High Energy Phosphates During Hibernation and Arousal in the
Ground Squirrel

MARILYN L. ZIMNY AND ROY GREGORY

From the Department of Anatomy, Louisiana State University School of Medicine,
New Orleans, Louisiana

ABSTRACT

ZIMNY, MARILYN L. AND ROY GREGORY. High energy phosphates during hibernation and arousal in the ground squirrel. Am. J. Physiol. 195(1): 233--236. 1958.—Biochemical levels of inorganic phosphate, adenosine triphosphate, phosphocreatine and glycogen were determined on cardiac muscle, skeletal muscle and liver samples from hibernated 13-striped ground squirrels and those allowed to awaken for intervals of 7.5, 15 and 30 minutes. Cardiac muscle glycogen increases during hibernation apparently at the expense of skeletal muscle and liver glycogen. Glycolysis occurs in these tissues during early arousal, followed later by glycogenesis. Adenosine triphosphate is maintained in both cardiac and skeletal muscle during hibernation and is used as an energy source during arousal. It appears that glycolysis is important in resynthesizing phosphocreatine. From this study of periods of relatively low and high metabolic demands we conclude that phosphocreatine is a 'transport' form of high-energy phosphate forming adenosine triphosphate from the phosphate pool when and where needed in the cycle of intermediary metabolism.

Previous investigations concerning hibernation (1) and the arousal process (2) in the 13-striped ground squirrel, Citellus tridecemlineatus, have indicated a storage of high-energy phosphate as phosphocreatine in cardiac and skeletal muscle during hibernation with a subsequent shift to adenosine triphosphate during arousal. Although the functional relationships of high-energy phosphate compounds to muscle contraction still remain somewhat obscure, the present study indicates that adenosine triphosphate and phosphocreatine should be considered as working together with phosphocreatine acting as a 'transport' form from both glycolytic processes and the phosphate pool. Apparently the enzymes related to these compounds react at such a rapid rate that those values once thought representative of hibernating levels actually represent the early stages of arousal.

With the data now available it has become evident that the high-energy phosphate compounds are relatively maintained during hibernation with rapid changes early in arousal followed by gradual adjustments upon return to the nonhibernating state.

MATERIALS AND METHODS

Cardiac muscle, skeletal muscle and liver samples were obtained for comparison from five groups of animals as follows: a) 21 control animals killed under Nembutal anesthesia, tissue frozen in situ with an ether-dry ice mixture and immediately removed; b) 11 hibernated (3–5 days) animals, tissue frozen in situ and removed within 30 seconds of handling; c) 15 animals removed from the cold room (3–5°C) to the laboratory (25–27°C) and killed 15 minutes later, constituting the 15-minute aroused group; d) 17 animals removed from the cold room (3–5°C) to the laboratory (25–27°C) and killed 30 minutes later, constituting the 30-minute aroused group; and e) 27 animals representing the experimental groups used previously for phos-
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Body weight, rectal temperature, determined by means of an iron-constantan thermocouple; heart rate, determined from an electrocardiographic tracing; and respiration rate, taken with a stopwatch, were recorded on all animals except those in the hibernated group b. Tissue inorganic phosphate (IP), adenosine polyphosphate, represented as adenosine triphosphate (ATP), and phosphocreatine (PC) were determined by the method of Wollenberger (3) and tissue glycogen by the method of Kemp and Heijningen (4).

RESULTS

Changes in phosphate and glycogen levels for cardiac muscle are given in figure 1. The total high-energy phosphate level is maintained during hibernation while IP significantly decreases 20% (P < .05). Although glycogen shows a 35% increase, it is not significant (P < .10) when the 7.5-minute aroused group is compared with the hibernated group. Inorganic phosphate increases 38%, PC increases 68%, ATP decreases 70% and glycogen decreases 55%. It seems that ATP is an immediate source of energy in the arousal process with glycolysis not only increasing PC, which will subsequently transfer high-energy phosphate to ATP, but also increasing IP.

When the 15-minute arousal period is compared with that of 7.5 minutes, both PC and IP show significant (P < .01) decreases, 60% and 23%, respectively. Adenosine triphosphate makes a 45% gain, which is not significant (P < .2), and the phosphate levels are beginning to resemble the control pattern.

Although ATP makes another gain of 40% in going from the 15-minute to the 30-minute arousal period it is not significant (P < .1). However, this second increase is bringing it closer to the control level. The increases of tissue glycogen seen in the 15-minute and 30-minute arousal groups may be related to processes of glycogenogenesis. Neither represents a significant change over the preceding value.
Changes in phosphates and glycogen for skeletal muscle are given in figure 2. Phosphocreatine significantly increases 56% ($P < .01$) during hibernation apparently at the expense of a 37% decrease in IP ($P < .01$) and a 25% decrease in glycogen ($P < .2$). All phosphate fractions once again show significant changes ($P < .01$) when the 7.5-minute arousal period is compared with that of hibernation. Both PC and ATP decrease, 25% and 61%, respectively, but ATP shows the greater decrease because it is the preferred form of high-energy phosphate during the early stages of arousal. In addition to this glycolysis may be aiding in maintaining the PC level.

During the 15-minute arousal period when compared with that of 7.5 minutes phosphocreatine significantly decreases 46% ($P < .01$) indicative of a transfer of high-energy phosphate which shows by 30 minutes of arousal with a 114% increase ($P < .01$) of ATP. The increase in glycogen during the later two arousal periods may once again be due to glyconeogenesis.

Liver phosphate levels related to the 7.5-minute arousal were not determined but these compounds can be compared among the other groups. Adenosine triphosphate increases significantly, 121% ($P < .01$) and PC decreases significantly, 62% ($P < .01$) during hibernation as compared with control animals.

When compared with hibernation 15 minute arousal shows ATP decreasing 47% ($P < .01$) while PC increases 44% ($P < .2$) but by 30 minutes ATP has increased 22% ($P < .3$) and PC has decreased 20% ($P < .4$). Although liver glycogen decreases during hibernation and increases during arousal no statistically significant changes were noted between the various groups. Considering the metabolic processes related to the liver it may be that during hibernation the lowered metabolic demand allows ATP to accumulate for use to stimulate these processes upon awakening.

In considering these phosphate and glycogen fluctuations it is also interesting to note the increases of body temperature, heart rate and respiration rate. After the 15-minute arousal period the following increases occurred from the cold room: body temperature 75%, a gain of six degrees; heart rate 192%, a gain of 71 beats; and respiration 260%, a gain of 44 inspirations. After the 30-minute arousal period the increases were as follows: body temperature 113%, a gain of 9 degrees; heart rate 644%, a gain of 174 beats; and respiration rate 543%, a gain of 76 inspirations.

**DISCUSSION**

Uncertainty still exists as to the active roles of ATP and PC in the contractile mechanisms of cardiac and skeletal muscle (5-7). This study of these high-energy phosphates during hibernation and arousal in the ground squirrel serves as a method for comparison of these compounds during periods of relatively low and high metabolic demands.

Cardiac and skeletal muscle are considered the main sources of heat during arousal from hibernation (8). Cardiac muscle glycogen increases during hibernation apparently at the expense of skeletal muscle and liver glycogen (9, 10). During arousal in the hamster it was found that when the body temperature rose rapidly there was a marked loss of glycogen from liver, muscle and heart (9). In our work on the ground squirrel we see this same pattern during the early stages of arousal but in later stages glycogen increases in those tissues and the explanation at present seems to be that of glyconeogenesis.

Cardiac muscle maintains its PC and ATP levels during hibernation, but probably does not while the animal is entering hibernation nor during arousal. Apparently even at a greatly reduced heart rate and work load, utilization and generation of phosphate bond
energy has reached an equilibrium. This follows the studies of Bing (11) on extracted heart muscle illustrating that changes in work can occur without alterations in the supply of high-energy phosphates and the studies of Wollenberger (3) with heart-lung preparations in which wide variations in ‘volume’ work had little effect on ATP and PC levels in the heart. Since ATP is considered the active form of high-energy phosphate (12) it is used preferentially during the early arousal period while glycolysis affects an increase in PC. We know from the work of Bing (11) that the heart can use carbohydrate as well as noncarbohydrate substances for metabolic energy and the utilization is not influenced by cardiac work but by their respective arterial concentrations. In working with normal muscle Mommaerts (13) found that in muscle activity the splitting of PC is the ‘energetic master reaction’ but soon after activity begins glycolysis increases and affects a steady resynthesis of PC. It has also been found that in the regulation of metabolism glycolysis is inversely dependent upon the concentration of ATP (14). Consequently in the 7.5-minute arousal group the immediate use of ATP may be accelerating glycolysis within the tissue. Following 15 minutes of arousal it appears that PC has transferred phosphate to ATP and possibly to other metabolic systems (15) which may contribute to glyconeogenesis. By 30 minutes of arousal the pattern is approaching that of the control.

Skeletal muscle also maintains ATP during hibernation but PC increases apparently as a result of glycolysis (13). Since it has been found that during the first few minutes of muscular work PC and ATP decrease and IP increases (16, 17) this may well account for the changes which occur in the first stages of arousal. Skeletal muscle activity lags behind that of cardiac muscle activity so that the 15-minute arousal period has the same pattern as that of the 7.5 and it is not until the 30-minute period that a phosphate transfer from PC to ATP takes place to maintain the increased activity at this time. A return to the control pattern is not yet seen by 30 minutes as in cardiac muscle. However, this is explainable on the basis of an increase of 644% in heart rate during the 30-minute period. Although electrical potential measurements were not made on the hind leg muscles, movement was in no way comparable.

Liver phosphates seem to fluctuate with the glycolytic-glycogenic-glyconeogenic mechanisms present within the organ. The inverse relationship between ATP concentration and glycolysis is represented in this pattern (14). During hibernation, with low metabolic demand, ATP increases as glycolysis is occurring slowly while during 15 minutes of arousal, with high metabolic demands, ATP decreases as glycolysis proceeds rapidly. This glycolysis aids in increasing the PC level and by 30 minutes of arousal it is more evident that PC is transferring phosphate to ATP. At this time glycolysis is reaching equilibrium and glyconeogenesis becomes apparent.

REFERENCES