Time Course of Changes in Phospholipid in Cardiac Mitochondria from Rats Recovering from Prolonged Swimming

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SUMMARY

The time course of the recovery of cardiac mitochondrial phospholipids was investigated in rats subjected to a 3 hour loaded swim. The 3 hour swim caused a 50% reduction in mitochondrial phospholipids compared with the level in sedentary controls. During the first 12 hours of the recovery period, phospholipids tended to increase but fell again before reaching control values by 120 hours after the swim ended. Phospholipid recovery in microsomes was completed in 12 hours. Total mRNA in cardiac tissue decreased by about 15% in rats subjected to a 3 hour loaded swim but recovered to control levels in the first 12 hours of the sedentary recovery period. Thus, fluctuations in cardiac mitochondrial phospholipids do not parallel the changes in total mRNA in cardiac tissue. (Jpn Heart J 35: 345–351, 1994)

Key words: Prolonged loaded swim Phospholipids Mitochondria Microsome Heart Recovery mRNA

Previous studies have shown that a 3 hour loaded swim produces approximately a 50% decrease in cardiac mitochondrial contents of phosphatidylethanolamine (PE) and phosphatidylcholine (PC) from the levels observed in sedentary rats.1) Phospholipase A2 activity in the microsomal fraction of cardiac tissue also increased following the prolonged swim. Preadministration of coenzyme Q10 partially suppressed both the decrease in phospholipids and the increase in phospholipase A2 activity after the swim.2)

Changes in phospholipid contents during total ischemia have been studied extensively. Total ischemia for several hours caused only a slight decrease in phospholipid content in canine hearts.3–6) The reperfusion, however, caused a 30% decrease in mitochondrial phospholipids in arrhythmically beating canine hearts.7) These results suggest a close relationship between cardiac function and membrane phospholipid contents. Some phospholipids are essential for the functions of membrane enzymes, and bulk phospholipids serve as a spacer for mem-
brane enzymes to protect them from aggregation and loss of function. Decreases in phospholipids may, therefore, result in depression of cardiac functions. In an analogy with the case of reperfusion, the phospholipid decrease after a prolonged swim may depress cardiac functions. Since slow recoveries in function after exhaustion are well described in skeletal muscle, it is important to determine whether the cardiac phospholipid recovery process after exhaustive exercise follows a similar time course. Several factors suggest that mitochondrial phospholipids are an important site to examine the recovery process after exhaustive exercise. Since oxygen radicals are produced near the inner membrane of mitochondria and the oxygen radical scavenger system is poor in mitochondria, mitochondrial membranes consisting of phospholipids may be open to peroxidation during severe exercise. Phospholipase A2 shows an easier access to peroxidized phospholipids. Moreover, mechanical stimulation of cultured skeletal muscle increases phospholipase A2 activity. Membrane phospholipids of mitochondria are synthesized by enzymes tightly bound to the endoplasmic reticulum, carried by specific proteins and inserted into mitochondrial membranes. Enzymes for synthesis of phospholipids are not coded on mitochondrial DNA. Changes in mitochondrial phospholipids, therefore, are of great interest in the cardiac tissues from animals exposed to exhaustive exercise.

Another area of interest was the question of whether mRNA responsible for the synthesis of biomolecules changed concomittantly with cardiac mitochondrial phospholipids during the recovery period. Relatively small effects on actin mRNA are observed in skeletal muscle after a single bout of concentric contraction. Similarly, cytochrome C mRNA showed only a small increase in skeletal muscle in response to exercise. Lipoprotein lipase mRNA was not increased in rat heart immediately after a 2 hour swim in contrast to a clear increase in skeletal muscles. On the other hand, a rapid and marked increase in cardiac mRNA has been found in response to catecholamine application to cultured cardiac cells and in the pressure overloaded heart. Measurement of mRNA levels in cardiac tissues after exercise are thus of interest. Since our exercise experiment protocol, a 3 hour loaded swim in which cardiac phospholipids markedly decreased, was more severe than the exercise experiments reported by other investigators, it seemed likely that clear changes in mRNA would be demonstrable under our experimental conditions. Since no method of measuring phospholipid mRNA is available in our laboratory at present, only total mRNA in cardiac tissue together with the time course of changes in mitochondrial phospholipids were measured in the present study.
**METHODS**

Eight-week-old male Wistar rats were used in the experiments. They were trained to swim for 30 minutes/day for 4 days in plastic barrels with water at 35°C. Thirty rats were subjected to loaded swim. Their loads were increased over the 4 day period from 0 to 3% of body weight. On the fifth day twenty-five rats were subjected to a 3 hour swim with a load representing 3% of body weight. They were sacrificed by decapitation at five time points: immediately after completion of the 3 hour swim, and after sedentary recovery periods of 12, 24, 48 and 120 hours after completion of the swim. The five remaining rats, trained for four days in the same way, were placed in 3 cm of water at 35°C for 3 hours in a barrel; they remained sedentary and were used as controls. Hearts were isolated after a quick decapitation and put in a buffer solution containing 0.25M sucrose and 5 mM Tris-HCl (pH 7.4) and then cut into small pieces, to which alkaline proteinase (5 mg/g wet-weight) was added. After 20 minutes the tissue suspension was homogenated with a Polytron (Kinematica GMBH, Littau, Switzerland) at power scale 5 for 0.5 sec and centrifuged at 900 g × 10 min. The mitochondrial pellet was obtained by centrifugation of the supernatant at 14,000 g × 10 min. The resulting supernatant was centrifuged at 80,000 g for 80 minutes and a microsomal pellet was obtained. Pellets were washed three times by resuspending and centrifuging, and then used for lipid measurements. Methods for lipid extraction, phospholipid separation, and phosphorus and protein measurements were as described previously.\textsuperscript{1,2} Cholesterol was measured according to the routine method.\textsuperscript{20}

Differences were assessed by the one way analysis of variance with time after exercise as a factor, followed by Dunnett’s test for multiple comparisons, and considered significant when \( p < 0.05 \).

Another set of experiments was done to measure mRNA levels. Nine rats received the same training. Three were sedentary controls. Six rats were subjected to a 3 hour loaded swim; three were sacrificed immediately after the swim ended, and two after a 12 hour recovery period (one sample was accidentally lost). A 0.5 g sample of left ventricular muscle from each isolated heart was minced and homogenized with a Polytron homogenizer. The level of mRNA was measured with a Quick Prep mRNA purification kit (Pharmacia LKB Biotechnology, Sweden) according to the instructions.

**RESULTS**

The mean PC and PE levels in mitochondria decreased by 46 and 45%, respectively, after the 3 hour swim. Levels tended to increase during the first 12
Figure 1. The phospholipid (PL) contents of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in rat cardiac mitochondria after a 3 hour loaded swim and after recovery periods of 12, 48 and 120 hours (mean ± SEM). *p < 0.05, **p < 0.01.

Figure 2. The phospholipid (PL) contents of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in rat cardiac microsomes after a 3 hour loaded swim and recovery periods of 6, 12, 24 and 48 hours (mean ± SEM). *p < 0.05, **p < 0.01.

Hours of the sedentary recovery period. However, they were reduced significantly at 24 hours, then slowly increased to the control value by the 120 hour time point (Figure 1). Cholesterol content decreased concomitantly to 0.14±0.01 nmol/mg protein from the control value of 0.31±0.05 nmol/mg protein (p < 0.05) and recovered to 0.33±0.04 and 0.31±0.02 nmol/mg-protein at 6 and 12 hours, respectively. Levels of PE and PC decreased in microsomes after the loaded swim but increased to values higher than in controls during a 12 hour sedentary recovery period (Figure 2).

Total mRNA showed only small fluctuations. The level tended to be slightly
Figure 3. The total mRNA level in rat cardiac tissue after a 3 hour loaded swim and a recovery period of 12 hours.

reduced after the 3 hour swim, but recovered to a level similar to the control value during the 12 hour sedentary period (Figure 3).

DISCUSSION

In agreement with our previous experiments, cardiac mitochondrial phospholipids decreased significantly after a 3 hour loaded swim.1,2) Following a marked fall, phospholipid levels recovered transiently, then fell before recovering to control values after 120 hours. The cause of this fluctuation remains unknown, but it is noteworthy that the time required for recovery greatly exceeds the duration of the exercise. Mitochondria have no DNA for phospholipid syntheses. By contrast, microsomal phospholipids recovered rather quickly in 6 to 12 hours. It is well documented that phospholipids are synthesized on endoplasmic reticulum and transported to mitochondria to repair membranes.13,14) Thus, it is rather reasonable that the recovery in mitochondrial phospholipids required a longer time than that in microsomes. Mechanisms of the rather quick recovery of cholesterol content needs study in the future.

The time courses of recovery of PC and PE seemed different in microsomes. The recovery process was quicker for PC than for PE. The cause for this difference may be attributed to the higher rate of synthesis for PC than PE in vivo.21)

We expected that the rapid and marked decrease in cardiac mitochondrial phospholipids might be accompanied by an increase in cardiac mRNA to accelerate phospholipid synthesis and transport to mitochondria. Contrary to this expectation, mRNA tended to show a slight decrease immediately after the 3 hour loaded swim. This result appears to be consistent with the reported decrease in protein synthesis in the 1 to 2 hour period following exercise22) and with the decrease in tyrosine hydroxylase mRNA produced in young rats by exercise.23) Booth22) hypothesized that mRNA decreases as cellular high-energy phosphates
decrease and cellular pH falls. Such metabolic changes may have occurred during the present exercise period and probably caused the slight decrease in mRNA level. In other words, the small decrease in mRNA may be a general phenomenon due to fatigue.

One might expect mRNA levels to increase during the recovery period to replace biomolecules, including phospholipids, degraded during exercise. This expectation, however, was not fulfilled. In the 12 hour sedentary recovery period, mRNA showed values similar to controls and apparently remained at that level thereafter. Clearly mRNA levels are more stable than phospholipid levels.

REFERENCES


