent solubilization, there are several reports that caveolin subcellular expression may change during stress (15, 31, 35). Movement of caveolin-3 from Triton-insoluble fractions enriched in caveolin to Triton-soluble fractions has been reported in aged myocardium, and myocardial infarction in the senescent rat further enhances this effect (31). There are also reports that other stresses in cardiomyocytes and cardiomyoblasts can lead to movement of caveolin-3 from light to heavy fractions (15, 35). The altered caveolin-3 distribution observed in this study and others (15, 31, 35) was not associated with a change in the total expression of this protein, suggesting a redistribution of caveolin-3 from one compartment to another.

In contrast to the cardiomyocyte caveolar membrane protein caveolin-3, expression of the endothelial cell caveolar protein caveolin-1 was reduced in the short-reperfusion protocol without a change in distribution. Decreased expression of caveolin-1 has also been reported in endothelial cells subjected to oxidative stress (27) or hypertrophic cardiomyopathy (28), in lung remodeling following myocardial infarction (18), and after vascular injury (4). Young et al. (50) reported that infusion of caveolin-1 peptide protects the heart against ischemia-reperfusion injury through enhanced endothelial cell nitric oxide release, which reduced neutrophil adherence to the coronary vasculature. Because endothelial nitric oxide synthase is localized in endothelial caveolae and caveolin-1 binding modulates the activity of this enzyme (1, 22, 50), it is possible that reduced caveolin-1 levels may be involved in the etiology of ischemia-reperfusion injury.

Early reperfusion after coronary occlusion was associated with a significant decrease in cholesterol levels in the light
buoyant fractions in the ischemic and nonischemic zones, which led to a decrease in the total cholesterol levels compared with the normal heart. Over 20 years ago, Rouslin et al. (33) reported an increase in the cholesterol content of mitochondria after myocardial ischemia. Venter et al. (45) subsequently reported that the ischemia-mediated increase in mitochondrial cholesterol was accompanied by a corresponding reduction in plasma membrane cholesterol. It is well known that cholesterol is concentrated in lipid rafts and caveolae (1, 22) and that cholesterol depletion disrupts caveolar structure (46). Frank et al. (11) found an ~50% reduction in the number of myocardial caveolae after 2 h of anoxia, with the remaining caveolae appearing flattened. These findings and the evidence that cholesterol depletion alters subcellular signaling (7, 8, 19) suggest that changes in caveolin and/or cholesterol levels may play a role in acute myocardial ischemia-reperfusion injury.

The results of the present study support the hypothesis that changes in the subcellular expression of caveolin and cholesterol may play a role in reperfusion-associated MAPK signaling. In the short-reperfusion protocol, p44 and p42 ERK were activated in the ischemic zone in the light buoyant and heavy fractions. The ischemia-reperfusion-mediated p44 ERK activation was not due to altered protein expression, because no change in the total amount of this ERK isoform was observed in the ischemic zone. By contrast, p42 protein was significantly reduced in fractions 8–10, indicating that a greater proportion of p42 in these fractions was active. The increase in ERK activation in the light fractions during ischemia-reperfusion was accompanied by a reduction in caveolin-1 and -3 levels in these fractions. Because caveolin binding may prevent the activation of certain signaling molecules, including ERK (9, 10), the activation of the ERK isoforms in the light fractions may be due to the removal of this inhibitory mechanism.

The role of enhanced ERK signaling during acute ischemia-reperfusion is unclear. Inhibition of ERK signaling blocks myocardial preconditioning, suggesting a cardioprotective role for the ERK isoforms (2, 13, 32). By contrast, an ERK pathway inhibitor has no effect on infarct size in the nonpreconditioned heart (13, 32). One possible reason for the disparity is that ischemia-reperfusion may activate ERK in distinct subcellular compartments, leading to the modulation of different downstream targets and, ultimately, localization-dependent physiological effects. Of particular relevance to our present findings is a report that Na+/H+ exchanger 1 (NHE1), the primary isoform in cardiomyocytes, is enriched in myocardial Triton-insoluble membranes (7). In this same study, transfection of a Chinese hamster ovary cell line with NHE1 resulted in expression of NHE1 in caveolin-enriched fractions, and the activity of NHE1 was increased by cholesterol depletion. These findings, combined with evidence that cardiomyocyte NHE1 ac-
Activity is increased by ERK phosphorylation (39) and activation of NHE1 during early reperfusion contributes to reperfusion injury (2, 39), suggest that ERK activation in caveolin-enriched fractions may be deleterious.

ERK activation after 2 h of reperfusion was isoform dependent. The p44 isoform was activated in the ischemic zone light fractions, an effect most likely due to the increase in p44 expression. The activation of p42 ERK in the light fractions, on the other hand, was independent of protein expression. There was no activation of p44 ERK in the ischemic zone heavy fractions, but there was a significant increase in phosphorylated p42 ERK in these same fractions, despite a reduction in p42 protein. Activation of p42 ERK also in the nonischemic zone heavy fractions was probably due to increased p42 expression. Thus it appears that the subcellular distribution and activation of the ERK isoforms differ after long-duration reperfusion. Differences in myocardial p44 and p42 ERK have also been reported by Lips et al. (23), who showed an increase in infarct size in p42 ERK-knockout mice but no difference in infarct size in p44 ERK-knockout mice. Hyperactivation of ERK and hypertrophic cardiomyopathy have been reported in caveolin-3-knockout mice (48). Because chronic ERK activation is associated with cardiac hypertrophy (5, 6), activation of the ERK isoforms after 2 h of reperfusion may ultimately lead to this cardiac abnormality.

In contrast to the robust activation of ERK, no significant activation of p38 MAPK was observed after 10 min of reperfusion in the ischemic zone, despite a significant decrease in p38α protein levels. The predominant p38 isoform that may be

Fig. 6. Distribution of ERKs after 2 h of reperfusion. A: representative Western blot showing p44/p42 ERK expression in time-matched control, ischemic, and nonischemic samples. B and C: densitometric summary of p44 and p42 ERK protein in time-matched, ischemic, and nonischemic samples. *P < 0.05 vs. time-matched control. +P < 0.05 vs. nonischemic zone.

Fig. 7. Activation of ERKs in the long-reperfusion protocol. A: representative blot depicting activation of p44/p42 ERKs as measured by the level of the dually phosphorylated form (p-ERK) in time-matched control, ischemic, and nonischemic samples. B and C: summary of densitometric results from anti-active p44 ERK and anti-active p42 ERK blots. Results were pooled into light fractions 4–7 and heavy fractions 8–10. *P < 0.05 vs. time-matched control. +P < 0.05 vs. nonischemic zone.
important in ischemia-reperfusion injury in the heart is p38α. However, p38β may also exist in the heart and contribute to phosphorylated p38 MAPK signal (2, 41). The disparate results suggest differential roles of p38 MAPK and ERK in ischemic myocardium. The pattern of p38 MAPK activation after 2 h of reperfusion was quite different from that after 10 min of reperfusion. Activation of p38 MAPK occurred in the nonischemic zone light and heavy fractions, whereas no activation was detected in any of the fractions in the ischemic zone. These results are consistent with those of Yoshida et al. (49), who reported p38 MAPK activation in the nonischemic myocardium after acute myocardial infarction. Furthermore, Simonis et al. (38) found that all cardiac PKC isoforms were activated in the nonischemic zone, suggesting that ischemia-reperfusion activates signaling pathways in ischemic and nonischemic zones. Activation of p38 MAPK in the nonischemic zone may be prolonged, inasmuch as Qin et al. (30) reported increased p38 activation in the remote noninfarcted myocardium at 1–12 wk after infarction. They also found a correlation between p38 MAPK activation and myocyte apoptosis in the remote myocardium that was temporally associated with cardiac remodeling. Other groups showed that myocardial remodeling can be blocked with a p38 MAPK inhibitor (21, 36). Thus the activation of p38 MAPK in the nonischemic zone after 2 h of reperfusion in the present study may ultimately induce myocardial remodeling.

A limitation of the long-reperfusion protocol was that the caveolin and cholesterol expression/distribution in the time-matched controls was significantly different from that in the normal heart sample during the short-reperfusion protocol. This observation may have been due to the effects of prolonged anesthesia, because ketamine anesthesia has been shown to alter plasma cholesterol levels (14). The time-control effect may also be due to stress resulting from the prolonged open-chest model, an idea supported by the greater baseline MAPK activation in the time-matched control than in the normal heart. In addition, catecholamines have been reported to downregulate caveolin expression (26). Despite the changes in caveolin and cholesterol in the time-matched controls, a significant activation of ERK and p38 MAPK occurred in the 2-h reperfusion samples. However, MAPK activation at 2 h of reperfusion could not be correlated with alterations in caveolin/cholesterol levels.

The results of the present study show that early reperfusion after myocardial ischemia is associated with reductions in caveolin isoform expressions as well as a decrease in total cholesterol levels. These alterations were associated with increased activation of ERK isoforms, but not p38 MAPK, in light buoyant and heavy subcellular fractions in the ischemic zone. After 2 h of reperfusion, ERK phosphorylation levels remained elevated in the ischemic zone, whereas p38 MAPK activation was evident only in the nonischemic zone. These results suggest that alterations in membrane lipid rafts and caveolae may regulate MAPK signaling during acute myocardial ischemia-reperfusion.

GRANTS

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