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Metabolic and physiological differences between zero-flow and low-flow myocardial ischemia: effects of L-acetylcarnitine

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Summary

The metabolic and physiologic differences between low-flow and zero-flow ischemia of varying duration were compared in the isolated perfused rat heart. Hearts subjected to 60 and 90 minutes of zero-flow ischemia recovered less cardiac work than hearts subjected to low-flow ischemia. Low-flow ischemia caused a build-up of both myocardial long-chain acyl coenzyme A and acyl carnitine esters, while zero-flow ischemia produced no change in long-chain acyl carnitine and only a transient increase in long-chain acyl coenzyme A. High energy phosphate depletion was greater in zero-flow ischemia. Perfusion with excess free fatty acids decreased the recovery of cardiac work after low-flow ischemia but had no effect after repeated episodes of zero-flow ischemia. L-Acetylcarnitine improved the recovery of cardiac work after low-flow ischemia in hearts perfused with 0.4 and 1.2 mM palmitate. With zero-flow ischemia, L-acetylcarnitine had no effect on the recovery of cardiac work in hearts perfused with 0.4 mM palmitate and a slight but statistically significant effect with 1.2 mM palmitate. Possible protective mechanisms of L-acetylcarnitine against ischemic damage are discussed.

Key words: carnitine, perfused rat heart, long-chain acyl coenzyme A

Introduction

Effects of ischemia on myocardial function and metabolism have been investigated in a number of studies using a variety of experimental models, including regional and global ischemia, animals with differing degrees of collateral coronary flow, perfused hearts, and *in vitro* total ischemia (4, 6, 8, 10, 12, 14, 16, 34). In these previous studies, myocardial ischemia has been associated with an increased NADH/NAD ratio, a transiently increased glycolytic rate, glycogen depletion, accumulation of lipid intermediates, inhibition of fatty acid metabolism, and decreased mitochondrial and contractile function (4, 6, 11, 12, 13, 16, 20, 23, 27, 34). Hearts exposed to excess free fatty acids during ischemia showed an enhanced deterioration of mechanical, electrical, and metabolic functions (10, 14, 16, 22, 24), and it has been suggested that carnitine protects against myocardial ischemia (8, 15, 17, 18, 29, 30). However, not all forms of myocardial ischemia may result in the same abnormalities in metabolism and function. The metabolism of cardiac tissue subjected to the cessation of coronary flow (zero-flow ischemia) may be quite different from that in areas of low-flow ischemia. Consequently, the effects of excess free fatty acids and of protective agents may also be altered.

We compared the recovery of cardiac work after 30, 60, and 90 minutes of zero-flow and low-flow ischemia and reperfusion in the isolated perfused rat heart. Changes in myocardial

levels of free carnitine, carnitine esters, long-chain acyl coenzyme A, and adenine nucleotides were measured during ischemia. The effects of high versus low fatty acid in the perfusion media were compared. In addition, we tested the effects of L-acetylcarnitine, a key myocardial metabolite, as an agent that may protect the myocardium during low-flow and/or zero-flow ischemia. The effects on heart function of excess free fatty acids and L-acetylcarnitine were determined by measuring the recovery of cardiac work in reperfused ischemic hearts.

Methods

Animals

Male Sprague-Dawley rats (King Animal Laboratories, Madison, WI), weighing 200–250 g each, were used. Rats were given free access to food and water prior to the experiment.

Heart Perfusion

The basic design of the rat heart apparatus used in this laboratory has been described previously (21). Each rat was anesthetized with sodium pentobarbital (40 mg/kg). The chest was opened and the heart was excised, placed in cold bicarbonate buffer, and mounted on the perfusion apparatus within 60 seconds by cannulation of the aorta. Initially, the heart was perfused in a retrograde fashion for 10 minutes from a reservoir located at a pressure of 100 cm H_2O above the heart (Langendorff perfusion). The left atrium was cannulated during this time. The perfusion medium was a modified Krebs-Henseleit bicarbonate buffer, pH 7.4, which contained 11 mM glucose and 2 mU/ml insulin (Eli Lilly Co.). The medium was gassed with 95 % oxygen and 5 % carbon dioxide and maintained at 37 °C. The heart was then converted to a working heart by perfusing buffered medium into the left atrium at 10 cm H_2O left atrial filling pressure. Buffer was spontaneously ejected from the left ventricle against an afterload of 85 cm of H_2O and was recirculated. Preparation of the medium containing fatty acid was described earlier (5). Hearts were paced at 315 beats/minute using a Grass S88 stimulator. After a 10-minute period of perfusion, control values of heart rate, aortic pressures, aortic and coronary flow, and left ventricular pressures and dp/dt were recorded. Pressures were measured with a Stathan pressure transducer and recorded on a Gilson 5/6 H recorder. Left ventricular dp/dt was monitored using a Gilson IC-DIFF differentiator. Aortic and coronary flows were measured by a timed collection. Pressure-work was calculated as described previously (21).

Zero-flow ischemia was induced after the initial 10-minute control period by clamping the aortic and left atrial lines and maintaining the heart at the zero-flow state at a temperature of 37 °C for the desired time. Low-flow ischemia was induced by clamping the aortic line and restricting left atrial flow, utilizing a bypass line from a reservoir located 100 cm H_2O above the heart. The rate of flow was set by a micrometer caliper so that a 90 % reduction in coronary flow (1.2 ml/min) was achieved. A constant level of coronary flow was maintained during the ischemic period by means of the 100 cm of H_2O hydrostatic pressure column to the left atrial line. Ischemia was induced after the initial 10 minutes of perfusion and maintained for the desired time. Some hearts were reperfused for a 15-minute period and recovery of cardiac work was measured.

Metabolic analysis

In the reperfused hearts assayed for carnitine, the coronary vasculature was washed out for 2 minutes by Langendorff perfusion with Krebs-Henseleit buffer which contained only 11 mM glucose. After perfusion, hearts were immediately freeze-clamped at the temperature of liquid nitrogen and stored at −70 °C until assayed. Free carnitine and its esters were assayed using the method of Parvin and Pande (25) with slight modification. Long-chain acyl CoA was extracted from myocardial tissue and determined by the enzymatic cycling method of Veloso and Veech (33). Nucleotides were measured by methods described previously (2, 28).

Expression of results and statistical analysis

All data were expressed as mean ± standard error. Statistical significance of difference was determined by using the independent Student's *t* test or oneway analysis of variance and the least significant difference test, depending on which test was more appropriate.

Results

Recovery of cardiac work in hearts exposed to various periods of zero-flow and low-flow ischemia is shown in figure 1. Each heart was perfused with 1.2 mM palmitate, 5.5 mM glucose, and 2 mU/ml insulin. The initial value for cardiac work was measured after a 10-minute working heart control period. After the ischemic period, each heart was reperfused for 5 minutes in a Langendorff manner followed by a 10-minute working heart perfusion. The recovery of cardiac work was measured at this point. In both low-flow and zero-flow ischemia, increasing the duration of ischemia produced a progressive decrease in the recovery of cardiac work. After 30 minutes of ischemia, no apparent differences were observed between the two models of ischemia, but with longer duration the hearts subjected to zero-flow ischemia showed less recovery of cardiac work.

Figure 2 compares the effects of low-flow and zero-flow ischemia on the myocardial levels of total carnitine, long-chain acyl carnitine, acid-soluble carnitine (free plus short-chain acyl carnitine), and long-chain acyl coenzyme A. Neither model of ischemia affected tissue levels of total carnitine. Low-flow ischemia produced a dramatic increase in long-chain acyl carnitine with a commensurate decrease in acid-soluble carnitine. In contrast, zero-flow ischemia produced no change in the levels of these carnitine compounds. Both low-flow and zero-flow ischemia produced an increase in long-chain acyl coenzyme A after 30 minutes of ischemia, but the increase was significantly greater in the low-flow ischemic hearts. Long-chain acyl coenzyme A levels remained elevated in low-flow hearts subjected to longer periods of ischemia, while in zero-flow ischemia the levels returned to the control value.

A comparison of the effects of the two models of ischemia on levels of total adenine nucleotide, ATP, ADP, and AMP is shown in figure 3. The initial nucleotide values were obtained from hearts perfused for 30 minutes under normal working heart conditions and are lower than values found *in vivo* (32). Both models of ischemia decreased total adenine nucleotides to the same degree. Zero-flow ischemia produced a significantly greater

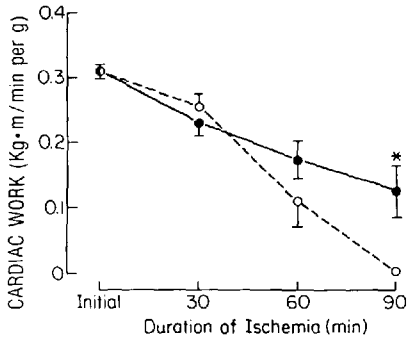

Fig. 1. Effects of 30, 60, and 90 minutes of low-flow (solid line) and zero-flow (dashed line) ischemia on the recovery of cardiac work after 5 minutes of Langendorff or 10 minutes of working heart reperfusion. Each point represents mean ± S.E. for 8 hearts. *$p < 0.01$ level of significance between two groups.

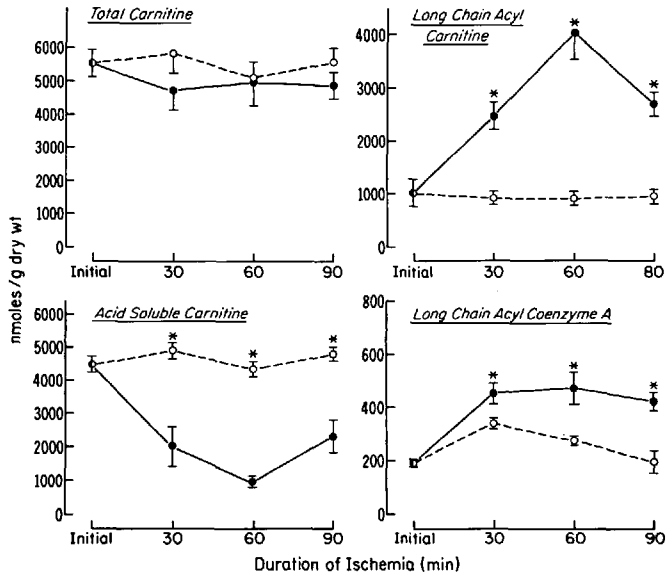

Fig. 2. Effects of low-flow (solid line) and zero-flow (dashed line) ischemia on the levels of total carnitine, long-chain acyl carnitine, acid-soluble carnitine and long-chain acyl coenzyme A. Each point represents mean ± S.E. for 8 hearts. *p < 0.01 level of significance between two groups.

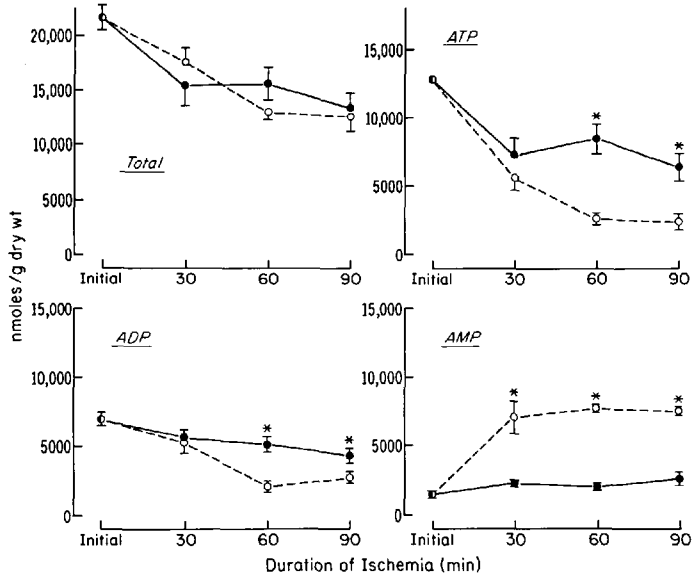

Fig. 3. Effects of low-flow (solid line) and zero-flow (dashed line) ischemia on the levels of total adenine nucleotides, ATP, ADP, and AMP. Each point represents mean ± S.E. for 8 hearts. *p < 0.01 level of significance between two groups.

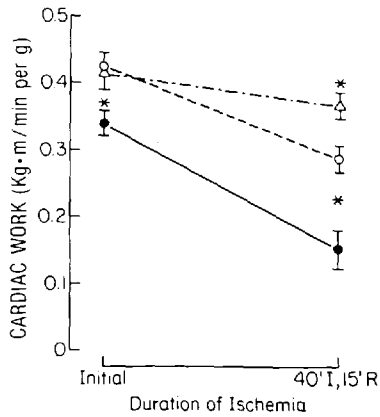

Fig. 4. Effects of 40 minutes of low-flow ischemia (I) and 15 minutes of reperfusion (R) on the recovery of cardiac work in hearts perfused with 5.5 mM glucose, 2 mU/ml insulin and 0.4 mM palmitate (dashed line) or 5.5 mM glucose, 2 mU/ml insulin, and 1.2 mM palmitate (solid line). The dashed-dot line shows the recovery of cardiac work in hearts perfused with 5.5 mM glucose, 2 mU/ml insulin, 0.4 mM palmitate, and 11 mM L-acetylcarnitine. Each point represents mean ± S.E. for 8 hearts. *$p < 0.01$ level of significance of difference between dashed lines and other groups.

decrease in ATP and ADP content after 60 and 90 minutes of ischemia than did low-flow ischemia. The levels of AMP were significantly greater after all durations of zero-flow ischemia. Thus, zero-flow ischemia produced a greater depletion of high-energy phosphates than did low-flow ischemia.

Figure 4 shows the effects of perfusion medium fatty acid concentration on the recovery of cardiac work in hearts subjected to low-flow ischemia for 40 minutes followed by 15 minutes of reperfusion. The initial point represents the cardiac work measured after an initial 10-minute control working heart perfusion. Hearts perfused with 0.4 mM palmitate, 5.5 mM glucose, and 2 mU/ml insulin had a significantly greater initial value for cardiac work than hearts perfused with elevated fatty acids (1.2 mM palmitate, 5.5 mM glucose,

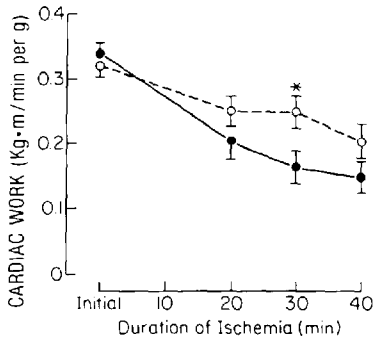

Fig. 5. Effects of L-acetylcarnitine on the recovery of cardiac work with reperfusion in hearts subjected to a low-flow ischemia for various time periods. All hearts were perfused with 1.2 mM palmitate, 5.5 mM glucose, and 2 mU/ml insulin with (dashed line) and without (solid line) 11 mM L-acetylcarnitine. Each point represents mean ± S.E. for 14 to 25 hearts.

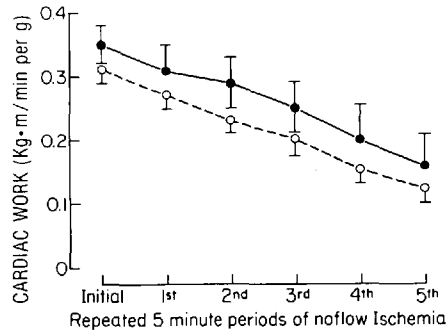

Fig. 6. Effects of repeated episodes of zero-flow ischemia on the recovery of cardiac work in hearts perfused with 5.5 mM glucose, 2 mU/ml insulin, and 0.4 mM palmitate (solid line, n = 20) or 5.5 mM glucose, 2 mU/ml insulin, and 1.2 mM palmitate (dashed line, n = 13). Each point represents mean ± S.E.

2 mU/ml insulin). The recovery of cardiac work after 40 minutes of low-flow ischemia was significantly less in hearts perfused with elevated free fatty acids. Figure 4 also shows the effects of 11 mM L-acetylcarnitine on cardiac work in hearts perfused with low levels of free fatty acids. L-acetylcarnitine-perfused hearts had significantly ($p < 0.01$) greater recovery of cardiac work than did controls. The effects of L-acetylcarnitine on the recovery of cardiac work in hearts perfused with elevated free fatty acids are shown in figure 5. L-acetylcarnitine increased the mean recovery of cardiac work after 20, 30, and 40 minutes of ischemia, but only at the 30-minute ischemic point was the recovery significantly ($p < 0.01$) greater. However, if we averaged and compared the recovery of cardiac work after all three durations of ischemia, the mean recovery of L-acetylcarnitine-perfused hearts was significantly ($p < 0.001$) greater than that of control hearts (0.239 ± 0.015 and 0.172 ± 0.017 kg/min/g, respectively).

To study the effects of elevated free fatty acid and L-acetylcarnitine on hearts subjected to zero-flow ischemia, a slightly different protocol was used. Hearts were subjected to five repeated 5-minute episodes of zero-flow ischemia. Each ischemic episode was followed by a

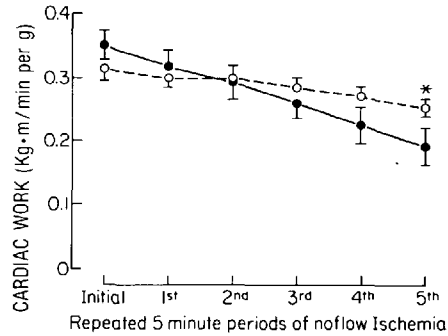

Fig. 7. Effects of repeated episodes of zero-flow ischemia and reperfusion on the recovery of cardiac work in hearts perfused with 1.2 mM palmitate, 5.5 mM glucose, 2 mU/ml insulin and 11 mM L-acetylcarnitine (dashed line) and without L-acetylcarnitine (solid line). Each point represents mean ± S.E. for 13 hearts. *$p < 0.05$ level of significance between two groups.

Table 1. Effects of L-acetylcarnitine (L-AC) on myocardial levels of carnitine and long-chain acyl CoA.

Group (n)	Carnitine			Long-chain Acyl CoA
	Acid-soluble	Long-chain acyl (nmoles/g dry wt.)	Total	
40′ WH[1]) (4)	3259 ± 279	980 ± 104	4241 ± 413	191 ± 12
30′ I control (14)	2051 ± 48[2])	2472 ± 243[2])	4708 ± 398	390 ± 26[2])
L-AC (14)	–	–	–	352 ± 26[2])
30′ I, 15′ R control (12)	3295 ± 266	1338 ± 154	4497 ± 242	274 ± 29[2])
L-AC (8)	4553 ± 500[2,3])	641 ± 112[3])	5202 ± 566	174 ± 24[3])

[1]) All hearts were perfused for the times indicated with 1.2 mM palmitate, 5.5 mM glucose, and 2 mU/ml insulin. WH, I, and R represent working heart, ischemia and reperfusion, respectively. The concentration of L-acetylcarnitine (L-AC) was 11 mM. All values are mean S.E. for number shown in parentheses.

[2]) $P < 0.05$ level of significance between 40′ WH group and experimental groups.

[3]) $P < 0.05$ levels of significance between control and L-AC groups.

5-minute Langendorff perfusion and a 10-minute working heart perfusion. Cardiac work was measured at the end of this 10-minute period. Figure 6 shows a progressive decline in the recovery of cardiac work after each episode of ischemia. The rate of decline was very consistent and uniform between hearts, as opposed to the more variable recovery of cardiac work observed when hearts were subjected to longer periods of zero-flow ischemia. The concentration of exogenous free fatty acid (0.4 versus 1.2 mM palmitate) had no effect on the rate of decline. Addition of L-acetylcarnitine to the perfusion media had no effect on the recovery of cardiac work in hearts perfused with low levels of exogenous free fatty acid (data not shown). In hearts perfused with elevated exogenous free fatty acid (1.2 mM palmitate), acetylcarnitine slightly but significantly ($p < 0.05$) improved the recovery of cardiac work after the fifth episode of zero-flow ischemia (fig. 7).

Table 1 shows the effects of L-acetyl carnitine on myocardial levels of acid-soluble (free plus short-chain acyl carnitine), long-chain acyl carnitine, total carnitine, and long-chain acyl coenzyme A. Thirty minutes of low-flow ischemia produced a significant ($p < 0.01$) increase in the levels of long-chain acyl carnitine and long-chain coenzyme A. L-acetylcarnitine had no significant effect on long-chain coenzyme A. Because of L-acetylcarnitine trapped in the ventricular chamber, coronary vasculature, and extracellular space, it was impossible to determine accurately the amount of intracellular carnitine in ischemic hearts perfused with 11 mM L-acetylcarnitine. Carnitine measurements were possible in reperfused hearts because these spaces were washed out by Langendorff perfusion (see Methods). Reperfusion of control ischemic hearts decreased long-chain acyl carnitine and long-chain acyl coenzyme A. L-acetylcarnitine produced an even greater reduction of these compounds. We also measured the myocardial levels of ATP, ADP, AMP, and total adenine nucleotide during low-flow ischemia and reperfusion and found no significant effect of L-acetylcarnitine.

Discussion

We have shown a number of metabolic and physiologic differences between zero-flow and low-flow ischemia. As we (27) and others (16, 34) have shown previously, low-flow

ischemia in the isolated perfused rat heart causes an accumulation of long-chain acyl carnitine and coenzyme A esters. *In vitro* experiments with isolated enzymes and subcellular fractions have shown that these compounds can inhibit a number of enzyme activities and mitochondrial energy production (3, 13, 26). In high concentrations, long-chain acyl CoA and acyl carnitine have detergent effects on cell membranes (13). Long-chain acyl carnitine inhibits sarcolemmal Na^+-K^+-ATPase, Ca^{++}-ATPase, Ca^{++} binding of sarcoplasmic reticulum (3). Long-chain acyl CoA inhibits a number of mitochondria-linked enzyme systems, including the adenine nucleotide translocator (26). These findings have led to the suggestion that accumulation of these intermediates of lipid metabolism by the ischemic myocardium may be detrimental and may contribute to the genesis of irreversible damage (13, 16, 26, 34).

We have shown that these esters do not accumulate in all forms of ischemia. Zero-flow ischemia caused no change in the levels of long-chain acyl carnitine and only a small increase in long-chain acyl coenzyme A, which was not maintained with longer durations of ischemia. Similar results were found by Neely et al. (20). Since long-chain acyl carnitine and coenzyme A do not build up in all models of ischemia, one possible interpretation could be that accumulation of these compounds is not harmful to the heart and that they may not contribute to irreversible damage during myocardial ischemia (20). However, accumulation of lipid intermediates, along with other biochemical changes, may cause irreversible damages during low-flow ischemia, while a different set of biochemical reactions may lead to cellular death in zero-flow ischemic tissue. For instance, the rapid and severe depletion of high-energy phosphate compounds during zero-flow ischemia may be the principal cause of irreversible damage. Jennings et al. have hypothesized that marked ATP depletion results in irreparable loss of membrane function and thereby mediates the onset of irreversible damage (12). The rapid depletion of high-energy phosphate compounds during zero-flow was most likely caused by elimination of oxygen delivery to the tissue, at which point all ATP produced must have come from anaerobic glycolysis. In low-flow ischemia, there is still some delivery of oxygen, which may allow for oxidative metabolism (23).

Several studies have suggested that excess free fatty acids are harmful to the heart during ischemia. Oliver et al. (22) showed that raised plasma levels of free fatty acids may induce or facilitate arrhythmias in patients with myocardial infarction, while Opie et al. (24) suggested that elevated plasma free fatty acid may extend the infarct area. These studies have been confirmed in a number of studies using experimental animals (10, 14, 16). We confirmed these results using low-flow ischemia in the isolated perfused rat heart. However, we found that elevated exogenous free fatty acids are not harmful during zero-flow ischemia. Excess free fatty acids during low-flow ischemia may be harmful because they cause a progressive build-up of long-chain fatty acyl coenzyme A, carnitine derivatives, and other lipid compounds which we believe are detrimental to the heart, as discussed previously (3, 13, 16, 27, 34). Since zero-flow ischemia does not cause an accumulation of these lipid compounds, we would not expect free fatty acids to be harmful in this case.

We also compared the possible protective effects of L-acetylcarnitine against low-flow and zero-flow ischemia and found that L-acetylcarnitine improved the recovery of cardiac work after low-flow ischemia using both high and low levels of exogenous free fatty acid. With repeated episodes of zero-flow ischemia, L-acetylcarnitine had no effect on the recovery of cardiac work in hearts perfused with low levels of exogenous free fatty acid and only a small but significant effect with high levels of free fatty acid.

There are two hypothetical mechanisms by which L-acetylcarnitine may have protected the ischemic myocardium: hemodynamically, by increasing coronary flow; or metabolically, by stimulating flux through the citric acid cycle and lowering accumulated fatty acid intermediates.

We have shown that L-acetylcarnitine, in millimolar concentration range, is a potent coronary vasodilator in the open-chest dog (unpublished observations).

In the isolated perfused working rat heart model, the oxygen supply to the heart is controlled by the rate of coronary flow (21). During the ischemic period, coronary flow was mechanically set at 1.2 ml/min. With reperfusion, no difference in coronary flow was found between acetylcarnitine and control hearts. Therefore the beneficial effect of acetylcarnitine is probably due not to a hemodynamic effect, but to metabolic changes.

Acetylcarnitine plays a central role in cardiac metabolism through the enzyme carnitine acetyltransferase (9), which permits the equilibration of mitochondrial acetyl-CoA/CoA and acetylcarnitine/carnitine couples. Through this reaction, acetylcarnitine can buffer the fluctuation in the acetyl CoA pool of mitochondria and thus may be required for normal cardiac function (31). Because L-acetylcarnitine can readily be converted to acetyl CoA, it may stimulate flux through the citric acid cycle and lower the accumulation of lipid intermediates during low-flow ischemia. Since these compounds do not accumulate during zero-flow ischemia, this may explain why L-acetylcarnitine was beneficial during low-flow ischemia but was only partially effective during zero-flow ischemia. Table 1 shows that L-acetylcarnitine had no effect on long-chain acyl coenzyme A levels during ischemia; but with reperfusion, it produced a significantly greater lowering of long-chain acyl coenzyme A. It was not possible to accurately measure carnitine esters during ischemia because of contamination with acetylcarnitine. Tissue levels were determined in reperfused hearts after a washout period, and L-acetylcarnitine-perfused hearts had significantly lower levels of long-chain acyl carnitine.

The uptake of free carnitine and acetylcarnitine is very slow (19) and may limit its protective effect. This may explain why no significant effect on tissue adenine nucleotide was found.

In summary, we have found a number of metabolic differences between zero-flow and low-flow ischemia in the isolated perfused rat heart. Low-flow ischemia results in an accumulation of long-chain acyl coenzyme A and acyl carnitine esters, while zero-flow ischemia causes no change in long-chain acyl carnitine and only a smaller transient increase in long-chain acyl coenzyme A. High-energy phosphate depletion was greater in zero-flow ischemia. Excess exogenous free fatty acids were harmful during low-flow ischemia but not in zero-flow ischemia. L-acetylcarnitine was shown to be beneficial during low-flow ischemia but only slightly effective during zero-flow ischemia. We were unable to determine precisely the mechanism of L-acetylcarnitine's protective effects, but we hypothesize that L-acetylcarnitine may decrease the accumulation of toxic lipid intermediates, at least during reperfusion, and may supply a more readily available energy substrate.

Acknowledgments

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